Semester – V

16MBU504A **MICROBIAL BIOTECHNOLOGY** **(3H – 3C)**

Instruction Hours / week: L: 3 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

This paper imparts knowledge on applications of microorganisms in various fields.

OBJECTIVE

- To make students understand the aspects of industrial, soil, environmental, agricultural microbiology.

Unit I – Microbial Biotechnology and its Applications

Microbial biotechnology: Scope and its applications in human therapeutics, agriculture (Biofertilizers, PGPR, Mycorrhizae), environmental, and food technology. Use of prokaryotic and eukaryotic microorganisms in biotechnological applications. Genetically engineered microbes for industrial application: Bacteria and yeast

Unit II – Therapeutic and Industrial Biotechnology

Recombinant microbial production processes in pharmaceutical industries - Streptokinase, recombinant vaccines (Hepatitis B vaccine). Microbial polysaccharides and polyesters, Microbial production of bio-pesticides, bioplastics, Microbial biosensors

Unit III – Applications of Microbes in Biotransformations

Microbial based transformation of steroids and sterols. Bio-catalytic processes and their industrial applications: Production of high fructose syrup and production of cocoa butter substitute

Unit IV – Microbial Products and their Recovery and RNAi

Microbial product purification: filtration, ion exchange & affinity chromatography techniques Immobilization methods and their application: Whole cell immobilization. RNAi and its applications in silencing genes, drug resistance, therapeutics and host pathogen interactions.

Unit V – Microbes for Bio-energy and Environment, Intellectual Property Rights

Bio-ethanol and bio-diesel production: commercial production from lignocellulosic waste and algal biomass, Biogas production: Methane and hydrogen production using microbial culture. Microorganisms in bioremediation: Degradation of xenobiotics, mineral recovery, removal of heavy metals from aqueous effluents. Patents, patenting fundamental requirements- patent multicellular organisms, IPR, Copyrights, Trademarks

SUGGESTED READINGS

1. Ratledge, C and Kristiansen, B. (2001). Basic Biotechnology, 2nd edition, Cambridge University Press.
2. Demain, A. L and Davies, J. E. (1999). Manual of Industrial Microbiology and Biotechnology, 2nd edition, ASM Press.
3. Swartz, J. R. (2001). Advances in Escherichia coli production of therapeutic proteins. Current Opinion in Biotechnology, 12, 195–201.
4. Prescott, Harley and Klein's Microbiology by Willey JM, Sherwood LM, Woolverton CJ (2014), 9th edition, Mc Graw Hill Publishers.
5. Gupta PK (2009) Elements of Biotechnology 2nd edition, Rastogi Publications.
6. Glazer AN and Nikaido H (2007) Microbial Biotechnology, 2nd edition, Cambridge University Press
7. Glick BR, Pasternak JJ, and Patten CL (2010) Molecular Biotechnology 4th edition, ASM Press.

8. Stanbury PF, Whitaker A, Hall SJ (1995) Principles of Fermentation Technology 2nd edition, Elsevier Science.
9. Crueger W, Crueger A (1990) Biotechnology: A text Book of Industrial Microbiology 2nd edition. Sinauer associates, Inc.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB COURSE NAME: MICROBIAL BIOTECHNOLOGY
COURSE CODE: 16MBU504A LECTURE PLAN BATCH-2016-2019

LECTURE PLAN

UNIT1

Duration	Topic	Reference
01	Microbial biotechnology. Scope and its application in human therapeutics	T1:149-159 T2: 189-198
02	Gene therapy	T3:483-486 T2:157-162
03	Agriculture: Biofertilizer, PGPR, Mycorrhizae & Transgenic plants	T3:9-13
04	Application in Environmental field wastewater treatment	R1: 235-243 T2:679-684
05	Bioremediation & metal extraction	T2:718-726
06	Application in food industry –Novel protein & Food products production	T1: 412-425
07	Use of prokaryotic & Eukaryotic microorganism in BT application	T2:81-82 R1:153-165
08	Genetic engineeringof Bacteria & Yeast	T3194-198
09	Unit revision	
	Total hours: 09	

T1: R.C.Dubey.2005.Text Book of Biotechnology,S.Chand.publishers
T2:U.Sathyanarayana.2005.Arunabhesen publisher.
T3: V.Kumaresan .2008. Biotechnology. Saras publication.
R1:Bernard.R.Glick and Jack J. Pasternak.Molecular Biotechnology .2002.Panima Publication.

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COURSE CODE: 16MBU504A LECTURE PLAN BATCH-2016-2019

LECTURE PLAN

UNIT2

Duration	Topic	Reference
01	Streptokinase production – Bacteria /Plasmid	W1
02	Recombinant Vaccine –Production, Hepatitis B vaccine	T2:200-201
03	Production of microbial polysaccharide ,biosynthesis& recovery	T2:382-386
04	Commercial polysaccharides/genetic engineered products	W2
05	Microbial polyesters -PHA& Future.	T2:386-388
06	Microbial production of biopesticide, BT Toxin & mode of action& product.	T3-415-420 T4503-505
07	Biodegradable bioplastic & Biosensor application in health & environment.	T2:390-39 T2:297-304
08	Unit Revision	
	Total hours: 08	

T4: P.K.Gupta,Elementsof Biotechnology.Rastogi Publication.2009.
R2:Prescott,Harley & Klein's Microbiology by Willey JM,SherwoodL.M.2014- McGraw Hill publishers.
W1: WWW.Omicsonline.org
W2: WWW.biologydiscussion.com

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CLASS: III B.Sc MB COURSE NAME: MICROBIAL BIOTECHNOLOGY
COURSE CODE: 16MBU504A LECTURE PLAN BATCH-2016-2019

LECTURE PLAN

UNIT3

Duration	Topic	Reference
01	Microbial based transformation of steroids and sterols	T2:308-310
02	Biotransformation by Microbial system	W3
03	Alternative process & application	W3
04	Production of High Fructose syrup	T2-296-297
05	Genetic engineering technology and future	T2-367-368
06	Safety of biotechnology Food product	R1-247-249
07	Coccoa Butter substitutes property and safety	W4
08	Unit Revision	
	Total hours: 08	

T2:U.Sathyanarayana.2005.Arunabhesen publisher.
R1:Bernard.R.Glick and Jack J. Pasternak.Molecular Biotechnology .2002.Panima Publication.
W1: WWW.Sciencedirect.org
W2: WWW.knowledgealprospector.com

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COURSE CODE: 16MBU504A LECTURE PLAN BATCH-2016-2019

LECTURE PLAN

UNIT4

Duration	Topic	Reference
01	Microbial product purification	T6:126-140
02	Ion exchange & Affinity chromatography	T5-122-135
03	Immobilization of whole cell & products	T3: 531-535
04	Method:Enzyme entrapment, adsorption,encapsulation,bonding	T1:515-519
05	Polymer materials & application	T1:51-519
06	RNAi application & silence gene	W5
07	Diagnostics& Therapeutics- Host pathogen interaction	W5
08	Unit Revision	
	Total hours: 08	

T6:Keithweilson,Text bookofanalyticalBiochemistry.2002.S.Chand publisher.
T5:Stanbury.P.F,Whitker A,Hall.SJ(1995), Principle of fermentation technology,4th edition.asmpress.
W1: WWW.Sciencedirect.org
W2: WWW.knowledgealprospector.com

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CLASS: III B.Sc MB COURSE NAME: MICROBIAL BIOTECHNOLOGY

COURSE CODE: 16MBU504A LECTURE PLAN BATCH-2016-2019

UNIT5

Duration	Topic	Reference
01	Production of bioethanol & Biodiesel	R1-240-256 T1-565-567
02	Lignocellulosic waste & Algal Biomass	R1-254-258
03	Biogas production;Methane & Hydrogen Production using microbes	T1 – 559-565
04	Bioremediation- Xenobiotics	T2-718-726
05	Mineral recovery& heavy metal removal from effluent.	R1- 236-240
06	Patents, copyright, IPR, Trade mark	R1-443-446
07	Patenting fundamental requiements	R1-446-448 W6
08	Unit Revision	
09	Unit Revision &Old qp	
	Total hours: 09	

W6:www.mondaq.com

UNIT-I

SYLLABUS

Microbial Biotechnology and its Applications

Microbial biotechnology: Scope and its applications in human therapeutics, agriculture (Biofertilizers, PGPR, Mycorrhizae), environmental, and food technology. Use of prokaryotic and eukaryotic microorganisms in biotechnological applications. Genetically engineered microbes for industrial application: Bacteria and yeast.

Microbial Biotechnology: Scope and Techniques

One can be a good biologist without necessarily knowing much about microorganisms, but one cannot be a good microbiologist without a fair basic knowledge of biology! – Stanier, R. Y., Doudoroff, M., and Adelberg, E. A. (1957).The Microbial World. p. vii, Englewood Cliffs, NJ: Prentice-Hall,

Microorganisms, whether cultured or represented only in environmental DNA samples, constitute the natural resource base of microbial biotechnology. Numerous prokaryotic and fungal genomes have been completely sequenced and the functions of many genes established. For a newly sequenced prokaryotic genome, functions for over 60% of the open reading frames can be provisionally assigned by sequence homology with genes of known function. Knowledge of the ecology, genetics, physiology, and metabolism of thousands of prokaryotes and fungi provides an indispensable complement to the sequence database. This is an era of explosive growth of analysis and manipulation of microbial genomes as well as of invention of many new, creative ways in which both microorganisms and their genetic endowment are utilized. Microbial biotechnology is riding the crest of the wave of genomics. The umbrella of microbial biotechnology covers many scientific activities, ranging from production of recombinant human hormones to that of microbial insecticides, from mineral leaching to bioremediation of toxic

wastes. In this post, we sketch the complex terrain of microbial biotechnology. The purpose of this post is to convey the impact, the extraordinary breadth of applications, and the multidisciplinary nature of this technology. The common denominator to the subjects discussed is that in all instances, prokaryotes or fungi provide the indispensable element.

HUMAN THERAPEUTICS

PRODUCTION OF HETEROLOGOUS PROTEINS:

One of the most dramatic and immediate impacts of genetic engineering was the production in bacteria of large amounts of proteins encoded by human genes. In 1982, insulin, expressed from human insulin genes on plasmids inserted into *Escherichia coli*, was the first genetically engineered therapeutic agent to be approved for clinical use in humans. Bacterially produced insulin, used widely in the treatment of diabetes, is indistinguishable in its structure and clinical effects from natural insulin. Human growth hormone (hGH), a protein made naturally by the pituitary gland, was the second such product. Inadequate secretion of hGH in children results in dwarfism. Before the advent of recombinant DNA technology, hGH was prepared from pituitaries removed from human cadavers. The supply of such preparations was limited and the cost prohibitive. Furthermore, there were dangers in their administration that led to withdrawal from the market.Some patients treated with injections of pituitary hGH developed a disease caused by a contaminating slow virus, Jakob–Creutzfeldt syndrome, which leads to dementia

and death. hGH can be produced in genetically engineered *E. coli* in large amounts, at relatively little cost, and free from such contaminants.

Human tissue plasminogen activator (tPA), a proteolytic enzyme (a “serine” protease) with an affinity for fibrin clots, is another therapeutic agent made available in large amounts as a consequence of recombinant DNA technology. At the surface of fibrin clots, tPA cleaves a single peptide bond in plasminogen to form another serine protease, plasmin, which then degrades the clots. This clot-degrading property of tPA makes it a life-saving drug in the treatment of patients

with acute myocardial infarction (damage to heart muscle due to arterial blockage). Recombinant human insulin and hGH offered impressive proof of the

clinical efficacy and safety of human proteins made by engineered microorganisms.

VACCINES

In the early 1990s, attention focused on the potential wide-ranging opportunities offered by DNA vaccines. DNA vaccines consist of appropriately engineered plasmid DNA prepared on a large scale inE. coli. The obvious advantages of DNA plasmid vaccines are that they are not infectious, do not replicate, and encode only the protein(s) of interest. Unlike other types of vaccines, there is no protein component, and hence induction of an immune response against subsequent immunizations is minimized.
A vaccine plasmid includes the following major components: a strong

promoter system for expression in eukaryotic cells of an antigenic protein(e.g., a viral coat protein), the immediate early promoter of cytomegalovirus is frequently used; a cloning site for the insertion of the gene encoding the antigenic protein; and an appropriately located polyadenylation termination sequence. Most eukaryotic mRNAs contain a polyadenylate (polyA) tail at 3'end that appears to be important to the translation efficiency and the stability of the mRNA.The plasmid also includes a prokaryotic origin of replication for its production in *E.coli* and a selectable marker, such as the ampicillin resistance gene, to allow selection of bacterial cells that contain the plasmid. DNA vaccines are generally introduced by intramuscular injection. It is still not known how cells internalize the DNA after the injection.The encoded antigen is then expressed in situ in the cells of the vaccine recipient and elicits an immune response
Such vaccines have attractive features. The immunizing antigens may be derived from viruses, bacteria, parasites, or tumors. Antigens can be expressed singly or in multiple combinations. In one case, the DNA vaccine contained multiple variants of a highly mutable gene, for example, the gene encoding gp120, a glycoprotein located on the external surface of HIV.
In other vaccines, the entire genome of the infectious microorganism was

introduced into a common plasmid backbone by "shotgun cloning." DNA vaccines induce both humoral responses (the appearance of serum

antibodies against the antigen) and cellular responses (activation of various T cells). These responses have been documented in animal models of disease in which protection is mediated by such responses. Important issues remain to be resolved before DNA vaccines can take a regular place alongside other types of vaccines. In clinical trials, vaccines for malaria, hepatitis B, HIV, and influenza elicited only moderate response in human volunteers. An assessment of DNA vaccines encoding certain highly conserved influenza virus proteins concluded that there is a need for considerable enhancement of the immune response to DNA immunization before such vaccines become a promising approach for humans.

Biotechnology in Medicine:

Biotechnology products for therapeutic use include a very diverse range of products, as outlined in Tables 22.4, 22.5. Some products are intended to mimic the human counterpart, whereas others are intended to differ from the human counterpart and may be analogues, chemically modified (e.g., pegylated) or novel products (e.g., single chain or fragment antibody products, gene transfer vectors, tissue-engineered products).

Most of these products are regulated as medicinal products; however, the regulatory status of others such as some cell therapies and tissue: organ-based products differs globally and falls within the borderline between the practice of medicine, medical devices and medicinal products. Different areas of medicine in which biotechnology is used to develop diagnostic kits and cure are presented in the Figure 22.1.

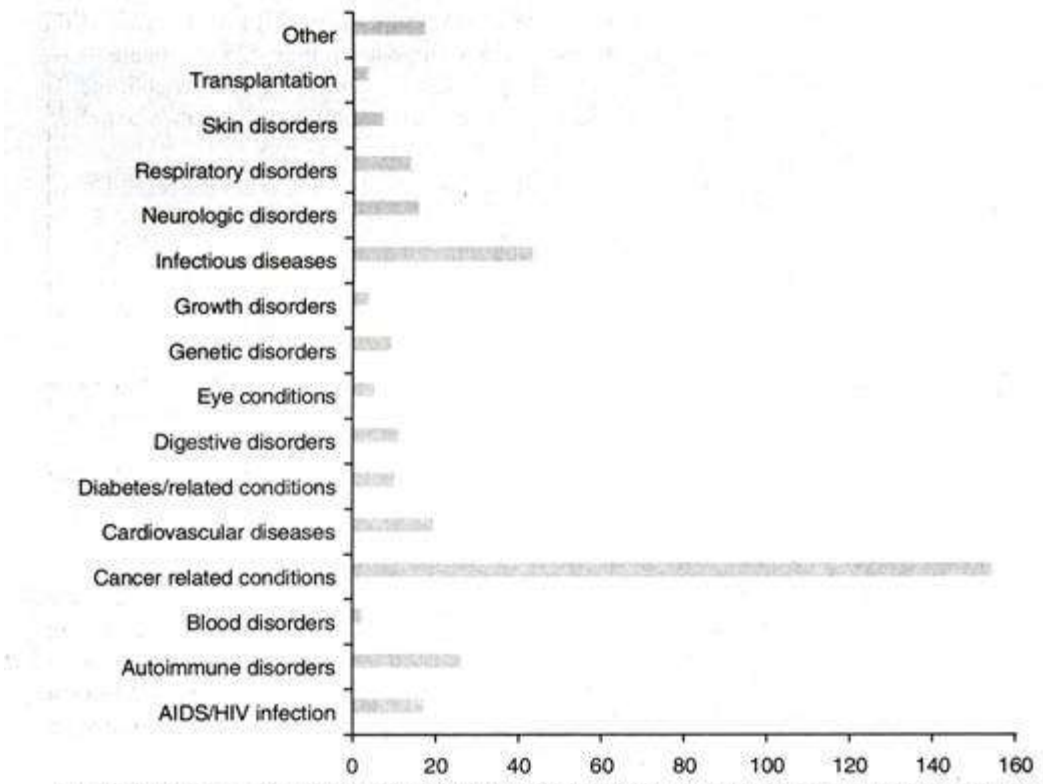

Fig. 22.1. Different areas of medicine in which efforts are being made with the help of biotechnology.

Biotechnology-derived pharmaceuticals may be derived from a variety of expression systems such as Escherichia coli, yeast, mammalian, insect or plant cells, transgenic animals or other organisms. The expressed protein or gene may have the identical amino acid or nucleotide sequence as the human endogenous form, or may be intentionally different in sequence to confer some technical advantage such as an optimized pharmacokinetic or pharmacodynamics profile.

The glycosylation pattern of protein products is likely to differ from the endogenous human form due to the different glycosylation preferences of the expression system used. Furthermore, intentional post-translation modifications or alterations may be made such as pegylation. It is important for the toxicologist to be aware of the nature of the product to be tested in terms of primary, secondary and tertiary structure, and any post-translational modifications such as glycosylation status, particularly as these may be altered if the manufacturing system is modified.

Table 22.4. Biotechnological products in medicine.

Class	Products
Hormones	Follicle stimulating hormone, growth hormone, insulin, insulin analogues
Growth factors	Platelet-derived growth factor, nerve growth factor, insulin growth factor-1
Cytokines	Interferones, interleukins, colony stimulating factor, erythropoietin
Vaccines	Conventional, recombinant protein antigen, modified bacteria or viruses
Nucleic acid based products	Gene therapy, DNA vaccines, ribozymes
Cell, tissue & organs	Autologous, xenoxenix
Others	Clotting factors, enzymes

Biopharmaceutical Drug Development:

In the field of biopharmaceutical drug development, it is the development of therapeutic human proteins by recombinant methods. (Table 22.5) for use as medicines that has the longest tradition. As mentioned above, recombinant human insulin was the first recombinant

medicine in the world, produced by Genentech and brought to market in 1982. Today, recombinant human insulin has almost completely driven the other preparation of insulin (isolated from human or animal tissues) from the market.

Table 22.5. Selected examples of recombinant proteins with indication and manufacturer.

Drug	Product name	Indication	Manufacturer
Human insulin	Humulin	Diabetes mellitus type I	Eli Lilly
Somatotropin	Humatrope	Inadequate growth	Eli Lilly
Erythropoietin alpha	Erypo/Epogen	Anemia	Jansen-Cilag/Amgen
Factor VIII	Bioclate/Kogenate	Hemophilia	Centeon/Bayer
Interferon alpha 2 a	Roferon A	Cancer	Roche
Interferon beta 1 b	Betaferon	Mulitples sclerosis	Schering
Tissue plasminogen activator tPA (alteplase)	Actilyse	Thrombolytic agent	Boehringer Ingelheim

The first therapeutic antibodies, especially monoclonal antibodies, have been on the market since the late 1990s. In 2002, antibodies were (along with vaccines) the most important therapeutic

class of drugs under development and there are also more recent market studies more than 100 antibodies or antibody fragments were at the clinical development stage in 2002 and research and development is being carried out on around 470 more in about 200 companies around the world (Table 22.6,7).

Since the introduction of therapeutic antibodies onto the market, they have achieved significant turnovers, which are growing continually. The market for 2008 is estimated at a volume of US $16.7 billion (from Data-monitor, November 2003). Today, in addition to proteins, which currently play the most significant role in the biopharmaceutical field, new types of drugs based on RNA (antisense drugs, ribozymes, aptamers, Spiegelmers and RNA interference) are also being developed on the basis of advances in knowledge on molecular biotechnology.

Table 22.6. Selected examples of approved monoclonal antibodies.

Drug	Product name	Indication	Manufacturer
Abciximab	Reopro	Anticoagulant	Eli Lilly Centocor Europe
Trastuzumab (anti-HERA2-a)	Herceptin	Breast cancer	Roche
Adalimumab (anti-TNF-alpha)	Humira	Rheumatoid arthritis	Abbott
Infliximab (anti-TNF-alpha)	Remicade	Crohn's disease	Centocor
Alematuzumab (anti CD52)	Campth	Leukemia	Millennium &Ilex

Table 22.7. Selected examples of therapeutic RNAs on the market or under development.

Principle of action	Product name/production stage	Indication	Company
Antisense	Vitravene/market	CMV retinitis	ISIS pharmaceuticals
Antisense	Affinitak/phase II	Cancer	ISIS pharmaceuticals
Antisense	Alicaforsen/phase III	Crohn's disease	ISIS pharmaceuticals
Antisense	AP 12009/phase II	Brain tumor	Antisense pharma
Ribozyme	ANGIOZYME/phase II	Intestinal cancer	Sirna therapeutics

Green Biotechnology:

Green biotechnology is the application of biotechnology processes in agriculture and food production. The main dominant forces in green biotechnology today are agro giants with a worldwide area of operation such as BASF, Bayer Crop-Science, Monsanto and Syngenta. They are concentrating considerable attention on molecular plant biotechnology, which is seen as a future growth factor in agro-industry. The traditional pesticide market, on the other hand has been stagnating for years.

Transgenic Plants:

The main emphasis in modern plant biotechnology is the production of transgenic plants. The first use of gene technology to bring about changes in plants became possible at the beginning of the 1980s, around ten years after the first experiment with bacteria. The market value of transgenic plants is estimated to be in excess of 2 billion euros, according to the calculation of

the German Federal Office for the Environment. These figures relate to transgenic crop plants, which were being grown on an area totaling about 40 million hectares worldwide in 1999 and 2000.

Novel and Functional food:

New types of foodstuffs with novel properties are often called functional food. Another category that is often mentioned in this context is nutraceuticals. These are foods that have a medicinal effect.

Livestock Breeding:

Modern biotechnology is being employed commercially to introduce novel performance features in productive livestock. The transgenic specimens then display for example different wool characteristics for sheep, or improved milk characteristics in cattle.

Grey/White Biotechnology:

The terms Grey and White Biotechnology have been coined for the application of biotechnological processes in environmental and industrial production contexts. The latter is primarily focused on the production of fine chemicals, in particular technical enzymes.

Technical Enzymes:

Modern biotechnology already dominates the technical enzymes market. They can be found as proteases, lipases, celluloses and amylases for example in modern detergents, where the serve, amongst other purposes as protein and fat solubilizes.

Safety Concerns:

There are a number of safety issues relating to biotechnology products that differ from those raised by low molecular weight products and need to be taken into account when designing the safety evaluation programme for a biotechnology derived pharmaceutical product.

The quality and consistency of the product requires careful control in terms of product identity, potency and purity because of concerns about microbiological safety, impurities arising from the manufacturing process (e.g., host-cell contaminants, endotoxin, residual DNA levels and process chemicals), and the fidelity of the protein sequence and post-translational modifications during process improvements and scale-up.

The immunogenic nature of heterologous proteins, vectors, cells, tissues and process contaminants must also be considered in the design of the safety evaluation programme and appropriate monitoring for anti-product antibodies, particularly neutralizing antibodies included in toxicity studies to aid interpretation of the findings. For gene transfer products, ther

The following points highlight the top eight applications of genetic engineering in industry. The applications are: 1. Protein Engineering 2. Metabolic Engineering 3. Pharmaceutical Industry4. Biodegradable Plastic Industry 5. Oil Industry 6. Bio-Hydrometallurgy 7. Bio-Mineralisation 8. Fuel Industry.

Application # 1. Protein Engineering:

Protein engineering involves insertion of chemically synthesised DNA into desired organisms to produce modified proteins. These techniques made it easy to alter one or few amino acids in a protein and thus alter its structure and behaviour.

In this way, enzymes and bioactive peptides used in different industries can be created with different characteristics. Table 9.6 summarizes a variety of modified proteins and bioactive peptides that has been created using bacteria.

Table 9.6 : Examples of modified proteins created through recombinant DNA technology

Modified characteristic	Bacterium used	Results	Mechanism involved
1. Glucose isomerase thermostability	*Actinoplanes*	Enhanced stability of soluble and immobilised forms.	Amino acid substitution of arginine for lysine at position 253.
2. Protein separation characteristics	*E. coli*	96-fold difference in modified partition coefficient.	Fusion with partitioning tetrapeptide.
3. Protein structure and crystallinity	*E. coli*	Unique macromolecular polymide copolymers.	Modification of leader region in message.
4. Biopesticide activity	*E. coli*	Modified moulting of fall armyworm.	Insertion of *Lac* Z gene and modification of hormone.
5. Anticoagulant polypeptide alteration	*E. coli*	Improve the 65 amino acid polypeptide hirudin.	Substitution of asparagine residue 47 with lysine or arginine.

In addition to bacteria, yeasts are becoming more important in the production of recombinant proteins that have particular biomedical importance. The most commonly used yeast is Saccharomyces cerevisiae. It is used for the production of vaccines for hepatitis B virus and malarial protozoa, therapeutic agents like insulin, insulin like growth factor, blood coagulating

factor, fibroblast growth factor, platelet-derived growth factor etc., diagnostic kits for hepatitis C virus protein, HIV-1 antigen etc.

*Application # **2. Metabolic Engineering:***

Application of genetic engineering in protein industry has progressed so much that an entirely new field has merged, called metabolic engineering. In this application of recombinant DNA technology metabolic networks are restructured by the recruitment of proteins from different cells.

It results in a change in pathway distribution and rate. By combining the metabolic capabilities from two organisms entirely new products and intermediates are being synthesised. Examples of some altered small metabolites and protein-related end products are given in Table 9.7.

Table 9.7 : Some altered small metabolites and protein end products

Host organism	Original metabolite	Source organism and enzyme added	New product
Erwinia herbicola	2, 5-diketo-D gluconic acid	*Corynebacterium*, 2, 5-DKG reductase	2-keto-L-gulonic acid, a vitamin C precursor in a single fermentation.
Acremonum chrysogenum	Cephalosporin C	*Fusarim solani*, D-amino acid oxidase, *Pseudomonas diminuta*, Cephalosporin acylase	7B-cephalosporanic acid, an antibiotic precursor by a single fermentation instead of 2 steps.
Chinese ovary cells hamster	Terminal β-galactosyl residues in cell surface glycoproteins	β-galactoside 2, 6-sialyctransferase (rat)	Increased β-galactosyl residues to better resemble human-derived forms.

*Application # **3. Pharmaceutical Industry:***

Through metabolic engineering a number of synthetic medical peptides have been produced with the help of recombinant DNA technology (Table 9.8). All such genetically engineered products show least harmful side-effects.

Table 9.8 : Some therapeutic synthetic peptides produced through recombinant technology

Peptide	Therapeutic use	Mechanism of action
1. LHRH agonists	Prostate cancer	Luteinizing hormone – releasing hormone affects pituitary receptor, and blocks release of LH.
2. Octreotide	Diarrhoea associated with intestinal tumors	Helps in controlling diarrhoea.
3. Immune stimulated	Various cancers	Stimulates immune system to control intra-cellular recognition of self and non-self.
4. Pentigetide	Allergy-related problems	Interacts with active peptides to control allergies.
5. Hirudin	Anticoagulant	Stops coagulation of blood.

*Application # **4. Biodegradable Plastic Industry:***

Biodegradable plastics like polyhydroxylbutyrate (PHB) can be obtained commercially by fermentation with the bacterium Alcaligenes eutrophus. But its production cost is very high. Recently three genes, viz., phbA, phbB and phbC encoding the enzymes 3- ketothiolase, acetoacetyl-CoA reductase and PHB synthase have been isolated from A. eutrophus.

These genes were introduced into a model plant Arabidopsis which produced PHB globules exclusively in their chloroplasts without affecting plant's growth and development. The amount of PHB in the leaves of this transgenic plant was up to 10 mg/g fresh weight. Industry has already started to explore the production of biodegradable plastics from other transgenic tree plants like poplar.

*Application # **5. Oil Industry:***

Plants store oil in their seeds (e.g., ground nut, mustard, rapeseed, sunflower, sesamum, soya bean etc.) or in fruits (e.g., olive, avocado, oil palm etc.). Such vegetable oils are used either as food or in industrial purposes. According to various requirements the fatty acid quality and yield can be improved by using genetic engineering technology.

For example, the wild type canola (rapeseed) contains 60% oleic acid along with some other fatty acids. A genetically engineered canola was constructed with a single gene from the California bay tree that encodes an enzyme involved in the synthesis of lauric acid. These transgenic canola seeds contain 60% lauric acid instead of 60% oleic acid; the new oil resembles the oil found in coconut and palm oil.

Similarly, a genetically engineered soya bean with desirable monounsaturated fatty acid content of about 25% oleic acid to over 85% in its oil has been developed. Such improved variety of soya bean oil is very good for health. The production of such genetically engineered oil has already been commercialized.

Application # ***6. Bio-Hydrometallurgy:***

Bio-hydrometallurgy is a new branch of metallurgy that amalgamate metallurgy and biotechnology together. Extraction of metals from ores through conventional metallurgy involves smelting ores at high temperature, a process which is very much polluting and energy intensive approach.

However, bio-metallurgy is an approach which is environment friendly and can be used to extract metals from low grade ores in contrast to high grade ores used in conventional metallurgy which are gradually becoming exhausted.

In bio-metallurgy, different bacterial species are being used for the extraction of metals from ores. For example, Thiobacillus ferrooxidans and related thiobacilli are used for copper extraction. Recently, genetically engineered strains of thiobacilli have been developed to increase the speed of extraction.

Such bacterial strains have increased resistance to arsenite and arsenate (which do not allow bacterial growth) and increased recovery of gold from arsenopyrite-pyrite ores.

*Application # **7. Bio-Mineralisation:***

It has been shown recently that some bacteria which are normal constituents of soil can deposit valuable metals like gold, silver etc. in soil. It is suggested that negatively charged polymer on the outside of the bacteria, attracts the positively charged gold particles in the soil. Thus, the metal particles clump together as grains that eventually form gold nuggets.

The process of gold deposition on the negatively charged polymers can continue even after the death of the bacteria. Naturally occurring bacteria involved in such biomineralisation include Bacillus cereus, podomicrobium-like budding bacteria and Chlorella vulgaris.

It is suggested that genes responsible for mineralization in a variety of microbes can be isolated, cloned and transferred into E. coli, which may then help in metal deposition more efficiently.

*Application # **8. Fuel Industry:***

In recent years, ethanol has found its use as an important chemical feedstock and as a fuel supplement. Ethanol is generally produced by fermentation of some sugar (starch, cellulose) rich products with the help of yeast, Saccharomyces cerevisiae or sometimes with Kluyveromyces fragilis.

At present E. coli and Klebsiella planticola carrying genes from Z. mobilis have been developed which could utilize glucose and xylose as the substrate to give maximum yield of ethanol.

Microorganisms, whether cultured or represented only in environmental DNA samples, constitute the natural resource base of microbial biotechnology. Numerous prokaryotic and fungal genomes have been completely sequenced and the functions of many genes established. For a newly sequenced prokaryotic genome, functions for over 60% of the open reading frames can be provisionally assigned by sequence homology with genes of known function. Knowledge of the ecology, genetics, physiology, and metabolism of thousands of prokaryotes and fungi provides an indispensable complement to the sequence database.

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ranging fromproduction of recombinanthumanhormones to that of microbial insecticides, frommineral leaching to bioremediation of toxic wastes.

In this chapter, we sketch the complex terrain of microbial biotechnology. The purpose of this chapter is to convey the impact, the extraordinary breadth of applications, and the multidisciplinary nature of this technology. The common denominator to the subjects discussed is that in all instances, prokaryotes or fungi provide the indispensable component.Topics addressed in later chapters of this book are treated briefly. Those not described elsewhere are discussed here in some detail.

AGRICULTURE

Methods dependent on microbial biotechnology greatly increase the diversity of genes that can be incorporated into crop plants and dramatically shorten the time required for the production of new varieties of plants. It is now possible to transfer foreign genes into plant cells. Transgenic

plants that are viable and fertile can be regenerated from these transformed cells, and the genes that have been introduced into these transgenic plants are as stable as other genes in the plant nuclei and show a normal pattern of inheritance. Transgenic plants are most commonly generated by exploiting a plasmid vector carried by *Agrobacterium tumefaciens*.. Foreign DNA carrying from one to 50 genes can be introduced into plants in this manner, with the donor DNA originating from different plant species, animal cells, or microorganisms.

Higher plants have genes whose expression shows precise temporal and spatial regulation in various parts of plants – for example, leaves, floral organs, and seeds that appear at specific times during plant development and/or at specific locations, or whose expression is regulated by light. Other plant genes respond to different stimuli, such as plant hormones, nutrients, lack of oxygen (anaerobiosis), heat shock, and wounding. It is therefore possible to insert the control sequence(s) fromsuch genes into transgenic plants to confine the expression of foreign genes to specific organelles or tissues and to determine the initiation anddurationofsuchexpression.Microorganisms that live on or within plants can be manipulated to contro l insect pests and fungal disease or to establish new symbioses, such as those between nitrogen-fixing bacteria and plants.

ABILITY TO GROW IN HARSH ENVIRONMENTS

Extending the habitat range for plants may be achieved by imparting traits such as cold, heat, and drought tolerance; ability to withstand high moisture or high salt concentrations; and resistance to iron deficiency in very alkaline soils. Tolerances toward environmental stresses are likely to be polygenic traits and as a consequence may be difficult to transfer from one kind of organism to another.

HERBICIDE TOLERANCE

Many otherwise effective broad-spectrum herbicides do not distinguish between weeds and crops. Crop plants can be modified to become resistant to particular herbicides. When applied to a weed-infested field of such genetically modified plants, these herbicides act as selective weed killers.

RESISTANCE TO INSECT PESTS

Certain strains of the bacterium *Bacillus thuringiensis* produce protein endotoxins that permeabilize the epithelial cells in the gut of the larvae of lepidopteran insects, moths, and butterflies. Genes encoding particular *B. thuringiensis* endotoxins have been transferred into and expressedintobacco,cotton,andtomato. Infieldtests,thetransgenictomato
and tobacco plants were only slightly damaged by caterpillar larvae under conditions that led to total defoliation of control plants.

CONTROL OF PATHOGENIC BACTERIA, FUNGI, AND PARASITIC NEMATODES

The cell walls of many plant pests, such as insects and fungi, contain chitin (poly-*N*-acetylglucosamine) as a major structural component. Many bacteria (e.g., species of *Serratia*, *Streptomyces*, and *Vibrio*) produce chitindegrading enzymes (chitinases). The control of some fungal diseases by such bacteria has been correlated with the production of chitinases. Genes encoding chitinases from several different soil bacteria have been cloned into *Pseudomonas fluorescens*, anefficient colonizer of plant roots.Theeffectiveness of these recombinant strains in controlling fungal disease is not yet known.

RESISTANCE TO VIRAL DISEASES

Plant virus diseases are difficult to control. Research in the mid-1980s showed that transgenic tobacco expressing the coat protein (capsid) gene of tobacco mosaic virus (TMV) is resistant to TMV, and it was speculated that the resistance is the result of the interference with virus uncoating by the expressed coat protein. Similar coat protein transgene-mediated protection was

reported for a number of other related plant RNA viruses, TMV, cucumber mosaic virus, alfalfa mosaic virus, and several potato viruses. The protection is now known to be the result of RNA silencing, a cell-based sequencespecific post transcriptional RNA degradation system that is programmedby the transgene-encoded RNA sequence (described in Chapter 6). In Hawaii, papaya ranks as the second most important fruit crop. This crop was subject to severe damage caused by papaya ringspot virus (PRSV). The introduction in 1998 of transgenic papaya cultivars with a transgene that expressed aPRSV coat protein saved the Hawaiian papaya industry.

NITROGEN FIXATION

Leguminous plants, including important crops such as soybeans, formsymbiotic associations with species of *Rhizobium*, *Bradyrhizobium*, and *Frankia* that fix atmospheric molecular nitrogen. Free-living rhizobia are found in the soil.Natural infection of host plants by the bacteria leads to formation of root nodules within which the rhizobia proliferate. It has been apractice for almost a hundred years to add commercially produced rhizobia to soil as legumeinoculants to reducetheneed for nitrogenous fertilizer. No adverse effects of such applications have been observed. Consequently, no adverse consequences should attend large-scale applications of genetically engineered strains of rhizobia.

FOOD TECHNOLOGY

PREPARATION OF FERMENTED FOODS

The use of microorganisms to produce fermented foods has a very long history.

Microbial fermentationis essential toproductionofwine,beer,bologna, buttermilk, cheeses, kefir, olives, salami, sauerkraut, and many more. The metabolic end products produced by the microorganisms flavor fermented foods. For example, mold-ripened cheeses owe their distinctive flavors to the mixture of aldehydes, ketones, and short-chain fatty acids produced by the fungi.

SINGLE-CELL PROTEIN

The term *single-cell protein*, or SCP, describes the protein-rich cell mass derived from microorganisms grown on a large scale for either animal or human consumption. SCP has a high content of protein containing all the essential amino acids. Microorganisms are an excellent source of SCP because of their rapid growth rate, their ability to use very inexpensive raw materials as carbon sources, and the uniquely high efficiency, expressed as grams of protein produced per kilogram of rawmaterial, with which they transformthese carbon sources to protein.

ENVIRONMENTAL APPLICATIONS OF MICROORGANISMS

Microorganisms mitigate a multitude of impacts that result from human use of the natural resources of the planet.

WASTEWATER TREATMENT

Wastewater originates from four primary sources: sewage, industrial effluents, agricultural runoff, and stormwater and urban runoff. Treatment of wastewater is essential to prevent contamination of drinking water and the entry of pathogens and contaminants into the food chain.

Bacteria of *Zoogloea* species play an important role in the aerobic secondary stage of sewage treatment. These

organisms produce abundant extracellular polysaccharide and, as a result, formaggregates called flocs. Such aggregates efficiently adsorb organic matter, part of which is then metabolized by the bacteria.

The flocs settle out and are transferred to an anaerobic digester, where other bacteria complete the degradation of the adsorbed organic matter. The microbial communities in a water treatment

plant convert organic carbon to carbon dioxide, water,andsludge; convertsome80%of theammonia and nitrate to molecular nitrogen; remove some soluble phosphate through incorporation into the sludge.

BIOREMEDIATION

Bioremediation dependsonthe activities of living organisms to cleanuppollutants dispersed in the environment. Physical or chemical treatments, such as vaporization, extraction, or adsorption, relocate rather than remove pollutants. In contrast, there are many instances in which biodegradation converts organic pollutants to harmless inorganic products, including carbon dioxide, water, and halide ions. Other advantages are that bioremediation is generally inexpensive and causes little disturbance to the environment. Naturally occurring consortia, frequently dominated by bacteria, have the capacity to degrade a wide spectrum of environmental pollutants.

BIOMINING: HEAVY METAL EXTRACTION USING MICROORGANISMS

Biomining utilizes naturally occurring prokaryotic communities. Here, microorganisms are used to leach metals, principally copper but also nickel and zinc, from low-grade sulfide- and/or iron-containing ores. The process exploits the energy metabolism of various acidophilic chemolithoautotrophs that utilize inorganic compounds as energy sources and CO2 as the source of carbon. These organisms use either ferrous iron or sulfide as an electron donor and oxygen as an electron acceptor with the formation of ferric iron or sulfuric acid. In the first case, the subsequent reaction of Fe3+ with insoluble metal sulfides yields soluble metal sulfates; in the second, metal sulfides are oxidized directly to metal sulfates. The metals are readily recovered from the leachate by electrolytic procedures, and the residual solution is recycled.

Gold is inert to microbial action. However, bioleaching of sulfidic goldcontaining ores under acidic conditions opens up the interior of the ore particles to solvent. After bioleaching, the ore is rinsed with water and the gold is solubilized with a cyanide solution.

Production of Proteins in Bacteria and Yeast

It is now possible to clone a DNA segment coding for a protein and introduce the cloned fragment into a suitable microorganism, such as *Escherichia coli* or the yeast *Saccharomyces cerevisiae*. The "engineered" microorganism then works as a living factory, producing very large amounts of rare peptides and proteins from the inexpensive ingredients of the culture medium. And with such products obtained in this way from pure cultures of microorganisms, there is no chance of contamination by viruses harmful to humans.

PRODUCTION OF PROTEINS IN BACTERIA

For several reasons, bacteria were the first microorganisms to be chosen for use as living factories. To begin with, a great deal was known about their genetics, physiology, and biochemistry. After *Homo sapiens*, the bacterium *E. coli* is the most thoroughly studied and best-understood organism in the living world. Furthermore, it is easy to culture bacteria in large amounts in inexpensive media, and bacteria can multiply very rapidly. For example, *E. coli* doubles its mass every 20 minutes or so in a rich medium.

INTRODUCTION OF DNA INTO BACTERIA

The field of bacterial genetics grew explosively in the mid-twentieth century, laying much of the groundwork for the development of procedures that efficiently introduce foreign DNA into bacteria. The three basic approaches take advantage of the three modes by which bacteria are known to exchange genetic information. There are two aspects of a genetic exchange: DNA (1)

leaves a donor cell and (2) enters a recipient cell. It is the latter process, the uptake of DNA by a cell, that is all-important to biotechnologists.

Direct Introduction by Transformation

In the classical process, *E. coli* cells are first converted into a competent state by resuspension in buffer solutions containing very high concentrations (typically 30mM)ofCaCl2 at 0◦C. The effect ofCa2+ on a membranebilayer with a high content of acidic lipids is to "freeze" thehydrocarboninterior, presumablybybindingtightly to thenegatively charged head groups of the lipids.Because the outermembrane of Gram-negative bacteria such as *E. coli* contains a large number of acidic groups in the form of lipopolysaccharide [LPS]) at a very high density, this membrane

becomes frozen and brittle, with cracks through which macromolecules, including DNA, can pass. After DNA is added to the suspension, the cells are heated to 42◦C and then chilled.Under these conditions, cells have been found to take up pieces ofDNAthrough the cytoplasmic membrane, but the molecular mechanisms of the process still remain obscure.

Introduction by Conjugation

The *conjugational transfer* of genes in bacteria was discovered by Joshua Lederberg and Edward L. Tatum in 1946. Subsequent work has shownit to be a unidirectional transfer from a cell containing a sex plasmid, or F-plasmid (for "fertility"), into a cell lacking that plasmid. The transfer of chromosomal genes by conjugation occurs only in rare donor cells, in which the sex plasmid has become integrated into the chromosome. Amore frequent process, which occurs with nearly100%efficiency, is the transfer of just the F-plasmid froma donor to a recipient (Figure 3.1). Conjugation requires that the donor and recipient cells join to forma stable pair connected, at least in the beginning, by a filamentous apparatus (sex pilus).

The first step in the cloning of a fragment of DNA is to insert it into a suitable *vector DNA,* and plasmids are the most frequently used vectors. However, the unmodified sex plasmids are *not* used as vectors. If they were, the job of transferring the recombinant plasmids to other strains and species would be easy, because all the proteins needed for such a transfer are encoded on the plasmid itself. But the procedure could also be potentially dangerous, because if a plasmid-containing strain were to escape into the environment, the recombinant plasmid with the foreign DNAcould conceivably start to spread into other, naturally occurring bacteria.

Injection of Bacteriophage DNA and Transduction

A problem with the transformation process is its lowefficiency. With *E. coli* as the recipient, the usual frequency of transformation suggests that only one out of hundreds of thousands of the exogenous DNA molecules enters the cell. In contrast, when bacteriophage (bacterial virus) infects bacterial cells, every virus particle adsorbs to a susceptible host cell and injects it with the DNA contained in the virus head at very high efficiency, often close to 100%.

Scientists have been able to take advantage of this natural process to inject foreign DNA into bacterial cells, thanks to a third type of genetic exchange in bacteria, transduction. In *generalized transduction*, a piece of bacterial chromosome is transferred into a recipient cell by means of a bacteriophage. The chromosomal DNA gets into the phage head.

Once there, the fragment is injected into the cytoplasm of a new host cell in exactly the same way as the phage DNA. The phage head simply injects any DNA it happens to be carrying, regardless of the nature or the source of that DNA. Recombinant DNA technologies utilize this feature of the virus infection process by packaging recombinant DNA into phage heads *in vitro*.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS:III B.Sc MB COURSE NAME: Microbial Biotechnology

COURSE CODE: 16MBU504A MCQ - UNIT:I BATCH-2016-2019

S.No	QUESTIONS	A	B	C	D	ANSWER
1	The cloned genes are expressed in ----------	Periplasm	Recombinant cell.	Host cell	Donor cell	Recombinant cell
2	A typical example for a head and tail phage	Phage M13	pHC79	lambda Vectors	pUC118	lambda Vectors
3	What are the reasons for considering the yeast as a vector?	length	short doubling time (90 minutes)	can be grown on complex media	tough selection	short doubling time (90 minutes)
4	Which are the shuttle vectors?	YE ps	PBR 322	LE u2	pUC118	YE ps
5	Which is the yeast chromosomal gene that codes for isopropyl malate dehydrozejase?	2um plasmid	pBR322	YEPs	LEU2	LEU2

6	Which is the one of the enzyme involved in the conversion of pyuvie acid to leucine?	Malate dehydrozenase	Isopropyl kinase	Isopropyl malate dehydrozenase	Pyruvate carboxylase	Isopropyl malate dehydrozenase
7	Origin of Replication in yeast chromosomes having 100 bp	LEU2	ARS	2μm plasmid	ANS	ARS
8	Bacterial plasmids carrying a yeast gene	YRPs	2 μm plasmid	YIPs	YAC	2 μm plasmid
9	Phages were first discovered by	Fredrick Twort	Edward Tautum	Flein d' Hericcle	Griffith	Fredrick Twort
10	The smallest known phage is	Phage M13	PUC 118	PUC 119	PHC 79	Phage M13
11	……. Can be used as an selectable marker in certain experiment to ensure the presence of plasmid present in a bacteria in a culture	DNA probe	Radiolabelled RNA	Antibiotics resistant plasmid	Radiolabelled DNA.	Antibiotics resistant plasmid

12	Origin of replication in yeast chromosomes having 100 bp	Minichromosome vectors	LEU2	ARS	2um plasmid	ARS
13	The insertion of DNA fragment is accompanied with deletion of all or the major part of non essential region of genome, the deleted region is called	Okazaki fragment	Stuffer fragment	Coding region	Non coding region	Stuffer fragment
14	Which plasmid is referred to as the 'work house' of gene cloning	pBR 322	Col El Plasmid DNA	Col El Amp Plasmid DNA	pBR 325 Plasmid DNA	pBR 322
15	The ampicillin resistant gene of pBR 322 was derived from	pSC 101	Ti Plasmid	pBR 313	RSF 2124	RSF 2124
16	The size of pBR 322 is	4,363 bp	6,600 bp	10,900 bp	5,300 bp	4,363 bp
17	A derivative of pBR 322 which confers resistance to ampicillin,	pBR 313	Col El Plasmid DNA	pBR 325	Col El Amp Plasmid	pBR 325

	tetracycline and chloramphenicol				DNA	
16	In Rhizobium leguminosarum, the genes for nitrogen fixation and module formations are located in	Plasmid	DNA	Bacteriophage	RNA	Plasmid
19	In EcoRI the first two letters are known as ____	Genus & specific name (species)	Genus name	Specific name	Inventor name	Genus & specific name (species)
20	Bacterial plasmids carrying a yeast gene	YRPs	2 um plasmid	YIPs	YAC	2 um plasmid
21	YAC has a approximate of …… DNA frequent	30 kbp	100 kbp	200 kbp	20kbp	30 kbp

22	Which is not the difference between YAC & BAC	BAC is circular and YAC is linear	YAC has telomere & BAC wont have	YAC has centromere and BAC wont	YAV would be bound to histone but BAC wont	BAC contain much larger DNA insert than a YAC
23	Vectors are ……..	Extrachromosomal DNA molecule	protein	BAC	YAC	Extrachromosomal DNA molecule
24	YAC differs from typical cloning vector is having	Several multiple cloning stes	A centromere sequence at each end	More than one origin of replication	Tolemere sequence	Several antibiotic resistance gene
25	Retroviral infection can	fish	mice	plant	bacteria	mice

	be applied to introduce the gene into					
26	Which of the following is an insertion vertor	λ EHBL 4	Charon 16A	λ GEM 12	Charon 4a.	Charon 16A
27	Among these which one is the replacement vector	Charon 16A	λ ZAP II	λ ZAP II	λ EHBL 4	λ EHBL 4
28	Enzyme used to cleave the appropriate site of the λ for the insertion of vector is	Endonucleases	lyases	Ligases	Translocase	Endonucleases
29	vector is involved in _____	cloning	cutting	joiing	screening	cloning
30	restriction enzymes mostly preferred for genetic engineering are of type	blue colour	green colour	colourless	greenish yellow	colourless
31	Achieving same copy by	blotting	electrophoresis	cloning	joining	cloning
32	The other name of r DNA is	Ribosomal DNA	Chimeric DNA	Bactriophage	Chromosomal	Chimeric DNA

					DNA	
33	In the method of identification of recombinants the method usually used is	Primary screening	Replica Plating	Secondary screening	Auxano graphy	Replica Plating
34	A lactose analogue which is involved in the screening of B galactosidase	Y-gal	X-gal	B-galactosidase	B-galactoside permease	X-gal
35	When X-gal added to the agar the cells of which synthesize B-galactosidase will be coloured	Yellow	Red	Blue	Black	Blue
36	Baculo virus is a	Parasite	Obligate parasite	Saprophyte	Pathogen	Obligate parasite

37	RNA4 which encodes the ----------	Virus coat protein	cell wall protein	Viral core protein	Glyco protein	Virus coat protein
38	Introduce the gene in to E.coli by _____	Transduction	Particle bambrt ment	Transformat ion	Micro injection	Transformation
39	Cells containing pBR will be resistant to …….. and sensitive to ……	Resistant to Ampicillin sensitive to tetracyclin	Resista nt to methro nine and sensitiv e to Arginin e	Resistant to tetracycline and sensitive to ampicillin	Resistant Argmine and sensitive to methroni ne	Resistant to Ampicillin sensitive to tetracyclin
40	Bacteriophage P 1 resembles bacteriophage _____.	λ	T7	φ	T3	λ
41	Name the animal virus used as vectors	SV 40 Virus	HIV	Rabbies	polio virus	SV 40 Virus
42	SV40 vectors are grown	Plant cells	E.coli	Bacteria	Animal	E.coli

	and manipulated using _____ as the host				cells	
43	cDNA clones are ligated to suitable vector are	m13 vectorand phage vector	Yac vector	Plasmid Vector		m13 vector and phage vector
44	λ phage lacks	Icosahedral	Base plate	Flexiblr fluid	Contracti le sheath	Contractile sheath
45	…….. phage display system is powerful technology for engineering proteins such as functional mutant proteins and peptides	φ×174	Colipha ge	M13 Phage	d)λ phage	M13 Phage
46	Ti plasmid is in …… size.	~ 200 kb	100 kb	50 kb	150 kb	~ 200 kb
47	The molecular weight of the cloning vector should ideally around	100	10	20	40	10

	…… kbp					
48	pBR 322 is first identified and developed by	A.Chan and N.cohen	Elinst Berlimer	T. Bolival ond Rodrigues	Ishiwata	T. Bolival ond Rodrigues
49	whch organism is infected by M13 bacteriophage	Pseudomonas	E.coli	Bacillus	Klebsiella	E.coli
50	In pBR 322 ,322 is stands for	No of genes	No of Restriction sites	No of base pairs	Number used to distinguish from other plasmid	Number used to distinguish from other plasmid

51	ARS is …….	Autonomous replicating plasmid	Automatic replicating sequence	Automatic reproducing sequence	Autonomous reproducing sequence.	Autonomous replicating plasmid
52	Advantage of lamda phage vector	Transformation efficiency	Easy to grow	low cost	Self treplication	Transformation efficiency
53	Most commenly used plasmid vector for cloning	pBR 322	pUC8	F plasmid	Ri plasmid	pBR 322
54	DNA ligase is synthesized from	E.coli and bacteriophage	E.coli & Staphylococcus	Klebsiella & bacteriophage	Bacteriophage	E.coli and bacteriophage
55	Plasmid vector can carry upto ……kb of fragment	40kb	8kb	20kb	75kb	8kb

56	Baculoviruses infect	Animal cells	Human cells	Insect cells	Plant cells	Insect cells
57	The digested DNA molecule are run agarose gel for	identify the change	Purification	Suitable range of length of DNA.	to remove impurities	Suitable range of length of DNA.
58	Transfer vector is a ……vector	Shuttle Vector	Plasmid Vector	Binary Vector	Cointegrate Vector	Shuttle Vector
59	Transfer vector contains __ cloning site	Single	Multiple	Double	Triple	Multiple

UNIT-II

SYLLABUS

Recombinant microbial production processes in pharmaceutical industries - Streptokinase, recombinant vaccines (Hepatitis B vaccine). Microbial polysaccharides and polyesters, Microbial production of bio-pesticides, bioplastics, Microbial biosensors

Recombinant microbial production of Streptokinase

Streptokinase (SK) is an extracellular protein produced by various strains of streptococci. Its activity was firstly reported by Tillet and Garner, 1933 who discovered the haemolytic activity of this protein. It is now well established that the fibrinolytic activity of streptokinase originates in its ability to activate plasma plasminogen. Streptokinase is one of the two protein components of the thrombolytic agent known as APSAC (anisoylated plasminogen streptokinase activator complex).

Streptokinase is produced by certain Streptococci and certain bacteria which contain appropriate genetic material derived from Streptococci of Lancefield groups A, C or G. Streptokinase which is to be used for clinical purposes is commonly prepared from cultures of*Streptococcus equisimilis* strain H46A, from which the secretion of streptokinase into the external medium is directed by a 26 amino acid signal peptide which is cleaved during the secretion process. The mature protein has a molecular weight of about 47 kilo Dalton (kD) and was found to be composed of 415 amino acid residues. The growth of a β-hemolytic streptococcus was studied in continuous culture with pH as a limiting factor. In these experiments, pH was controlled only by addition of buffer to the medium. The yield of cells and of some extra cellular antigens was investigated. Rosenberger and Elsden studied the effect of

both glucose and tryptophan limitation on growth in continuous cultures of a*Streptococcus faecalis* strain.

Numerous methods of purifying streptokinase have been described which are based on quantitative differences in solubility, electrical charge, molecular size and shape or non specific physical interactions with surfaces. Recently we have produced a fusion recombinant streptokinase and purified it in a single step affinity chromatography using glutathione as the ligand and by affinity chromatographatography on acylated plasminogen with ?-nitro phenyl guanidinobenzoate (NPGB). Unlike the contaminating proteins which make up the impurities, such as streptolysin or streptodornase in a culture product of H46A, streptokinase is a single chain protein that does not contain the amino acids cysteine or cystine. This structural difference was employed to provide a method for the purification of streptokinase from the fermentation broth.

Thrombosis, the blockage of blood vessels with clots, can lead to acute myocardial infarction and ischemic stroke, both leading causes of death. Other than surgical interventions to remove or by pass the blockage, or the generation of collateral vessels to provide a new blood supply, the only treatment available is the administration of thrombolytic agents to dissolve the blood clot.

The choice of a thrombolytic agent during therapy is dictated by a number of factors, which depends essentially upon the relative merits and demerits of individual PG activators. These include the cost of the drug, the side-effects and their severity, *in vivo* stability and specificity towards fibrin clots and immunological reactivity.

Streptokinase (SK) is a potent plasminogen activator with widespread clinical use as a thrombolytic agent. It is naturally secreted by several strains of beta-haemolytic streptococci. The enzyme streptokinase has been most frequently associated with hemolytic streptococci of the Lancefield group A, human C and G, with the C group being preferred. In particular, the strain H46A (identified by the American Type Culture Collection,

Rockville, Md. As No. 12449, Rebecca C. Lancefield strain H46A, 1956) is the most frequently employed strain. Native streptokinase is a single chain polypeptide with a molecular weight of 47 kDa. The protein consists of 415 **amino acid** residues in a single polypeptide chain.

The gene coding for streptokinase skc, from *S. equisimilis* H46A was expressed in several heterologous gram positive and gram negative bacteria..

Some studies have focused on recombinant production or optimizing the vector and the level of expression of recombinant streptokinase with low level. Therefore, in this study, we produced recombinant streptokinase with some modification in method.

Microbial Production

Bacterial strains and plasmids:

Streptococcus dysgalactiae subsp. *equisimilis* used as the source of chromosomal DNA for the Polymerase Chain Reaction (PCR). *E. coli* DH5α (Stratagene) (f-gyr A96 Nalr, recA1 relA1 Thi-1 hsdR17 $r^{-}k$ m+k) was used as the primary host for the construction and propagation of plasmids. For recombinant protein production, a prokaryotic expression vector pET32a (Novagene) was used. This vector enables to expresses a fusion protein with a six histidines tag, a thrombin recognition site and a T7 tag at the N-terminus. These additional **amino acid**s increase the size of expressed protein near 15 kDa. The recombinant pET32a (pET32a-SKC) is transformed in *E. coli*, BL21 (DE3) pLysS (f^{-}ompt hsdB, rB^{-} mB^{-}, dcm gal, DE3, pLYsS cmr) as host strain. LB agar and broth used for routine bacterial culture. The required antibiotics were added to media according to references recommendation.

Discussion

A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infarction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and streptokinase.

The low yields obtained in SK production and the pathogenesis of its natural host has been the principal reasons to search for a recombinant source for this important therapeutic protein.

In several study, recombinant streptokinase were produced by different vectors and hosts. The expression and subsequent secretion of SK have been studied in several heterologous hosts like ***Escherichia coli***, **Bacillus subtilis** and Pichia pastoris.

Selection of an appropriate expression system is dependent on the characteristics and intended application of the recombinant protein and is essential to produce sufficient quantities of the protein. Over the last 30 years, there have been considerable advances in the technologies for expressing recombinant proteins. ***Escherichia coli*** is widely used as an expression host for the production of recombinant proteins, both in research and industry.

Vaccines

In developing countries, infectious diseases still cause 30% to 50% of all deaths. Effective chemotherapeutic agents simply do not exist for many of the diseases that plague these regions, and many of the agents that do exist are far too costly for much of the population to afford. Vaccines thus have become the most important tool for fighting infectious diseases in those parts of the world. The situation is very different in developed countries, where infectious diseases account for only 4% to 8% of all deaths. This is not to say, however, that vaccines are not important in those parts of the world. The low rate of infectious diseases in industrialized nations is in fact largely the result of the

widespread use of vaccination

PROBLEMS WITH TRADITIONAL VACCINES

Traditional vaccines are of two types, live and killed. Most *live vaccines* consist of attenuated (weakened) viral or bacterial strains, usually obtained by totally empirical procedures, such as prolonged storage or cultivation under sub optimal conditions. *Killed vaccines* are either killed whole cells of bacteria or inactivated toxin proteins, which are called *toxoids*.Many traditional vaccines are quite effective,but new vaccines,and new techniques for producing vaccines, are desperately needed.

Among the problems encountered with traditional live vaccines is the danger of reversion to the virulent state. For instance, the oral (Sabin) vaccine was thought to be generally safe and was used as the primary means of vaccination against polio in the United States and Europe since the mid-1960s.However, when the nucleotide
sequences of the vaccine strains became available, they were found to be quite similar to those of the parent virulent strains, with one of the vaccine strains showing only two nucleotide substitutions.

Mutant strains with such slight alterations do revert from time to time, and indeed the use of Sabin vaccine produced an estimated one case of poliomyelitis (VAPP, for vaccine-associated paralytic poliomyelitis) for every 520,000 administrations of the first dose. Poliomyelitis from infection by wild-type virus has been essentially eradicated in the United States since around 1981, and all subsequent new cases were caused by the vaccination.

In view of this situation, the U.S. Department of Health and Human Services recommended in 2000 that all childhood vaccinations against polio now be done with the inactivated polio vaccine (which is similar to the vaccines used before the advent of the live polio vaccine.

Another danger is that the viruses used in traditional live vaccines have to be grown in tissue culture cells, which poses the risk of introducing hidden viruses from those host cells. In one well-known case, a cell line used for propagation of the polio vaccine was found to contain a virus capable of producing tumors in experimental animals. Still another drawback is that even attenuated pathogens can produce severe diseases in individuals with immune system deficiencies. This could be a serious problem in developing countries, wheremanymalnourished children suffer fromsuch deficiencies.

The chief problem with the traditional killed vaccines is that they themselves can cause severe reactions. For example, the "whole-cell" vaccine for pertussis consists of whole killed cells of Gram-negative bacteria. Such preparations contain the principal component of the bacteria's outer membrane, lipopolysaccharide (LPS), also called endotoxin. Even very small amounts of endotoxinmayelicitastrongtoxic response. Insensitiveanimals, such as rabbits, endotoxin in amounts as low as 1 ng/kg of body weight can produce a measurable increase inbodytemperature –and the consequences. Crude killed-cell preparations usually contain other toxic materials as well. Because of widespread fear of side effects caused by such killed–bacterial cell preparations, many governments have had to change the status of pertussis vaccination of infants from compulsory (or highly recommended) to voluntary.

A second problem is the direct risk run by the workers who cultivate dangerous pathogens in large amounts to manufacture the vaccines.

A third is the possibility that the organism or toxin in the vaccine may not be completely killed or inactivated.

A RECOMBINANT SUBUNIT VACCINE FOR HEPATITIS B

The hepatitis B virus, transmitted through contaminated needles and sexual contact, infects an estimated 200,000 Americans every year.Of the 20,000 who subsequently become carriers, one in five dies of cirrhosis of the liver and one in 20 develops liver cancer. Surprisingly, the virus does not grow in tissue culture cells and until recently was available only from the plasma of carriers. Vaccines were made either by purifying the viral surface antigen

or by inactivating living virus through chemical treatment (e.g., with formaldehyde). This source of the virus was quite limited, however, and use of the killed vaccine always carried the risk that not all the particles were inactivated. Fortunately, the surface antigen (the surface glycoprotein) of the virus was known to be an effective vaccine. The first step in producing a subunit vaccine was therefore to clone the gene for this protein from the viral genome.

The hepatitis B virus genome consists of largely double-stranded DNA that codes for a core protein aswell as for the major surface protein (S protein); most of the protein subunits making up the viral envelope are S protein molecules (226 residues). TheDNAcoding for the S protein was inserted into a YEp plasmid vector behind an effective yeast promoter and before a terminator (the construction of the first-generation recombinant plasmids.

Presumably, one reason the yeast was chosen as the host was the expectation that it would glycosylate the envelope protein. It did not do so, but the protein seemed to have folded properly nevertheless; it self-assembled into a form that resembled an empty virus envelope 22 nm in diameter and nearly indistinguishable from those found in the plasma of patients. This yeast-produced vaccine, although lacking the oligosaccharides, was as effective as the vaccine derived from human plasma, and in 1986, it became the first recombinant DNA–based vaccine licensed for use in the United States. With earlier methodologies, about 40 L of infected human serum were required to produce

a single dose of hepatitis B vaccine; now we can obtain many doses of the recombinant vaccine from the same volume of yeast culture.

Although the original hepatitis B recombinant vaccine was highly successful, there was room for improvement. A newer generation of vaccines is now produced usingDNAthat codes for PreS2 and PreS1 regions in addition to theSprotein,becausethese N-terminalparts of theprotein appear to help in the buildup of immunity. The plasmid also contains a promoter effective in animal cells and is introduced into a mammalian cell line (often a Chinese hamster ovary line). Under these conditions, the translation occurs on ribosomes attached to the endoplasmic reticulum, so that the protein products (some including PreS2 and PreS1 domains) are exported through the natural secretion pathway of the endoplasmic reticulum–Golgi apparatus, are glycosylated in the normal manner, and enter the medium as empty vesicles.

Microbial polysaccharides and polyesters

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.

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.

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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 preventionn 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 vinelandii*	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.

Xanthan or more frequently referred to as xanthan gum was the first polysaccharide available commercially. It is a well-studied and most widely used hexopolysaccharide.

Chemistry

Xanthan has a molecular weight in the range of 2-15 × 10^4 daltons. The basic repeating unit of xanthan is a pentasaccharide containing glucose (Glc), mannose (Man) and glucuronic acid (GIcA) with acetate (Ac) and pyruvate (Pyr)

Applications:

Xanthan gum is used as a food additive for the preparation of soft foods (ice cream, cheese). It is also used in oil industry for enhancing oil recovery. Further, xanthan is useful for the preparation of tooth pastes and water based paints.

Biosynthesis:

For the biosynthesis of xanthan, the monomers are bound to a carrier lipid molecule and then transferred to a growing polymer chain. The activated monosaccharide nucleotides (e.g. uridine diphosphate glucose, UDP-glucose) supply energy for the formation of glycosidic bonds between adjacent units. The biosynthesis of other exopolysaccharides is comparable with that of xanthan. Dextran synthesis however is much simpler as described later.

Production:

Xanthan is commercially produced by the Gram-negative bacterium, Xanthomonas campestris. The culture medium usually consists of 4-5% carbohydrate (glucose, sucrose, corn starch hydrolysate), 0.05-0.1% nitrogen (ammonium nitrate, urea, yeast extract) and salts.

The pH is maintained around 7.0, and the fermentation is carried out by batch culture for 2-3 days. Xanthan in the culture broth is precipitated by isopropanol or methanol. These agents also kill the microorganisms. The precipitated xanthan can be dried and used for commercial purposes.

Genetic engineering of Xanthomonas campestris for xanthan production:

The wild type X. campestris can efficiently utilize glucose, sucrose or starch as a carbon source. They are however, unable to use lactose as a carbon substrate. Whey is a byproduct obtained in the manufacture of cheese. Disposal of large quantities of whey is a major problem in dairy

industry. Fortunately, whey is rich ‘ in lactose, besides containing small quantities of proteins, vitamins and minerals. Attempts are made to use whey in fermentation industries.

Genetically engineered X. campestris have been developed that can utilize lactose (from whey) for the production of xanthan. For this purpose, the E. coli lazy genes (encoding the enzyme β-galactosidase and lactose permease respectively) were cloned under the transcriptional control of X. campestris bacteriophage promoter. This construct was first introduced into E. coli, and then transferred to X. campestris.

The genetically engineered strains of X. campestris expressed the genes and produced high quantities of the enzymes β-galactosidase and lactose permease. These new strains utilize lactose or whey very efficiently for the industrial production of xanthan. This is a good example of successfully converting a waste product (whey) into a commercially important and valuable product (a biopolymer namely xanthan gum).

Dextran:

Chemically, dextrans are glucans (polymers of glucose) containing 1→6 glycosidic linkages. Some dextrans also have α 1→2, α 1→3 and a 1→4 linkages. The molecular weights of dextrans are in the range of 15,000-500,000.

Applications:

Dextrans are used as blood plasma expanders, for the prevention of thrombosis and in wound dressing. In addition, dextrans are useful in the laboratory analytical techniques for purification of biomolecules.

Production:

Dextrans can be produced by a wide range of Gram-positive and Gram-negative bacteria e.g. Leuconostoc mesenteroides and Streptococcus mutans. In contrast to other exopolysaccharides (which are synthesized within the cells), dextrans are produced by extracellular enzyme in the medium. The enzyme is dextransucrase (a transglucosidase) which acts on sucrose and brings

about polymerisation of glucose residues, and simultaneously liberates free fructose into the medium.

The commercial production is carried out by using lactic acid bacterium, L. mesenteroides by a batch fermentation process. Besides sucrose, the culture medium contains organic nitrogen source and inorganic phosphate. The crude dextran produced is precipitated by alcohol and then subjected to acid hydrolysis.

In recent years, the alcohol precipitated polymeric dextran is subjected to enzymatic hydrolysis by using exo- or endo-a dextranases to get dextrans of desired molecular weight. The resultant dextrans can be fractionated and dried.

It is also possible to use a cell free system for the production of dextrans. The extracellular enzyme dextrasucrase can transform sucrose into dextran in a cell-free nutrient solution. This reaction is optimum at pH 5.0-5.5 and temperature 25-30°C.

Alginate:

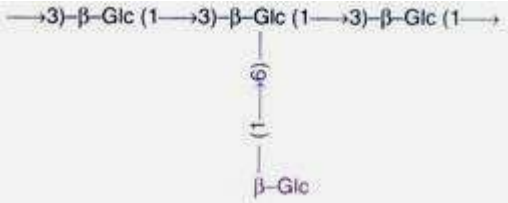
Alginate is a linear polymer composed of mannuronic acid and glucuronic acid (both of them being uronic acids) in a proportion ranging from 4: 1 to 20: 1. Some of the mannuronic acid residues are acetylated. Alginate is commercially produced by Gram-negative bacteria, Pseudomonas aeruginosa and Azobacter vinelandii.

The type of organism used and the culture conditions determine the relative proportion of mannuronic acid and glucuronic acid residues and the degree of acetylation in alginate. Alginates with high contents of mannuronic acid are elastic in nature while those with high concentration of glucuronic acid are strong and brittle.

Algal (seaweed) alginates are also polymers of mannuronic acid and glucuronic acid, and comparable in structure with bacterial alginates. However, algal alginates lack acetylation. For commercial purposes, seaweed alginates are more commonly used than bacterial alginates. This is mainly because bacterial alginates are relatively unstable and get easily degraded. Alginates are useful as thickening agents in food industry, and for immobilization of cells and enzymes.

Scleroglucan:

Scleroglucan is a glucose polymer (glucomer). It is a neutral polysaccharide with β 1→3 glucan backbone and single glucose (Glc) residue branches (β 1→6 linkage). The branching occurs at a regular sequence at every third glucose unit in the polymer backbone chain.



Scleroglucan is a fungal heoxpolysaccharide. It is commercially produced by Sclerotium glucanicum, S. rolfsii and S. delphinii. Scleroglucan is useful for stabilizing latex paints, printing inks and drilling muds.

Gellan:

Gellan is a linear heteropolysaccharide. The repeating unit of gellan is composed of two glucose, one glucuronic acid and one rhamnose molecules. Gellan is produced by Pseudomonas el odea. A deacetylated gellan which forms firm and brittle gels under the trade name Celrite has been developed by a reputed company in USA (Kalco Inc). Gellan is used in food industry. Even at a low concentration, it is a thicker.

Pollulan:

Pollulan is an α-glucose polymer (α-glucan) with α 1→4, and a few α, 1→6 glycosidic bonds. Pollulan is produced by using the fungus, Aureobasidium pollulans. It is estimated that about 70% of glucose (the substrate) is converted to pollulan during fermentation, although the time taken is rather long (5-7 days). Pollulan is mainly used in food coating and packaging.

Curdlan:

Curdlan is a β-glucose polymer (β-glucan). The glucose residues are held together by β 1 —>3 glycosidic bonds. The exopolysaccharide curdlan is commercially produced by employing Alcaligenes faecalis. Curdlan-like polysaccharides are also produced by other microorganisms

such as Agrobacterium rhizogenes and Rhizobium trifolii. Curdlan forms strong gels when heated to above 55°C. Therefore, it is used as a gelling agent for cooked foods. In addition, curdlan is also employed for immobilization of enzymes.

MICROBIAL POLYESTERS

Numerous microorganisms accumulate polyesters classified as polyhydroxyalkanoates (PHAs) as carbon and energy storage material when the growth condition is unfavorable in the presence of excess carbon source. Natural PHAs typically consist of various (*R*)-hydroxycarboxylic acids, and exhibit different material properties depending on the monomer composition. Such diversity comes from different metabolic pathways operating in the cell, and thus generating different monomers. Even more diverse PHAs can be produced by metabolically engineered microorganisms, which leads to the biosynthesis of non-natural polyesters containing lactate as a monomer. In order to make PHAs as useful polymers in our daily life, their production cost should be significantly lowered and material properties should be compatible with those produced by petrochemical industries. Metabolic engineering can address these issues by developing microbial strains capable of producing PHAs of desired material properties with high productivity and yield from inexpensive carbon sources.

Plastics we use every day are made from fossil oil and natural gas through petroleum refinery process. They are light, durable, inexpensive, easy to make various articles, and long lasting, which made them so popular in manufacturing various articles from simple containers and fibers to engineering plastics. As fossil resources will ultimately be depleted, it is necessary to develop alternative processes for the production of future plastics. Climate change and other environmental problems are also warning us that more sustainable ways of manufacturing plastics need to be developed.

Polyhydroxyalkanoates (PHAs) are polyesters synthesized by numerous microorganisms. Different microorganisms were found to accumulate various PHAs comprises (*R*)-

hydroxycarboxylic acids, having a carboxyl group at the end and a hydroxyl group at 3-, 4-, 5-, or 6-position; to date, more than 150 kinds of hydroxycarboxylic acids have been found as the monomers of PHAs . For convenience reasons, researchers classified PHAs into two groups depending on the total number of carbons in the monomer: short-chain-length (SCL)-PHAs having 3 to 5 carbon atoms and medium-chain-length (MCL)-PHAs having 6 to 14 carbon atoms . SCL-PHAs show thermoplastic material properties similar to polypropylene, while MCL-PHAs possess elastic material properties similar to rubber. Interestingly, some microorganisms synthesize PHAs having both SCL and MCL-monomers. Such SCL-MCL-PHAs exhibit material properties similar to low density polyethylene. Recently, some PHA homopolymers and block copolymers have been produced by metabolically engineered bacteria, further extending the PHA diversity. Thus, it is possible to produce diverse family of PHAs possessing material properties similar to many polymers we currently use.

Even though PHAs are such great materials, they are not being used widely. Two major reasons are relatively high cost of production and inferior material properties compared to the petroleum-based plastics. In this commentary, we suggest some strategies that can be taken to address these problems.

Lowering the production costs of PHAs

PHAs can be produced by fermentation of microorganisms, whether they are natural isolates or engineered ones. Factors affecting the overall cost of PHA production include the cost of raw materials including carbon source, the PHA yield on carbon source, PHA productivity and recovery and other downstream costs [10]. Thus, the solution to the high cost problem can be theoretically solved by using inexpensive carbon source, achieving high PHA yield and productivity and low downstream costs [2,10]. Interestingly, these are interlinked and can be solved by metabolic engineering.

First, the cheap raw materials can be chosen depending on the region where PHA production plant is in operation. Raw materials that are abundant in that region of production site can often

be, but not always though, inexpensive substrates for PHA production; for example, sucrose (sugar cane) is one of the best substrates in Brazil and Queensland, Australia. What will happen if the microorganism capable of producing desired PHA cannot utilize the most inexpensive carbon source? Let's assume that recombinant *Escherichia coli* K12 strain was developed by metabolic engineering to produce SCL-MCL PHA. If sucrose is the carbon source to be used, two options can be considered to address the inability of *E. coli* K12 strain to utilize sucrose. First, the sucrose utilization pathways can be introduced into the strain producing PHAs. Second, the strain already constructed can be discarded and a new sucrose-utilizing strain can be engineered to biosynthesize PHAs. If the lignocellulosic hydrolysate is the preferred carbon substrate, the strain should be developed to utilize both glucose and xylose equally efficiently, while making it tolerant to many toxic chemicals present therein.

For the production of PHA with high yield and productivity, more serious metabolic engineering needs to be performed. In the case of poly(3-hydroxybutyrate), (P3HB), the main metabolite precursor is acetyl-CoA as it is condensed to form acetoacetyl-CoA followed by reduction and polymerization [2,4]. Since byproducts formation needs to be minimized to increase the PHA yield, metabolic engineering is performed to eliminate the formation of acetic acid, lactic acid, formic acid and others that are produced during the cultivation. One interesting point is the generation of carbon dioxide during the conversion of pyruvate to acetyl-CoA. In the case of P3HB, for example, the theoretical maximum carbon yield of P3HB on glucose is 66.7%. Although the strategy for utilizing the evolved carbon dioxide is not clear at this moment, it will be desirable to fix carbon dioxide through a new enzyme and pathway, which can consequently be converted to acetyl-CoA.

PHA production in industrial-scale fermentation will be performed in a fed-batch mode to increase the productivity and concentration, as in the cases for many other industrial fermentation processes [2]. The PHA productivity can be maximized by the optimization of cell mass formation and the specific PHA productivity at the same time. It is particularly important to

closely examine the relationship between cell growth and PHA formation because PHA is an intracellular product which physically occupies the cytosol. Because of this, accumulation of large amounts of PHA inhibits cell growth and often negatively affects normal cell metabolism [2,4]. If the developed strain synthesizes PHA too efficiently (fast), cells will be full of PHA granules too early, resulting in lower overall productivity. If the developed strain synthesizes PHA too slowly, much carbon source will be wasted to cell growth with less PHA accumulation inside the cell. Optimization of cell growth and PHA biosynthesis rates will vary depending on the microorganism employed, and thus needs to be determined by experiments. Therefore, metabolic engineering of microorganism should be performed in the context of optimal fermentation.

Since PHAs are accumulated inside the cell, cells need to be disrupted to recover PHAs after fermentation [2,4]. Extraction of PHAs with solvents such as chloroform does not make much sense considering that PHAs are environmentally friendly polymer while chloroform is one of the environmentally worst solvents. It is interesting to note that PHAs could be efficiently purified by simple treatment with mild alkaline solution from recombinant *E. coli* accumulating a large amount of PHAs (e.g., greater than 85% of dry cell weight as PHAs) [11]. This was possible due to that cells became extremely fragile after the accumulation of such large amounts of polymer inside the cell. Thus, it is clear that metabolic engineering of strains and fermentation need to be performed to allow maximum accumulation of PHA granules inside the cell at the end of fermentation; again, too early accumulation of that much PHA will result in lower overall productivity. On the other hand, metabolic engineering approach can be taken to construct induced lysis for the PHA producing cells to release its PHA granules, allowing cost saving for PHA purification. Also, it should be mentioned that PHAs can be produced in plants directly from carbon dioxide and sunlight as having been studied for more than a decade [12]. Although the PHA contents achieved in plants to date are rather low, it will be interesting to see how much PHA accumulation can be increased as more metabolic engineering effort is exerted.

In summary, the production cost of PHA can be lowered by using inexpensive carbon substrates, developing a strain that is capable of producing PHA at the optimal rate so that the high PHA content and high overall productivity can be achieved at the end of fed-batch culture, and establishing a simple yet environmentally friendly recovery processes of low operating costs. Towards these goals, it is important to develop the improved strains by metabolic engineering and fermentation-recovery processes in an integrated manner

Future perspectives

Plastics are one of the greatest inventions of humans. It is difficult to think our world without plastics. However, will we be able to produce plastics in year 2500 in the same way as we do through petroleum refineries now? Probably not, as all the fossil resources will be depleted by then. Thus, it is essential for us to develop more sustainable processes for the production of plastics. Bio-based production of plastics from renewable biomass, and maybe directly from carbon dioxide in the future, can be realized in general in two ways. One is the bio-based production of monomers, followed by chemical polymerization process we use nowadays. Although this topic was not covered in this commentary, it is indeed a great strategy for more sustainable production of plastics as nicely reviewed previously [13,16,17]. The other is the fermentative production of plastics by metabolically engineered microorganisms as discussed in this paper. Chemical companies have not yet been willing to produce plastics by bio-based processes due to the high production cost and inferior material properties; although it seems to be slowly changing now [1]. As discussed earlier, metabolic engineering is allowing us to develop high performance microorganisms capable of producing chemicals and materials cost effectively. When combined with bioprocess development, many economically feasible bioprocesses for the production of plastics will be realized. Once the process becomes economical, the bio-based production of plastics indeed forms a complete, environment-friendly carbon cycle (Figure 1).

As readers will mostly agree, we are responsible for the future of humankind and our environment. After all, *"…We have not inherited the land from our fathers, we have borrowed it from our children …*].

Production of microbial polyesters by fermentation and its complete carbon cycle. Microorganisms isolated from nature are metabolically engineered to accumulate a large amount of plastics with high yield and specific productivity (left). Biomass obtained from carbon dioxide and sunlight is converted to fermentable sugars, and used as a substrate for lab-scale fermentation for the examination of the performance and further strain optimization. Once a high performance microorganism is developed, industrial-scale fermentation, after process optimization to give the highest possible yield and productivity, is performed to produce large amounts of plastics. After fermentation, polymers inside cells are purified and used to make articles we use everyday. When they are disposed after use, they will be degraded to carbon dioxide (and methane under anaerobic condition). Thus, the carbon cycle becomes closed, providing environmentally friendly sustainable way of producing plastics without using fossil oil.

MICROBIAL PRODUCTION OF BIOPESTICIDES

Microbial insect control utilizes pathogenic microorganisms isolated from diseased insects during naturally occurring epidemics. Typically, such epidemics only occur when pest population densities are high and usually after appreciable damage have been done to crops. Over 400 species of fungi and more than 90 species of bacteria which infect insects have been described including *Bacillus thuringiensis*, varieties of which are manufactured and sold throughout the world primarily for the control of caterpillar pests and more recently mosquitoes and black flies.

Among fungal pesticides, five have been introduced since 1979, and three in 1981. Many countries with centrally planned economies have been using fungal pesticides successfully for

many years. Sofar, more than 40,000 species of *Bacillus thuringiensis* have been isolated and identified as belonging to 39 serotypes. These organisms are active against either *Lepidoptera*, or *Diptera* or *Coleoptera*.

Bacillus Thuringiensis

Maximizing the potential for successfully developing and deploying a biocontrol product begins with a carefully crafted microbial screening procedure, proceeds with developing mass production protocols that optimize product quantity and quality, and ends with devising a product formulation that preserves shelf-life, aids product delivery, and enhances bioactivity.

Microbial selection procedures that require prospective biocontrol agents to possess both efficacy and amenability to production in liquid culture increase the likelihood of selecting agents with enhanced commercial development potential.

Scale-up of biomass production procedures must optimize product quantity without compromise of product efficacy or amenability to stabilization and formulation. Formulation of Bacillus spp. for use against plant pathogens is an enormous topic in general terms but limited in published specifics regarding formulations used in commercially available products.

Types of formulations include dry products such as wettable powders, dusts, and granules, and liquid products including cell suspensions in water, oils, and emulsions. Cells can also be microencapsulated. Considerations critical to designing successful formulations of microbial biomass are many fold and include preserving biomass viability during stabilization, drying, and rehydration; aiding biomass delivery, target coverage, and target adhesion; and enhancing biomass survival and efficacy after delivery to the target.

Bacillus thuringiensis (B.t.) is a gram positive bacterium characterized by a parasporal crystalline protein inclusion (Figure 1). The proteins are highly toxic to pests and specific in their activity over the past 40 years The commercial use of *B.thuringiensis* as a pesticide has been largely restricted to a narrow range of *Lepidopteran* (Caterpillar) pests. In recent years, however, investigators have discovered *B.t.* pesticides with specificities over a much broader range of pests.

The toxin genes have been isolated and sequenced, and recombinant DNA-based *B.t.* products produced and approved. Many of the newly discovered strains have activities that would extend the use of *Bacillus thuringiensis* beyond traditional agricultural markets.

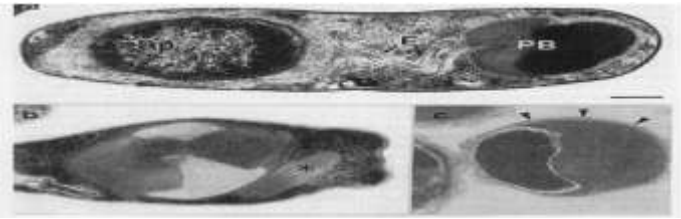


Figure 1: Electron micrograph of *Bacillus thuringiensis* parasporal body.

Bacillus thuringiensis products account for 90-95 percent of the total biopesticide market which has grown from $ 24 M in 1980 to $ 107 M in 1989, and is forecast to expand at an annual rate of 11 percent. The availability of a large number of diverse *B.t.* toxins may also enable farmers to adopt usage strategies that minimize the risk of *B.t*. resistant pests.

The *B.t.* strains known are classified according to their H antigens into 27 groups and 7 subgroups and according to structure and molecular organization of the genes coding for the parasporal delta-endotoxins (Table 1 and 2). Only a few strains are used commercially as control

agents. The main bacteria are different varieties of *Bacillus thuringiensis, Bacillus sphaericus* and *Bacillus popilliae*.

Mode of Action

Numerous moth and butterfly larvae and some beetle and fly larvae are susceptible to infection. Formulations of *Bt* variety *kurstaki* are available for the control of many caterpillar pests including imported cabbageworm, cabbage looper, hornworms, European corn borer, cutworms, some armyworms, diamond-back moth, spruce budworm, bagworms, tent caterpillars, gypsy moth caterpillars and other forest caterpillars, and Indianmeal moth larvae in stored grain.

Less well controlled are corn earworms on corn, codling moth, peach tree and squash vine borers.

Formulations of *Bt* variety *tenebrionis* and variety *san diego* have been registered for the use against the Colorado potato beetle larvae and elm leaf beetle adults and larvae.

Bt variety *israelensis* is marketed for use against black flies and mosquitoes, fungus gnats. Unless used on a community-wide basis, it is probably more effective to eliminate standing water and control weeds at the edges of ponds. *Bt* variety *aizawai* is used to control wax moth larvae in bee hives and various caterpillars. It is important for the control of diamondback moth caterpillar which has developed resistance to *Bt* variety *kurstaki* in some areas.

The toxic crystal *Bt* protein in commercial formulations is only effective when eaten by insects with a specific (usually alkaline) gut pH and the specific gut membrane structures required to bind the toxin. Not only must the insect have the correct physiology and be at a susceptible stage of development, but the bacterium must be eaten in sufficient quantity. When ingested by a susceptible insect, the protein toxin damages the gut lining, leading to gut paralysis. The affected insects stop feeding and die from the combined effects of starvation and tissue damage. *Bt* spores

do not usually spread to other insects or cause disease outbreaks on their own as occurs with many pathogens.

Bioplastics

The term "bioplastic" represents a plastic substance that is based (wholly or in part) on organic biomass rather than petroleum. Many bioplastics are biodegradable, which is - in theory - one of their greatest advantages (more on this later). However, it is easy to confuse some of the common terms in use; although they sound similar, many terms regarding bioplastics are not interchangeable. Here are three distinct descriptors:

What does *bio-based* plastic mean?

This is a very broad term that basically means a substance was derived from plant-based material, whether wholly or in part. Starch and cellulose are two of the most common renewable feedstocks used to create bioplastics; these typically come from corn and sugarcane. Bio-based plastics are distinguished from much more common petroleum-based polymers

***Biodegradable* plastic**

Whether a plastic is biomass- or petroleum-based is a different question than whether it will biodegrade (a process by which microbes break down material if conditions are suitable). Technically, all materials are biodegradable, but for practical purposes, only those that degrade within a relatively short period of time (weeks to months, usually) are considered biodegradable. As mentioned in the previous bullet, not all bio-based plastics are biodegradable; bioplastics that don't degrade within a few months or years are sometimes called "durable." Conversely, there are petroleum-based plastics that will degrade faster under optimal conditions than will their organic biomass counterparts.

BIOSENSORS

Biosensors are analytical devices that convert a biological response into an electrical signal. Quintessentially biosensors must be highly specific, independent of physical parameters such as

pH and temperature and should be reusable. The term ‘‘biosensor’’ was coined by Cammann,1 and its definition was introduced by IUPAC.2–4 Fabrication of biosensors, its materials, transducing devices, and immobilization methods requires multidisciplinary research in chemistry, biology, and engineering. The materials used in biosensors are categorized into three groups based on their mechanisms: biocatalytic group comprising enzymes, bioaffinity group including antibodies and nucleic acids, and microbe based containing microorganisms.

Applications of biosensors Biosensors have been applied in many fields namely food industry, medical field, marine sector etc., and they provide better stability and sensitivity as compared with the traditional methods. 3.2.1. In food processing, monitoring, food authenticity, quality and safety) An arduous quandary in food processing industry is of quality and safety, maintenance of food products and processing. Traditional techniques performing chemical experiments and spectroscopy have shortcomings due to human fatigue, are expensive and time consuming. Alternatives for food authentication and monitoring with objective and consistent measurement of food products, in a cost effective manner, are desirable for the food industry. Thus development of biosensors in response to the demand for simple, real-time, selective and inexpensive techniques is seemingly propitious.21 Ghasemi-Varnamkhasti et al.22 worked on the monitoring of ageing of beer using enzymatic biosensors, based on cobalt phthalocyanine. These biosensors evinced a good capability to monitor the ageing of beer during storage. Biosensors are used for the detection of pathogens in food. Presence of Escherichia coli in vegetables, is a bioindicator of faecal contamination in food.23,24 E. coli has been measured by detecting variation in pH caused by ammonia (produced by urease–E. coli antibody conjugate) using potentiometric alternating biosensing systems. Washing the vegetables such as sliced carrots and lettuce with peptone water provides us with the liquid phase. It is then separated by amalgamating it in a sonicator, to disaffiliate bacterial cells from food items.25 Enzymatic

biosensors are also employed in the dairy industry. A biosensor, based on a screen-printed carbon electrode, was integrated into a flow cell.26 Enzymes were immobilized on electrodes by engulfment in a photocrosslinkable polymer. The automated flow-based biosensor could quantify the three organophosphate pesticides in milk. One of the popular food additives extensively used today are sweeteners, which are adversely causing undesirable diseases including dental caries, cardiovascular diseases, obesity and type-2 diabetes. It is believed that artificial sweeteners are addictive and coax us to eat more high-energy food unconsciously, inadvertently causing weight gain. Thus their detection and quantification are of prime importance. Traditional methods to distinguish the two types of sweeteners are ion chromatographic methods, which are complicated and laborious. A more efficacious method, which combined lipid films with electrochemical techniques as biosensors for speedy and sensitive screening of sweeteners has been explored by multichannel biosensor, which detect the electrophysiological activities of the taste epithelium. The signals are analyzed using spatiotemporal techniques, on MATLAB, where glucose and sucrose represent natural sugars while saccharin and cyclamate comprise artificial sweeteners. Since all sweeteners are mediated by heterodimeric G-protein coupled receptors in Type-II cells in the bud, they have a plurality of binding sites to identify sweet stimuli of different structures respectively. Studies suggest two types of sweet stimuli: cyclic adenosine 154 j o u r n a l o f o r a l b i o l o g y a n d c r a n i o f a c i a l r e s e a r c h 6 (2 0 1 6) 1 5 3 – 1 5 9 monophosphate pathway which utilizes natural sugars such as sucrose, and the second is, inositol triphosphate and diacylglycerol pathway exploited by artificial sweeteners for purpose of signal transduction. The response to artificial sweeteners greatly depends on residues in the amino terminal domains of taste receptors as ligand binding sites. The signal responses of taste receptor cells towards natural and artificial sweeteners are discrete. The taste epithelium biosensor delivered sparse signals with positive waveforms, when glucose was applied whereas sucrose sustained signals with negative spikes. The taste epithelium responded to artificial sweeteners with more intensive signals, showing that the responses to artificial sweeteners were quite different from those of

natural sugars, in both time and frequency domains. 3.2.2. In fermentation processes In fermentation industries, process safety and product quality are crucial. Thus effective monitoring of the fermentation process is imperative to develop, optimize and maintain biological reactors at maximum efficacy. Biosensors can be utilized to monitor the presence of products, biomass, enzyme, antibody or by-products of the process to indirectly measure the process conditions. Biosensors precisely control the fermentation industry and produce reproducible results due to their simple instrumentation, formidable selectivity, low prices and easy automation. Nowadays, several kinds of commercial biosensors are accessible; capable of detecting biochemical parameters (glucose, lactate, lysine, ethanol etc.) and are widely used in China, occupying about 90% of its market. In fermentation process, saccharification was monitored by traditional Fehiing's method. Since this method involves titration of reducing sugar, its outcomes were inaccurate. However, since the launch of glucose biosensor commercially in 1975, the fermentation industries have been benefited. Now the factories successfully use glucose biosensors to control production in the saccharification and fermentation workshop and utilize the bioenzymatic method to produce glucose. Biosensors are also employed in ion exchange retrieval, where detection of change of biochemical composition is carried out. For instance, glutamate biosensor has been used to conduct experiments on ion exchange retrieval of an isoelectric liquor supernatant of glutamate. The fermentation process is a byzantine process with multiple pivotal variables, most of which are laborious to measure in real-time. On-line monitoring of critical metabolites is essential to facilitate quick optimization and to control biological processes. In past years, biosensors have attracted a lot of attention in online monitoring in fermentation process due to its simplicity and quick response.27 3.2.3. Biosensing technology for sustainable food safety The term food quality refers to the appearance, taste, smell, nutritional value, freshness, flavour, texture and chemicals.11 Smart monitoring of nutrients and fast screening of biological and chemical contaminants are of paramount importance, when it comes to food quality and safety. Material science, nanotechnology,

electromechanical and microfluidic systems are striding in to make sensing technology imminent for use in market. Efforts are being made for developing control systems.

ensuring food quality and safety and, as a consequence, human health. Glucose monitoring becomes indispensable as during storage the food content and composition may get altered.28 German29 studied the electrochemistry of glucose oxidase immobilized on a graphite rod, altered by gold nanoparticles (AuNPs), which improved its sensitivity. Glutamine is the nitty-gritty of crucial functions such as (signalling, transport and precursor in biosynthesis of nucleic acids, amino sugars and proteins). Patients deficient in glutamine suffer from pathologies such as malabsorptive disorders and have to be supplemented, to improve immune functions, preserve intestinal functionality and lessen bacterial translocation.30 Glutaminase-based microfluidic biosensor chip with a flow-injection analysis for electrochemical detection has been used for detection in fermentation process.31 Biosensors are being employed to perceive general toxicity and specific toxic metals, due to their capability to react with only the hazardous fractions of metal ions.32 Pesticides pose grave threats to the environment. The common pesticides used are organophosphates and carbamic insecticide species. Immunosensors have proved their merit as sensitive, highspeed agrifood and environmental monitoring. AChE and butyrylcholinesterase biosensors have been devised for aldicarb, carbaryl, paraoxon, chlorpyrifosmethyl etc. Oxon utilizing screen-printed electrodes was developed by Arduini and colleagues.33 A similar type of biosensor is used to detect pesticides in wine and orange juice.5,34,35 Arsenic can be measured with the help of bacteria-based bioassays.36 3.2.4. In medical field In the discipline of medical science, the applications of biosensors are growing rapidly. Glucose biosensors are widely used in clinical applications for diagnosis of diabetes mellitus, which requires precise control over blood-glucose levels.37 Blood-glucose biosensors usage at home accounts for 85% of the gigantic world market.38 Biosensors are being used pervasively in the medical field to diagnose infectious diseases. A promising biosensor technology for urinary tract infection (UTI) diagnosis

along with pathogen identification and anti-microbial susceptibility is under study. Identifying end-stage heart failure patients, prone to adverse outcomes during the early phase of left ventricular assisted device implantation, is important. A novel biosensor, based on hafnium oxide (HfO2), has been used for early stage detection of human interleukin (IL)-10.39 Interaction between recombinant human IL-10 with corresponding monoclonal antibody is studied for early cytokine detection after device implantation. Fluorescence patterns and electromechanical impedance spectroscopy characterize the interaction between the antibody–antigen and bio-recognition of the protein is achieved by fluorescence pattern. Chen et al. applied HfO2 as a greatly sensitive bio-field-effect transistor.40 HfO2 biosensor has been functionalized for antibody deposition with detection of a human antigen by electrochemical impedance spectroscopy.

biosensor is an analytical device containing an immobilized biological material (enzyme, antibody, nucleic acid, hormone, organelle or whole cell) which can specifically interact with an analyte and produce physical, chemical or electrical signals that can be measured. An analyte is a compound (e.g. glucose, urea, drug, pesticide) whose concentration has to be measured.

Biosensors basically involve the quantitative analysis of various substances by converting their biological actions into measurable signals. A great majority of biosensors have immobilized enzymes. The performance of the biosensors is mostly dependent on the specificity and sensitivity of the biological reaction, besides the stability of the enzyme.

General Features of Biosensors:

A biosensor has two distinct components (Fig. 21.13).

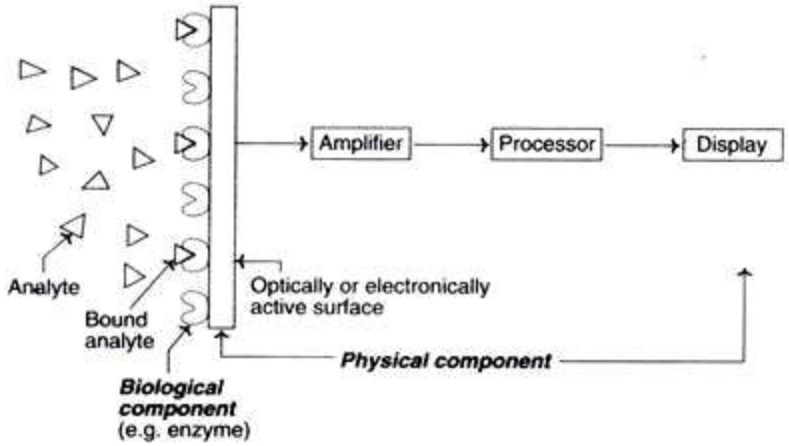


Fig. 21.13 : A diagrammatic representation of a biosensor.

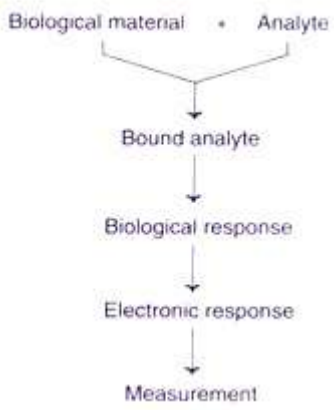
1. Biological component—enzyme, cell etc.

2. Physical component—transducer, amplifier etc.

The biological component recognises and interacts with the analyte to produce a physical change (a signal) that can be detected, by the transducer. In practice, the biological material is appropriately immobilized on to the transducer and the so prepared biosensors can be repeatedly used several times (may be around 10,000 times) for a long period (many months).

Principle of a Biosensor:

The desired biological material (usually a specific enzyme) is immobilized by conventional methods (physical or membrane entrapment, non- covalent or covalent binding). This immobilized biological material is in intimate contact with the transducer. The analyte binds to the biological material to form a bound analyte which in turn produces the electronic response that can be measured.



In some instances, the analyte is converted to a product which may be associated with the release of heat, gas (oxygen), electrons or hydrogen ions. The transducer can convert the product linked changes into electrical signals which can be amplified and measured.

Types of Biosensors:

There are several types of biosensors based on the sensor devices and the type of biological materials used. A selected few of them are discussed below.

Electrochemical Biosensors:

Electrochemical biosensors are simple devices based on the measurements of electric current, ionic or conductance changes carried out by bio electrodes.

Amperometric Biosensors:

These biosensors are based on the movement of electrons (i.e. determination of electric current) as a result of enzyme-catalysed redox reactions. Normally, a constant voltage passes between the electrodes which can be determined. In an enzymatic reaction that occurs, the substrate or product can transfer an electron with the electrode surface to be oxidised or reduced (Fig. 21.14).

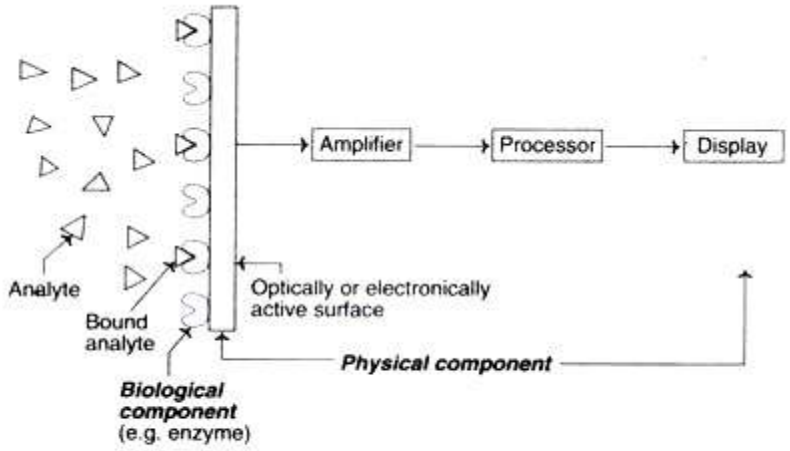


Fig. 21.13 : A diagrammatic representation of a biosensor.

This results in an altered current flow that can be measured. The magnitude of the current is proportional to the substrate concentration. Clark oxygen electrode which determines reduction of O_2, is the simplest form of amperometric biosensor. Determination of glucose by glucose oxidase is a good example.

In the first generation amperometric biosensors (described above), there is a direct transfer of the electrons released to the electrode which may pose some practical difficulties. A second generation amperometric biosensors have been developed wherein a mediator (e.g. ferrocenes) takes up the electrons and then transfers them to electrode. These biosensors however, are yet to become popular.

Applications in Medicine and Health:

Biosensors are successfully used for the quantitative estimation of several biologically important substances in body fluids e.g. glucose, cholesterol, urea. Glucose biosensor is a boon for diabetic patients for regular monitoring of blood glucose. Blood gas monitoring for pH, pCO_2 and pO_2 is carried out during critical care and surgical monitoring of patients. Mutagenicity of several

chemicals can be determined by using biosensors. Several toxic compounds produced in the body can also be detected.

2. Applications in Industry:

Biosensors can be used for monitoring of fermentation products and estimation of various ions. Thus, biosensors help for improving the fermentation conditions for a better yield. Now a days, biosensors are employed to measure the odour and freshness of foods. For instance, freshness of stored fish can be detected by ATPase. ATP is not found in spoiled fish and this can be detected by using ATPase. One pharmaceutical company has developed immobilized cholesterol oxidase system for measurement of cholesterol concentration in foods (e.g. butter).

Biosensor

Biosensor = bioreceptor + transducer. A biosensor consists of two components: a bioreceptor and a transducer. The bioreceptor is a biomolecule that recognizes the target analyte, and the transducer converts the recognition event into a measurable signal. The uniqueness of a biosensor is that the two components are integrated into one single sensor. This combination enables one to measure the target analyte without using reagents. For example, the glucose concentration in a blood sample can be measured directly by a biosensor made specifically for glucose measurement, by simply dipping the sensor in the sample.

This is in contrast to the commonly performed assays, in which many sample preparation steps are necessary and each step may require a reagent to treat the sample. The simplicity and the speed of measurements that require no specialized laboratory skills are the main advantages of a biosensor.

Enzyme is a Bioreceptor.

When we eat food such as hamburgers and french fries, it is broken down into small molecules in our body via many reaction steps (these breakdown reactions are called catabolism). These small molecules are then used to make the building blocks of our body, such as proteins (these synthesis reactions are called anabolism). Each of these catabolism and anabolism reactions (the

combination is called metabolism) are catalyzed by a specific enzyme. Therefore, an enzyme is capable of recognizing a specific target molecule.

Biosensor Characteristics.

Biosensors are characterized by eight parameters. These are: (1) Sensitivity is the response of the sensor to per unit change in analyte concentration. (2) Selectivity is the ability of the sensor to respond only to the target analyte. That is, lack of response to other interfering chemicals is the desired feature. (3) Range is the concentration range over which the sensitivity of the sensor is good. Sometimes this is called dynamic range or linearity. (4) Response time is the time required for the sensor to indicate 63% of its final response due to a step change in analyte concentration. (5) Reproducibility is the accuracy with which the sensor's output can be obtained. (6) Detection limit is the lowest concentration of the analyte to which there is a measurable response. (7) Life time is the time period over which the sensor can be used without significant deterioration in performance characteristics. (8) Stability characterizes the change in its baseline or sensitivity over a fixed period of time.

Applications of Biosensors

Health Care

Measurement of Metabolites. The initial impetus for advancing sensor technology came from the health care area, where it is now generally recognized that measurements of blood chemistry are essential and allow a better estimation of the metabolic state of a patient. In intensive care units, for example, patients frequently show rapid variations in biochemical composition and levels that require urgent remedial action. Also, in less severe patient handling, more successful treatment can be achieved by obtaining instant assays. At present, available instant analyses are not extensive. In practice, these assays are performed by analytical laboratories, where discrete samples are collected and shipped for analysis, frequently using the more traditional analytical techniques.

Market Potential.

There is an increasing demand for inexpensive and reliable sensors for use in doctor's offices, emergency rooms, and operating rooms. Ultimately, patients themselves should be able to use biosensors in the monitoring of a clinical condition, such as diabetes. It is probably true that the major biosensor market may be found where an immediate assay is required. If the costs of laboratory instrument maintenance are included, then low-cost biosensor devices can be desirable in the whole spectrum of analytical applications from hospital to home.

Diabetes.

The "classic" and most widely explored example of closed-loop drug control is found in the development of an artificial pancreas. Diabetic patients have a relative or absolute lack of insulin, a polypeptide hormone produced by the beta cells of the pancreas, which is essential for glucose uptake. Lack of insulin secretion causes various metabolic abnormalities, including higher than normal blood glucose levels. In patients who have lost insulin-secreting islets of Langerhan, insulin is supplied by subcutaneous injection. However, fine control is difficult to achieve and hyperglycaemia is often encountered. Further, even hypoglycaemia is sometimes induced, causing impaired consciousness and the serious long-term complications to tissue associated with this intermittent low glucose condition.

Insulin Therapy.

Better methods for the treatment of insulin-dependent diabetes have been sought and infusion systems for continuous insulin delivery have been developed. However, regardless of the method of insulin therapy, its induction must be made in response to information on the current blood glucose levels in the patient. Three schemes are possible, the first two dependent on discrete manual glucose measurement and the third a "closed-loop" system, where insulin delivery is controlled by the output of a glucose sensor which is integrated with the insulin infuser. In the former case, glucose is estimated based on analysis of finger-prick blood samples with a colorimetric test strip or more recently with an amperometric pensize biosensor device by the patients themselves.

Prepared by Dr.R.Usha,Associate Professor, Dept of Microbiology, KAHE, CBE.

Clearly, these diagnostic kits must be easily portable, simple to use and require minimal skill and easy interpretation. However, even with the ability to monitor current glucose levels, intensive conventional insulin therapy requires multiple daily injections. This open-loop approach does not anticipate insulin dosage due to changes in diet and exercise. For example, it was shown that administration of glucose by subcutaneous injection, 60 minutes before a meal provides the best glucose/insulin management.

S.NO	Questions	Option A	Option B	Option C	Option D	**Answer**
1	_____ are the enzymes are used to cut the target DNA fragments.	Ligases	Restriction endonucleases	Methylases	Exo Nucleases	**Restriction endonucleases**
2	System of naming restriction enzymes was proposed by	Smith	Nathans	Wilcox	Smith and Nathans	**Smith and Nathans**
3	In southern blotting, the kind of filter paper used for blotting is	Whatmann No.1	Aminobenzyloxymethyl	Nitrocellulose filter paper	Whatmann No.509	**Nitrocellulose filter paper**
4	The blotted filter paper is baked at …….	90 °C	82 °C	100 °C	80 °C	**80 °C**
5	In southern blotting, the kind of filter paper used for blotting is ……..	Whatmann No.1	Nitrocellulose filter paper	Aminobenzyloxymethyl	Whatmann No.509	**Nitrocellulose filter paper**

Prepared by Dr.R.Usha, Associate Professor, Dept of Microbiology, KAHE, CBE

6	._____ enzymes enable the breakage in internal phosphodiester bonds within a DNA molecule	Endonucleases	. Exonucleases	DNaseI	S1 nucleases	**Endonucleases**
7	DNA ligases are isolated from	**.** Bovine pancreas	Avian mycloblastosis	*E.coli* infected with phage T4	sheep	***E.coli* infected with phage T4**
8	_____ are also known as DNA ligases	Klenow fragment	. Molecular sutures	Molecular scissors	Holo enzyme	**Molecular sutures**
9	Southern blotting technique helps in detecting fragments of	RNA	DNA & RNA	DNA	Potein	**DNA**
10	The southern blotting technique can be used for the	Separation of DNA	Screening of recombinants	Denaturation of DNA	DNA sequencing	**Screening of recombinants**
11	Northern blotting is used for _____	Detection of RNA	Detection of DNA	Detection of protein	Detection of plasmid	**Detection of RNA**
12	_____ is the restriction site of E.coRI.	5’ AAGCTT 3’ 3’ TTCGAA 5’	5’GAATTC 3’ 3’CTTAAG 5’	5’ CCCGGG 3’ 3’ GGGCCC 5’	5’ GATC 3’ 3’ CTAG 5’	**5’GAATTC 3’ 3’CTTAAG 5’**
13	_____ enzyme mediates Nick	DNA Polymerase I	DNA Polymerase II	. DNA Polymerase III	RNA Polymerase	**DNA Polymerase I**

	translation					
14	Stain used in gel electrophoresis for the detection of DNA	Ethidium bromide	Crystal violet	Malachite green	Bromothymol blue	**Ethidium bromide**
15	Western blotting involves ----------	DNA probe	RNA probe	. Protein probe	Antibody probe	**Antibody probe**
16	…….. gel electrophoresis is used for the separation of DNA fragments	Agarose	PAGE	SDS-PAGE	Agarose & SDS-PAGE	**Agarose**
17	Enzyme used to remove unannealed regions of RNA from DNA:RNA hybrids	Exonuclease III	Ribonuclease T1		Endonuclease	**Ribonuclease T1**
18	RNA is tightly associated with	L ipids	Amino acids	Proteins	carbohydrates	**Proteins**
19	Precipitation of RNA can be taken place by	Ethanol	Alcohol	Formaldehyde	methane	**Ethanol**
20	Commonly used reagent in RNA	EDTA	Guanadinium Thiocyanate	NAOH	SDS	**Guanadinium Thiocyanate**

	extraction is					
21	The process by which the foreign DNA escapes host restriction is	Cloning	Host control restriction & modification	Sequencing	Blotting	**Host control restriction & modification**
22	_____ are the enzymes are used to cut the target DNA fragments.	Ligases	Restriction endonucleases	Methylases	Exo Nucleases	**Restriction endonucleases**
23	Alkali treatment of DNA fragment results in the ……	Disruption	Denaturation	depurination	none of the above	**Denaturation**
24	The blotted filter paper is baked at …….	90 °C	82 °C	100 °C	80 °C	**80 °C**
25	In southern blotting, the kind of filter paper used for blotting is ……..	Whatmann No.1	Nitrocellulose filter paper	Aminobenzyloxymethyl	Whatmann No.509	**Nitrocellulose filter paper**
26	Western blotting is used for the identification of	DNA fragment	RNA fragment	Antibodies	Protein	**Protein**
27	Solvent used in	SDS	Ethanol	Chloroform	None of the	**SDS**

	western blotting involve				above	
28	Western blotting is based on the principle	Ag-Ab reaction	Electrophoresis	Hybridisation	Translocation	**Ag-Ab reaction**
29	The Type I restriction enzyme need ……	Methyl gps	Sulfur gps	Fe+	S	**Methyl gps**
30	Western blotting detects protein even at	low as 5 ug	low as 10 ug	less than or equal to 15 ug	less than 0.5 ug	**low as 5 ug**
31	The cutting of DNA takes place with the enzyme at _____ sites.	identification site	Cleavage site	Restriction site	clear site	**Restriction site**
32	Site specific clearage is carried out by	Type III	Type I	Type II	Type IV	**Type II**
33	…… are the enzymes are used to cut the target DNA fragments	Ligases	Methylases	Restriction endonucleases	Exo Nucleases	**Restriction endonucleases**
34	The cleavage site of	30 kbp	24 – 26 kbp	24 – 28 kbp	40 kbp	**24 – 26 kbp**

	Type III enzymes is about _____ away from the recognition site.					
35	The symbol for type I restriction systems	Mod	hsd	Res	sap	**hsd**
36	Cofactor for type II system	mn 2+	ca 2+	mg 2+	fe 3+	**mg 2+**
37	The presence of restriction enzyme was postulated by	Werner Arber	Watson	Smith	Nathan	**Nathan**
38	Restriction enzymes mostly preferred for genetic engineering are of type	Type I	Type II	Type III	Type IV	**Type II**
39	Use of only one single enzyme for DNA digestion during the construction of restriction map is called	Single digestion	Double digestion	restrictive digestion	end labeling	**Single digestion**
40	Altering the optimal conditions for the activity of restriction enzymes	Single digestion	Double digestion	Partial digestion	End labeling	**Partial digestion**

	as to skip some of their restriction sites during the construction of restriction maps is called					
41	Two restriction enztmes which have the same recognition sequence but leave at different sites ……	Neoschizomers	Isoschizomers	Epimers	Isomers.	**Neoschizomers**
42	The recognition / cleavage site of Type II enzymes have ___	Same site	Different site	Adjacent site	modified site	**Same site**
43	In supercoiled DNA, if both polynucleotide strands are intact, they are describes as	CAD	Open circular DNA	Covalently closed circle DNA	supercoiled DNA	**Open circular DNA**
44	CsCI2 density gradient centrifugation is to separate	nucleus	DNA	aminoacid	membrane	**DNA**

45	Mostly density gradient centrifugation can be used to separate supercoiled DNA from non-supercoiled DNA by using intercalating agent is	EtBr(ethidium bromide)	CsCI2	EtrBr- CsCI2	CsNo2	**EtBr(ethidium bromide)**
46	_____ will yield multiple copies of plasmid	Plasmid amplification	Plasmid purification	plasmid denaturation	plasmid multiplication	**Plasmid amplification**
47	Radiolabelling of nucleic acid is done by	Horse radish peroxidose system	DIG labelling system	Nick translation	Biotin-Streptavidin labelling system	**Horse radish peroxidose system**
48	In vitro labeling of nucleic acid is done by	Probe preparation by PCR	Using 32 P	DIG labelling system	Horse radish peroxidose system	**DIG labelling system**
49	Gel electrophoresis separates DNA molecules according to their	Shape	Size	Volume	Structure	**Size**
50	Stain used in gel electrophoresis for the detection of DNA	Ethidium bromide	Crystal violet	Malachite green	Bromothymol blue	**Ethidium bromide**

51	DNA molecules in gel electrophoresis is labeled by radioactive isotope of	Phosphorous	Sulfur	Carbon	Iodine	**Phosphorous**
52	The polymerization of acrylamide is initiated by the addition of	Ammonium persulfate	Ammonium persulfate and TEMED	Riboflavin	None of the above	**Ammonium persulfate and TEMED**
53	Which is the effective methodology for separation of intact proteins by molecular weight	1D-SDS PAGE	2D-PAGE	Agarose gel electrophoresis	Nanospray – MS/MS	**1D-SDS PAGE**
54	The most commonly used stain for protein detection is	Methylene blue	Coomassic Brilliant blue	Ethidium bromide	Crystal violet.	**Coomassic Brilliant blue**
55	Photochemical polymerization of polyacrylamids gel is initiated by	Riboflavin	Vitamin C	Thymine	Vitamin A	**Riboflavin**
	Which is the tracking dye used in SDS-PAGE	Bromophenol blue	Coomassic Brilliant blue	Ethidium bromide	Crystal violet	**Bromophenol blue**

56						
57	What is the role of SDS in SDS-PAGE	Denative the proteins	Separate the proteins	Stain the protein	None of the above	**Denative the proteins**
58	Agarose is made up of	Glucose	Lactose	Agarobiose	Polyarylamide	**Agarobiose**
59	The polymerization of acrylamide is initiated by the addition of	Ammonium persulfate	Ammonium persulfate and TEMED	Riboflavin	Nacl	**Ammonium persulfate and TEMED**
	PH of stacking gel is	7.8	6.8	5.8	4.8	**6.8**

UNIT-III

SYLLABUS

Microbial based transformation of steroids and sterols. Bio-catalytic processes and their industrial applications: Production of high fructose syrup and production of cocoa butter substitute.

Microbial based transformation of steroids and sterols

The production of steroid drugs and hormones is one of the best examples of the successful application of microbial technology in large scale industrial processes. Steroids are structurally derived from cyclopentanoperhydrophenantrene (sterane) (Fig. 1). The research efforts in this field were triggered around 1950, with the announcement of the pharmacological effects of cortisol and progesterone, two endogenous steroids, and with the identification of the 11-hydroxylation activity of a Rhizopus species, a decisive step in the development of the practical synthesis of steroids with useful biological activity. Several microbial bioconversion of steroids and sterols have been reported ever since, focusing mainly on steroid hydroxylations, 1-dehydrogenation and sterol side-chain cleavage. These biotransformations, mostly associated to chemical synthesis steps, have provided adequate tools for the large scale production of natural or modified steroid analogues. The latter are currently favored when compared to their natural counterparts due to some therapeutic advantages, such as an increased potency, longer half-lives in the blood stream,

simpler delivery methods and reduced side effects. The preferential use of whole cells over enzymes as biocatalysts for the production of these pharmaceutical derivatives mostly results from the costs of the latter enzyme isolation, purifi- cation and stabilization. The manufactured steroid compounds have a wide range of therapeutic purposes, namely as anti-inflammatory, immunosuppressive, progestational, diuretic, anabolic and contraceptive agents . They have also

been successfully applied for the treatment of some forms of breast and prostate cancer and osteoporosis, as replacement agents in the treatment of adrenal insuffi- ciencies, in the prevention of coronary heart disease, as anti-fungal agents, as active ingredients in anti-obesity agents , and in the inhibition of HIV integrase, prevention and treatment of infection by HIV and in the treatment of declared AIDS. Recently, a steroidal glycoside, torvoside H, isolated from the fruits of Solanum turvum, exhibited anti-viral activity on herpes simplex virus type. The therapeutic action of steroid hormones has been traditionally associated to their binding to the respective intracellular receptors, which act as transcription factors in the regulation of gene expression. In the last decade, however, considerable evidence has emerged suggesting that some steroids, such as dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), progesterone, pregnenolone and their sulfate derivatives.

Perspectives

Expected developments in biological production of steroid compounds range from the identification of novel and improvement of existing biocatalysts to the improvement of the biotransformation process. The recent technological developments, associated with combinatorial biocatalysis will allow faster and wider screening of new steroids, to ultimately generate libraries of therapeutically relevant compounds. Novel steroid-like compounds are also increasing the field of their applications. Non-aqueous biocatalysis will benefit from the knowledge gathered on the response mechanisms of microbial cells to the presence of organic solvents. The understanding of these defense mechanisms, combined with recombinant DNA technology may lead to the development of highly effective and stable biocatalysts for use in non-conventional media. Genetic manipulation is expected to lead to biocatalysts with constitutive, rather than inducible expression of activity, as well as with enhanced activity levels. Directed evolution and DNA shuffling rapidly emerging technologies can lead to the development of tailored, highly selective, enzyme activities, able to perform the intended bioconversion in virtually any suitable medium. The development of efficient methods for in vitro coenzyme recycling will expand the use of enzyme preparations in steroid bioconversions. Rational design of fermentation/bioconversion media, combined with the developments

in process monitoring may lead to the implementation of robust, highly effective biotransformation processes.

Steroid transformations by microbial systems

A great deal of work has been carfiiied QUIon production of fats with many yeasts and molds. At present, however, industrial applications have been limited. Nonetheless. since the worldwide demand for oils is increasing faster than production from conventional plant sources. the dis-' tinct possibility exists thai more definite commercialization of microbial systems will be undertaken. Yeasts and molds may also prove to be useful commercial sources for sterols. While the conditions for optimal production have 10 be established, there have been considerable developments on microbial transformations of individual steroid subsrrares into useful steroid drugs and hormones or their useful intermediates. A variety of steroids are widely used as antiinffammatory, diuretic. anabolic, contraceptive. anti androgenic. prosgesrauonal and anticancer agents as well as in other applications. The importance of microbial transformations was realized for the first time in 1952 when Murray and Peterson of Upjohn Company patented the process of I l u-hydrexylation of progesterone by a Rhizopus species. The chemical methods for preparation of progesterone from widely available plant steroids, e.g. diosgenin (from the Mexican yam) and stigmasterol (from soybeans), were well standardized. In addition. conversion of progesterone to corticosteroids requires introduction of a specific oxygen function which can be achieved by a combination of chemical methods only with great difficulty and at a high cost. As such the single-step high-yielding microbial process immediately was considered for industrial exploitation. Since then microbial reactions for the transformation of steroids have proliferated and specific microbial transformation steps have been incorporated into numerous paruat syntheses of new steroids for evaluation as drugs and hormones. The synthetic protocol of a steroid drug or hormone, which generally comprises chemicals as well as microbial steps. is so designed that microbial transformation is con-ducted at the terminal or near terminal step. According to a present estimate, the production of steroid drugs and hormones to the value of $400 million involves biotechnological procedures. Biotechnology applications dealing with steroids may be classified broadly into two categories: (a) processing raw materials into useful intermediates for general steroid production,

e.g , microbial hydrolysis of plant steroid saponins to aglycones and microbial degradation of widely occurring sterols to useful steroid intermediates; and (b) transformation of specific steroid intermediates to desired products, e.g. microbial conversion of hydrocortisone to its .1'- dehydrogenated analogue. prednisolone, a highly selective antiinIlammatory commercial product.

Industrial applications The 11c-. 11 b- and 16a-hydroxylalions. 6'-tlehydrogenation and sidechain degradations are now exclusively achieved in the steroid industry by microbial transformations. The three hydroxylations and d'-dehydrogenation are used in manufacture of conicosrerolds and their analogues. The

typical microorganisms that are industrially used for Itu-hydroxylauon are Rhizopus nigricans and Aspergillus niger whereas strains of Cunninghamella blakesleena and Curvularia lunata are employed for II~-hydroxytenon. e.g. in the transformation of Substance S to hydrocortisone. 16aHydroxylation. which has become important for production of the steroid hormone analogue triamcinolone. is best achieved commercially by Streptomyces roseocbromogrnes. Scrutiny of the structural features of the widely used corticosteroids reveals that all of them contain the .11 -double bond. This dehydrogenation step is best achieved by microbial biotechnology and the frequently used microorganism for this purpose is Arthrobaaer simplex. Although the efficient removal of the side chain of cholesterol by a chemical method was reported in 1975. a more promising approach employing microorganisms has been applied to various sterols. Processes for the commercial conversion of sterols to 17 kerosterotds by mutants of Mycobacteria and Corynbacteria have been developed and are in practical use. The 17-ketOSleroids viz androst-4-ene-3. l7-dione (androst-enedione) or androsta-I.4-diene-3.I7-dione are useful products for chemical modification to finished products or intermediates. Moreover, chemical conversion of these l7-ketosteroids to pregnane analogues by reconstruction of the pregnane side chain has replaced partially the use of diosgenin and solasodine as base materials. A partial side-chain degradation of sterols to 2O-carboxylic acid structures also has been developed and is in commercial use. Selective cleavage of the acetyl side chain of pregnanes by microorganisms to the C- 19 steroids also is important commer cially as 16-dehydropregnenolone

Alternative processes

The commercial success of microbial steroid rranstormeuons so far has been achieved by nourishing vegetative cell cultures. However, developmental studies on several alternative processes have been conducted for the purpose of improving product yield, controlling side reactions. simplifying working procedures and thereby reducing product cost. These enemarive processes include employment of cell-free enzymes. immobilized enzymes. spores. immobilized spores, and intact microbial cells and immobilized cells. Much of the recent interest centers on the development of processes using immobilized cells for industrial exploitation. The industrially used microbial steroid transformations are based on enzymes with low stability and as such the immobilization technique is employed for immobilization of intact cells. Microbial cells can be immobilized by covalent attachment to or adsorption on solid supports. entrapment in polymeric gels. or by encapsulation and crosslinking. The perfonnance of the whole cells in steroid transformation can often be improved by using immobilization techniques. Several encouraging results have been obtained. Thus, sequential conversion of ccrtexclone to prednisolone by immobilized mycelia of Curvutaria lunata and immobilized cells of Arthrobacter simplex has been report ed. Immobilized living mycelia of C. lunata having a high 11~·hydro:c.ylation activity were prepared by in situ germination of spores entrapped in phoro-crosstinked resin gels of a suitable network structure. Acetone dried cells of A. simplex having an induced steroid 6'-dehydrogenase activity aJso were entrapped with photo-crosslinkable resin polymers and used for 0'- dehydrogenation of hydrocortisone 10 prednisolone. The production of prednisolone from conexotcne was achieved convenienliy by the ccmbination of sequential steps.

Biotransformation

Biotransformation is a process by which organic compounds are transformed from one form to another to reduce the persistence and toxicity of the chemical compounds. This process is aided by major range of microorganisms and their products such as bacteria, fungi and enzymes. Biotransformations can also be used to synthesize compounds or materials, if synthetic approaches are challenging. Natural transformation process is slow, nonspecific and less productive. Microbial biotransformations or microbial biotechnology are gaining importance and extensively utilized to generate metabolites in bulk

amounts with more specificity. This review was conceived to assess the impact of microbial biotransformation of steroids, antibiotics, various pollutants and xenobiotic compounds.

Biotransformations are structural modifications in a chemical compound by organisms /enzyme systems that lead to the formation of molecules with relatively greater polarity [1,2]. This mechanism has been developed by microbes to acclimatize to environmental changes and it is useful in a wide range of biotechnological processes [3]. The most significant aspect of biotransformation is that it maintains the original carbon skeleton after obtaining the products [4]. Biotransformation is of two types: Enzymatic and Nonenzymatic. Enzymatic are further divided into Microsomal and Non-microsomal [5]. Enzymatic Elimination is the biotransformation occurring due to various enzymes present in the body. Microsomal biotransformation is caused by enzymes present within the lipophilic membranes of smooth endoplasmic reticulum [6]. Non-Microsomal Biotransformation involves the enzymes which are present within the mitochondria. Examples include: Alcohol dehydrogenase responsible for metabolism of ethanol into acetaldehyde and Tyrosine hydrolases enzymes, Xanthine oxidase converting hypoxanthine into xanthine etc. Spontaneous, non-catalyzed and non-enzymatic types of biotransformation are for highly active, unstable compounds taking place at physiological pH. Some of these include Chlorazepate converted into Desmethyl diazepam, Mustin HCl converted into Ethyleneimonium, Atracurium converted into Laudanosine and Quartenary acid, Hexamine converted into Formaldehyde. Microbial biotransformation is widely used in the transformation of various pollutants or a large variety of compounds including hydrocarbons, pharmaceutical substances and metals. These transformations can be congregated under the categories: oxidation, reduction, hydrolysis, isomerisation, condensation, formation of new carbon bonds, and introduction of functional groups. For centuries microbial biotransformation has proved to be an imperative tool in alleviating the production of various chemicals used in food, pharmaceutical, agrochemical and other industries. In the field of pharmaceutical research and development, biotransformation studies have been extensively applied to investigate the metabolism of compounds using animal models. The microbial biotransformation phenomenon is then commonly employed in comparing metabolic pathways of drugs and scaling up the metabolites of interest discovered in these animal models for further pharmacological and toxicological evaluation .The White biotechnology involves the use of microbial biotransformation for generating products of interest. Living cells such as

bacteria, filamentous fungi, animals, plants, algae, yeast and actinomycetes are used. Microbial cells are ideal choice for biotransformation due to certain reasons like: I. Surface-volume ratio: Microbial biotransformation has high surface-volume ratio. II. Growth Rate: Higher growth rate of microbial cells reduces the time of biomass transformation. III. Metabolism Rate: Higher rate of the metabolism in microbes leads to efficient transformation of substrate. IV. Sterility: It is easier to maintain sterile conditions when microbes are used.

Transformation of steroids and sterols Steroids constitute a natural product class of compounds that is widely distributed throughout nature present in bile salts, adrenal-cortical and sex-hormones, insect molting hormones, sapogenins, alkaloids and some antibiotics. In 1937 the first microbial biotransformation of steroids was carried out. Testosterone was produced from dehydroepiandrosterone by using Corynebacterium sp. Subsequently cholesterol was produced from 4-dehydroeticholanic and 7-hydroxycholestrol using Nocardia spp.. All steroids have the same basic structure, a cyclopentanoperhydrophenanthrene which consists of four fused rings (Figure 1). Cortisone is very useful because of its anti-inflammatory action against rheumatold arthritis and skin diseases. By changing the structure, specifically by incorporating a 1,2 double bond in ring A of the cortisone molecule to produce prednisone which have the property of markedly increased antiinflammatory effect. The steroid molecule has several asymmetric centres and it makes the total synthesis of steroid very difficult. Preliminary research on the 11 alpha-hydroxylation of progesterone pointed to the possibility of the microbial introduction of oxygen into the steroid nucleus in a site specific and stereospecific manner without prior activation (Figure2). These reactions worked well and cost-effective production of cortisone became possible. The above microbial step reactions are of great economic significance. Progesterone transformation of a C-19 steroid is used industrially in the production of testosterone and estrogen and the microbial dehydration of ring A is used in estrogen production. The breakdown product 3-hydroxy-9,10-secoandrostatriene- 9,17-dione is produced from cholesterol via an opening of the B ring, with the production of two useful intermediate products, androstendione and androstadiendione with the help of an enzyme named as Arthrobacter simplex.

Production of high fructose syrup

High fructose corn syrup (HFCS) is a liquid alternative sweetener to sucrose that is made from corn, the "king of crops" using chemicals (caustic soda, hydrochloric acid) and enzymes (-amylase and glucoamylase) to hydrolyze corn starch to corn syrup containing mostly glucose and a third enzyme (glucose isomerase) to isomerize glucose in corn syrup to fructose to yield HFCS products classified according to their fructose content: HFCS-90, HFCS-42, and HFCS-55. HFCS-90 is the major product of these chemical reactions and is blended with glucose syrup to obtain HFCS-42 and HFCS-55. HFCS has become a major sweetener and additive used extensively in a wide variety of processed foods and beverages ranging from soft and fruit drinks to yogurts and breads. HFCS has many advantages compared to sucrose that make it attractive to food manufacturers. These include its sweetness, solubility, acidity and its relative cheapness in the United States (US). The use of HFCS in the food and beverage industry has increased over the years in the US. The increase in its consumption in the US has coincided with the increase in incidence of obesity, diabetes, and other cardiovascular diseases and metabolic syndromes. This study examines literature on the production and properties of HFCS and the possible health concerns of HFCS consequent to its consumption in a wide variety of foods and beverages in the typical US diet.

PRODUCTION AND USES OF HFCS

The schematic of HFCS production is shown in Figure 1. HFCS is produced from corn. The corn grain undergoes several unit processes starting with steeping to soften the hard corn kernel followed by wet milling and physical separation into corn starch (from the endosperm); corn hull (bran) and protein and oil (from the germ). Corn starch composed of glucose molecules of infinite length, consists of amylose and amylopectin and requires heat, caustic soda and/or hydrochloric acid plus the activity of three different enzymes to break it down into the simple sugars glucose and fructose present in HFCS. An industrial enzyme, -amylase produced from Bacillus spp., hydrolyzes corn starch to short chain dextrins and oligosaccharides. A second enzyme, glucoamylase (also called amyloglucosidase), produced from fungi such as Apergillus, breaks dextrins and oligosaccharides to the simple sugar glucose. The product of these two enzymes is corn syrup also called glucose syrup. The third and relatively expensive enzyme used in the process is glucose isomerase (also called D-glucose ketoisomerase or D-xylose ketolisomerase), that converts glucose to fructose. While -amylase and glucoamylase are added directly to the processing slurry, pricey glucose isomerase is immobilized by package into columns where the glucose syrup is

passed over in a liquid chromatography step that isomerizes glucose to a mixture of 90% fructose and 10% glucose (HFCS-90). Whereas inexpensive -amylase and glucoamylase are used only once, glucose isomerase is reused until it loses most of its enzymatic activity. The - amylase and glucoamylase used in HFCS processing have been genetically modified to improve their heat stability for the production of HFCS. In the US, four companies control 85% of the $2.6 billion HFCS business—Archer Daniels Midland, Cargill, Staley Manufacturing Co, and CPC International. With clarification and removal of impurities, HFCS-90 is blended with glucose syrup to produce HFCS-55 (55% fructose) and HFCS-42 (42% fructose). Both HFCS-55 and HFCS-42 have several functional advantages in common, but each has unique properties that make them attractive to specific food manufacturers. Because of its higher fructose content, HFCS-55 is sweeter than sucrose and is thus used extensively as sweetener in soft, juice, and carbonated drinks. HFCS-42 has a mild sweetness and does not mask the natural flavors of food. Thus it is used extensively in canned fruits, sauces, soups, condiments, baked goods, and many other processed foods. It is also used heavily by the dairy industry in yogurt, eggnog, flavored milks, ice cream, and other frozen desserts. The use of HFCS has increased since its introduction as a sweetener (Figure 2). Although, its use peaked in 1999, it rivals sucrose as the major sweetener in processed foods. The US is the major user of HFCS in the world, but HFCS is manufactured and used in many countries around the world (Vuilleumier, 1993). HFCS has functional advantages relative to sucrose. These include HFCS's relative cheapness (at 32 cents/lb versus 52 cents/lb for sucrose); greater sweetness with HFCS being sweeter than sucrose (Table 1), better solubility than sucrose (Table 2) and ability to remain in solution and not crystallize as can sucrose under certain conditions. Moreover, HFCS is liquid and thus is easier to transport and use in soft drink formulations (Hanover and White, 1993). It is also acidic and thus has preservative ability that reduces the use of other preservatives. HFCS has little to no nutritional value other than calories from sugar (Table 3). Analysis of food consumption patterns using USDA (2008) food consumption tables for the US from 1967 to 2000 (Bray et al., 2004) showed that HFCS consumption increased

1000% between 1970 and 1999 with HFCS representing greater than 40% of all sweeteners added to foods and beverages and the sole sweetener in soft drinks. The average daily consumption of HFCS for all Americans 2 years or older is about 50 g/person or about 132 kcal/person with the top 20% of HCFS consumers ingesting as much as 316 kcal/day. Thus HFCS is a major source of dietary fructose.

PUBLIC HEALTH CONCERNS There are three major concerns about the use of HFCS related to public health. The first is its possible role in obesity, cardiovascular disease, and other metabolic syndromes. The second is mercury contamination of HFCS samples during production and the third its toxicity to honey bees with possible contribution to colony collapse disorder (CCD) of honey bees.

Food items that contain HFCS Grocery foods items found to contain HFCS are numerous. These include baked goods such as pastries; biscuits, breads, cookies, and shortcakes; soft drinks; juice drinks; carbonated drinks; jams and jellies; dairy products including ice creams, flavored milks, eggnog, yogurts and frozen desserts; canned ready to eat foods including sauces and condiments; cereals and cereal bars; and many other processed foods. Majority of processed foods in the US contain HFCS to meet some functionality in the foods.

CONCLUSION Fructose and glucose are monosaccharides found in equal proportion in sucrose but in slightly unequal amounts in HFCS. The metabolism of glucose is well understood while that of fructose requires further research especially in light of its over consumption through HFCS in the US diet. Makers of HFCS under the banner of the corn refiners association have mounted very strong advertising blitz to assure the public that HFCS is safe especially since the use of HFCS peeked and started to decline in 1999. The public largely remains skeptical and there has been push back from health conscious individuals in the US against the ubiquitous presence of HFCS in the US diet. Several companies are responding to the push back and some are starting to offer foods and beverages without added HCFS giving individuals choices in selecting sweeteners in their diets.

High fructose corn syrup (HFCS) is, as the name implies, corn syrup whose glucose has been partially changed into a different sugar, fructose. To make HFCS, you start with corn, then mill it to produce starch -corn starch. Starch, the most important carbohydrate in the human diet, consists of long chains of glucose. To make corn syrup, you mix the corn starch with water and

then add an enzyme, produced by a bacterium, that breaks the starch down into shorter chains of glucose. Then you add another enzyme, produced by a fungus, that breaks the short chains down into glucose molecules. At that point, you have regular corn syrup.

To make the corn syrup into high fructose corn syrup, you turn some of its glucose molecules into fructose molecules by exposing the syrup to yet another enzyme, again produced by bacteria. This enzyme converts the glucose to a mixture of about 42 percent fructose and 53 percent glucose, with some other sugars as well. This syrup, called HFCS 42, is about as sweet as natural sugar (sucrose) and is used in foods and bakery items. HFCS 55, which contains approximately 55 percent fructose and 42 percent glucose, is sweeter than sucrose and is used mostly in soft drinks.

Cocoa butter

Cocoa butter, also called **theobroma oil**, is a pale-yellow, edible vegetable fat extracted from the cocoa bean. It is used to make chocolate, as well as some ointments, toiletries, and pharmaceuticals.[Cocoa butter has a cocoaflavor and aroma. Its best-known attribute is its melting point, which is just below human body temperature.

Cocoa butter is obtained from wholecocoa beans. For use in chocolate manufacture, the beans are fermentedbefore being dried. The beans are then roasted and separated from their hulls to produce cocoa nibs[4]. About 54–58% of the cocoa nibs is cocoa butter. The cocoa nibs are ground to form cocoa mass, which is liquid at temperatures above the melting point of cocoa butter and is known as cocoa liquor or chocolate liquor. Chocolate liquor is pressed to separate the cocoa butter from the non-fat cocoa solids. Cocoa butter is sometimes deodorized to remove strong or undesirable tastes.

Cocoa butter contains a high proportion of saturated fats as well as monounsaturated oleic acid, which typically occurs in each triglyceride. The predominant triglycerides are POS, SOS, POP, where P = palmitic, O

= oleic, and S = stearic acid residues. Cocoa butter, unlike non-fat cocoa solids, contains only traces of caffeine and theobromine.

Adulterants[

Some food manufacturers substitute less expensive materials such as vegetable oils and fats in place of cocoa butter. Several analytical methods exist for testing for diluted cocoa butter. Adulterated cocoa butter is indicated by its lighter color and its diminished fluorescence upon ultraviolet illumination. Unlike cocoa butter, adulterated fat tends to smear and have a higher non-saponifiable content.[13]

Substitutes

Cocoa butter is becoming increasingly costly. Substitutes have been designed to use as alternatives. In the United States, 100% cocoa butter must be used for the product to be called chocolate. The EU requires that alternative fats not exceed 5% of the total fat content.[1]

Substitutes include: coconut, palm, soybean, rapeseed, cottonseed and illipe oils; and shea butter, mango kernel fat[14]and a mixture of mango kernel fat and palm oil, and PGPR.

Uses

Cocoa butter is a major ingredient in practically all types of chocolates (white chocolate, milk chocolate, and dark chocolate). This application continues to dominate consumption of cocoa butter. Cocoa butter can be found in most supermarkets, and the process of preparing small amounts of chocolate from cocoa butter and cocoa powder means that the practice of making chocolate at home has become relatively popular.

Pharmaceutical companies use cocoa butter's physical properties extensively. As a nontoxic solid at room temperature that melts at body temperature, it is considered an ideal base for medicinal suppositories.[16]

Personal care

For a fat melting around body temperature, cocoa has good stability. This quality, coupled with natural antioxidants, prevents rancidity – giving it a storage life of two to five years. The velvety texture, pleasant fragrance andemollient properties of cocoa butter have made it a popular ingredient in products for the skin, such as soaps and lotions.

The moisturizing abilities of cocoa butter are frequently recommended for prevention of stretch marks in pregnant women, treatment of chapped or burned skin and lips, and as a daily moisturizer to prevent dry, itchy

skin. Cocoa butter's moisturizing properties are also said to be effective for treating mouth sores. However, the largest clinical study regarding the effects of cocoa butter on stretch marks in pregnant women found that results were no different from a placebo.

Physical properties

Cocoa butter typically has a melting point of around 34–38 °C (93–101 °F), so chocolate is solid at room temperature but readily melts once inside the mouth. Cocoa butter displays polymorphism, having different crystalline forms with different melting points. Conventionally the assignment of cocoa butter crystalline forms uses the nomenclature of Wille and Lutton [19] with forms I, II, III, IV, V and VI having melting points 17.3, 23.3, 25.5, 27.5, 33.8 and 36.3 °C respectively. The production of chocolate aims to crystallise the chocolate so that the cocoa butter is predominantly in form V, this is the most stable form that can be obtained from melted cocoa butter. (Form VI either develops in solid cocoa butter after long storage, or is obtained by crystallisation from solvents). A uniform form V crystal structure will result in smooth texture, sheen, and snap. This structure is obtained by chocolate tempering. Melting the cocoa butter in chocolate and then allowing it to solidify without tempering leads to the formation of unstable polymorphic forms of cocoa butter. This can easily happen when chocolate bars are allowed to melt in a hot room and leads to the formation of white patches on the surface of the chocolate called fat bloom or chocolate bloom.

1	.---------- converts the protoxin into active toxin.	Nuclease	Urease	Protease.	Lipase.	**Protease**
2	Convertion of protoxin to active toxin require both protease and ----------	Alkaline pH	Urease	Acid pH	Lipase.	**Alkaline pH**
3	Parasporal crystals sensitive to ----------	Sunlight	Chemicals	Acid pH	Lipase.	**Sunlight**
4	Most effective and most often utilized microbial insecticides are toxins synthesized from .	.B.amyloliquefaciens	B.thuringiensis	B.subtilis	B.licheniform	***B.thuringiensis***

5	B.thuringienis subspecies kurstaki is toxic to	bugs	worms	cabbage worm	small worms	cabbage worm
6	B.thuringiensis subspecies israelensis kills .	cabbage worm	black flies	lepidopteron larvae	none of the above	**black flies**
7	_____ is also known as sandeigo.	B.thuringiensis subsp israelensis	B.thuringiensis subsp kurstaki	B.thuringiensis	B.thuringiensis stubs tenebrionis	**B.thuringiensis stubs tenebrionis**
8	The parasporal crystal is _____ .	not the active form	a protoxin	precursor of active toxin	all the above	**all the above**

9	The insecticidal activity of B.thuringiensis is contained within a very large structure called .	paraspor al crystal	parabasal crystal	perisporal crystal	sporal crystal	**parasporal crystal**
10	The subunits of the parasporal crystal can be dissociated invitro by treatment with	alcohol	ethylene	β-mercaptoetha nol	.xylene	**β-mercaptoethanol**
11	Parasporal crystals are lived in the environment	short	long	.moderately	limited	**short**

12	To enhance production rate for B.thuringiensis are used.	promoters active in all phase	promoters active in sporulation	promoters active in vegitation	promoters active in one phase	**promoters active in all phase**
13	When tenebrionis toxin gene is transformed into B.thuringiensis subsp israelensis the transformants was toxic to .	brassicae	cabbage white butterfly	catterpiller	butterfly	cabbage white butterfly
14	The parasporal crystals of B.thuringiensis subsp israelensis shows as an insecticide	more efficacy	sinks rapidly when sprayed	sinks slowly when sprayed	medium efficacy	sinks rapidly when sprayed

15	The alternative bacterium used for B.thuringiensis toxin is	Caulobacter crescentus	Zymomonas	A. niger	none of the above	**Caulobacter crescentus**
16	Which one of the following can proliferate well in water surface near mosquito larvae?	Synechocystis	Synechovibrio	both a and b	Synechococcus6	**both a and b**
17	Which one of the following can be used as a biocontrol agent?	Baculovirus	retrovirus	A. niger	Penicillium sp	**Baculovirus**
18	Baculovirus are pathogenic to	Neuroptera	Trichoptera	Diptera	all the above	**all the above**
19	. The gene that encodes insect specific neurotoxin was produced by	Androctonus australis	Synechocystis	Caulobacter	Synechovibrio	**Androctonus australis**

20	The insect specific neurotoxin disrupts the .	normal life cycle of insect	flow of Na ions	sporulation	flow of chloride ions	**flow of Na ions**
21	When the parasporal crystal is ingested by a target insect, the protoxin is activated by _____ .	alkaline pH	specific digestive proteases	both a and b	None of the above	**both a and b**
22	The mode of action of B.thuringiensis toxins imposes certain constraints in application. They are	for killing the insect it must be ingested	insects of plant roots are less likely ingesting Bt toxin.	kills insects during a specific development al stage	all the above	**all the above**

23	Chemical insecticides has the following disadvantage	specificity	insects become sensitive easily	beneficial insects being killed	simple degradation	beneficial insects being killed
24	The steps taken to kill insects in plant roots is	to inject Bt toxin into roots	to introduce Bt gene into cells of root	introduce Bt toxin gene into bacterial species of rhizosphere	spray the Bt toxin	**introduce Bt toxin gene into bacterial species of rhizosphere**
25	Methods for biological protection of plants	transgenic plants	chemical insecticides	trimming of plants	avoid plant damage	**transgenic plants**

26	Baculo virus is a	Parasite	Obligate parasite	Saprophyte	Pathogen	**Obligate parasite**
27	Biologiacal insecticides are usually for a number of insect species.	less specific	Highly specific	resistant	highly resistant	**highly resistant**
28	The biological insecticides are to humans and other animals	hazardous	.useless	non-economical	non-hazardous	**non-hazardous**
29	Bt toxin is safe because	persist in the environment	hazardous to mammals	does not persist in the environment	non degradable	**does not persist in the environment**
30	gene transfer to animal by ----------	Transformation	microinjection	vector Ti	transduction	microinjection

		efficienc y				
31	Site directed mutagenesis refer to	change of whole genome	changes in a single base	change of whole DNA sequence	none	**changes in a single base**
32	The transgenic plant transformed with highly modified synthetic protoxin gene had level of expression than wild type.	10 fold	100 fold	1000 fold	10000	**100 fold**

33	What is antisense RNA?	RNA molecule complementary to gene transcript (mRNA)	RNA molecule complementary to DNA	DNA molecule complementary to RNA.	Type of RNA	**RNA molecule complementary to gene transcript (mRNA)**
34	Sense RNA is	the translate m RNA	the translate DNA	both a and b	none	**the translate m RNA**
35	Chemical herbicides are .	No side effects	discriminate weeds from crop	persist in the environment	all of the above	**persist in the environment.**

36	The different biological manipulations that would cause a crop plant to be herbicide resistant are	overproduction of herbicide sensitive target protein	improve the ability of herbicide	resistant protein to bind to herbicide	metabolic activation of herbicide	overproduction of herbicide sensitive target protein
37	Glyphosate is .	environment friendly	hazardous	toxic to living beings	none of the above	**environment friendly**
38	The EPSPS plays important role in the synthesis of amino acids in both bacteria and plants	aromatic	aliphatic	both a and b	none	**aromatic**

39	Bromoxynil, a herbicide acts by inhibiting .	chlorophyll content	photosynthesis	uptake of water and nutrients	utilization of carbon dioxide	**photosynthesis**
40	can inactivate bromoxynil	nitrilase	denitrilase	salicylase	all of the above	**nitrilase**
41	The gene for enzyme nitrilase was isolated from .	Klebsiella ozaenae	E.coli	Pseudomonas	Staphylococcus aureus	**Klebsiella ozaenae**
42	Chemical herbicides are .	No side effects	discriminate weeds from crop	persist in the environment	disapppear fm soil	persist in the environment

43	. The length of the T-DNA region can vary from .	12 to 24 Kb	10 to 20 Kb	17 to 26 Kb	9 to 19 Kb	12 to 24 Kb
44	Delay of fruit ripening can be done by .	antisense RNA technology	biofarming	refrigeration	incubation	**antisense RNA technology**
45	The plant growth promoter induces the experience of a number of genes involved in fruit ripening and senescence.	auxin	cytokinin	gibberellins	ethylene	**ethylene**

46	Ethylene is synthesized from .	S-adenosyl methionine	S-thymidine methionine	S-guanosyl methionine	S-cyclosine methionine	**s-adenosyl methionine.**
47	The length of the T-DNA region can vary from .	12 to 24 Kb	10 to 20 Kb	17 to 26 Kb	9 to 19 Kb	**12 to 24 Kb**
48	Crown gall tumor is induced by .	E.coli	A.tumefaciens	Pseudomonas	Acinetobacter	**A.tumefaciens.**
49	_____ are encoded on the Ti plasmid of A.tumefaciens.	ras genes	Vir genes	coz genes	nif genes	**vir genes**

50	In the T-DNA region the gene which encodes isopentenyl transferase is .	tmr gene	tms 1 gene	tms 2 gene	nif gene	**tmr gene**
51	Permits the Ti plasmid to be stably maintained in A.tumefaciens	ori region	vir gene	opine catabolism region	Nopaline	**ori region.**
52	Hairy root disease in higher plants is caused by .	A.rhizogenes	A.tumefaciens	E.coli	Bacillus	**A.rhizogenes**
53	.---------- - produce parasporal crystal which kills insects	B.thuringinsis	B.rhizogenes	Heliothis virescens	Autographa californica	**B.thuringinsis**

54	DNA virus ----------	Baculo virus	Mosaic virus	Simian virus	Satellite virus	**Baculo virus**
55	Baculo virus particle consist of cylindrical ----------that surrounds viral DNA	Nucleocapsid	Nucleus	Nucleosome	Proteosome	**Nucleocapsid**
56	The Cry I proteins are toxic to ----------	Diptera	Coleoptera	Lepidoptera	Both a & b	**Diptera**
57	The Cry IV protein is toxic to ----------	Diptera	Coleoptera	Lepidoptera	d) Both a & b	**Lepidoptera**
58	Parasporal crystal does not usually contain the active form of the ----------	Insecticide	Pesticide	Herbicide	Both b& c	**Insecticide**

59	The B. thuringiensis subsp ---------- - insecticidal protein is highly toxic when injested by mosquito larvae.	israelensis	kurstaki	tenebrionis	aizawai	**kurstaki**
60	Possible attractive host for the expression of mosquitocidal Cry genes ----------	Bacillus sphaericus	B.thuringinsis	B.rhizogenes	Asticcacaulis excentricus	**B.thuringinsis**
61	Protoxin is activated with in the ---------- ---------	Gut	Lungs	Respiratory tract	Stomach	**Gut**

62	Antisense therapy is .	prevents the expression of the defective gene	delivery of a remedial gene into organ	cells taken from organ after the correction it transplanted back	remedial gene is introduced into an embryo.	prevents the expression of the defective gene
63	Testing of newborn for children for genetic disease is	parental screening	postnatal screening	antenatal screening	prenatal genetic screening	postnatal screening
64	The microinjected transgene construct is in form and free of vector DNA sequences	linear and prokaryotic	Circle and prokaryotic	Circle and eukaryotic	linear and eukaryotic	linear and prokaryotic

UNIT-IV

SYLLABUS

Microbial product purification: filtration, ion exchange & affinity chromatography techniques Immobilization methods and their application: Whole cell immobilization. RNAi and its applications in silencing genes, drug resistance, therapeutics and host pathogen interactions.

Microbial biotechnology involves the exploitation, genetic manipulation and alterations of micro-organisms to make commercial valuable products and that also involves fermentationand various upstream and downstream processes.

Microorganisms produce an amazing array of valuable products such as macromolecules (e.g. proteins, nucleic acids, carbohydrate polymers, even cells) or smaller molecules and are usually divided into metabolites that are essential for vegetative growth (primary metabolites) and those which give advantages over adverse environment (secondary metabolites). They usually produce these compounds in small amounts that are needed for their own benefit.

Genetic Engineering of Microorganisms for Biotechnology

Molecular genetics can be used to manipulate genes in order to alter the expression and production of microbial products, including the expression of novel recombinant proteins.

The compounds that are isolated from plants or animals can be synthesized by genetic manipulation of different micro-organisms to enhance the production and by environmental and other manipulations, even up to 1000-fold for small metabolites can be increased.

The advent of recombinant DNA technology (also referred to as gene cloning or *in vitro genetic manipulation)* has dramatically broadened the spectrum of microbial genetic manipulations. With the advancement of recombinant DNA technology, many novel host systems have been explored to produce commercially important products like therapeutic proteins, antibiotics, small molecules, biosimilars etc.

The basis of this technology is the use of restriction endonucleases, polymerases and DNA ligases as a means to specifically cut and paste fragments of DNA. Similarly, foreign DNA fragments can be introduced into a vector molecule (a plasmid or a bacteriophage), which enables the DNA to replicate after introduction into a bacterial cell.

The ability to modify and clone genes accelerated the rate of discovery and the development in biotech industries.

The basic steps in DNA cloning involves the following,

- A fragment of DNA is inserted into a carrier DNA molecule, called a vector, to produce a recombinant DNA.
- The recombinant DNA is then introduced into a host cell, where it can multiply and produce numerous copies of itself within the host. The most commonly used

host is the bacteria, although other hosts can also be used to propagate the recombinant DNA.

- Further amplification of the recombinant DNA is achieved when the host cell divides, carrying the recombinant DNA in their progenies, where further vector replication can occur.
- After a large number of divisions and replications, a colony or clone of identical host cell is produced, carrying one or more copies of the recombinant DNA.
- The colony carrying the recombinant DNA of interest is then identified, isolated, analyzed sub-cultured and maintained as a recombinant strain.

Expression of a foreign protein in a microbe

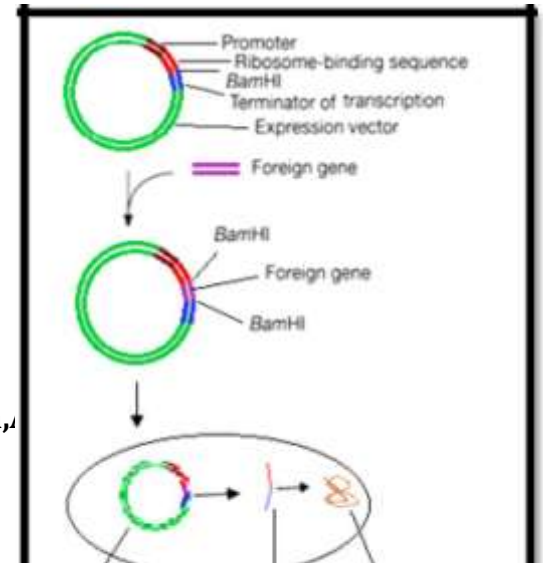


Figure7-1.3. Foreign protein expression in a microbe

There are several methods by which genetic alterations of producer microbial strains can be done for maximization of products or metabolites.

Traditional Method of Strain Improvement

- The remarkable increases in antibiotic productivity and the resulting decreases in costs have resulted due to mutation and screening for higher producing microbial strains. In recent years, efforts have been devoted to miniaturize and automate the screening procedures and to enhance the frequency of improved strains by selection procedures, e.g., the isolation of anti-metabolite-resistant mutants in cases where the natural metabolite is a precursor, an inhibitor or a co-repressor of a biosynthetic pathway.
- Mutation has also served to shift the proportion of metabolites production in a fermentation broth to a more favorable distribution, to elucidate the pathways of secondary metabolism, and to yield new antibiotics.
- Targeted mutagenesis**:** It involves introduction of mutations at a specific location in DNA. As many antibiotics, growth hormones, regulatory factors production genes

have now been cloned, targeted mutagenesis of the cloned DNA can be performed *in vitro*, followed by transformation of the recipient organism.

- The major problems of the classic strain improvement procedure based on random mutagenesis were the very low probability of introducing mutations into relevant genes and high rate of unwanted mutations in other, unrelated genes.

Classical Genetics

The most effective use of classical genetics in the past was the backcrossing of overproducing strains with parent strains to improve the vigor of mutant strains.

- After backcrossing such a strain with the wild type, progeny cells are produced that have inherited the overproducing traits from the mutant parent and the wild-type hardiness and vigor from the wild-type parent.

- Priorly this was not possible with *Penicillium*, which lacks a true sexual cycle. However, a parasexual cycle resulting in the production of heterokaryons was discovered in *Penicillium* in 1958 and was used to improve the strains.

- Protoplast fusion is relatively a new versatile technique to induce or promote genetic recombination in a variety of prokaryotic and eukaryotic cell especially, industrially useful microorganisms such as *Streptomycete ambofaciens*, *Micromonsporae chinospora*, because it breaks the barriers to genetic exchange. Another use of protoplast fusion has been the recombination of different strains from the same or different species to yield new antibiotics such as anthracyclines, aminoglycosides and rifamycins. Protoplast

fusion has also been useful in elimination of an undesirable component from penicillin broths imposed by conventional mating systems.

Rational Selection

It involves selecting an improved producer out of a very large population of progeny. For example, some antibiotics, notably penicillin and tetracyclines, are chelators of heavy metal ions. The more of these antibiotics an organism produces, the more resistant it will be to heavy metals in the medium. Thus, selection for mutants resistant to heavy metals was used in the improvement of the penicillin producers.

Cloning of the candidate genes

Recombinant DNA technology can be used to introduce genes coding for antibiotic synthetases into producers of other antibiotics or into non-producing strains to obtain modified or hybrid antibiotics. The use of recombinant DNA technology in antibiotic improvement and discovery has been enhanced by the finding that some streptomycetes antibiotic biosynthetic pathways are coded by plasmid genes, eg. methylenomycin A. Even when the antibiotic biosynthetic pathway genes of streptomycetes are chromosomal, they appear to be clustered into operons which facilitate transfer of an entire pathway in a single manipulation.

The genes encoding individual enzymes of antibiotic biosynthesis which have already been cloned include those of the cephalosporin, clavulanic acid, prodigiosin, undecylprodigiosin, actinomycin, and candicidin pathways. The isopenicillin N

synthetase ("CyClaSe") gene of *Cephalosporium acremonium* has been cloned in *Escherichia coli* and expressed at a level of 20% of total cell protein. Cyclase gene of *Penicillium chrysogenum* and *Streptomyces clavuligerus* has also been cloned in *E.coli* system. The expandase/ hydroxylase gene of *C. acremonium* has been cloned in *E. coli*. The protein accumulated as inclusion bodies in *E. coli* near to 15% of total cell protein

Similar to *E.coli*, now a days *Bacillus subtilis*, *Pichia pastoris*, *Saccharomyces cerevisiae* have also emerged as a promising heterologous expression system for prokaryotic and eukaryotic candidate genes.

There are several factors which govern the production of recombinant therapeutic proteins in

B. subtilis. The factors are as follows:

a) Well understood transcription and translation machinery including different regulatory factors responsible for extracellular product.
b) Generation of stable recombinant plasmids.

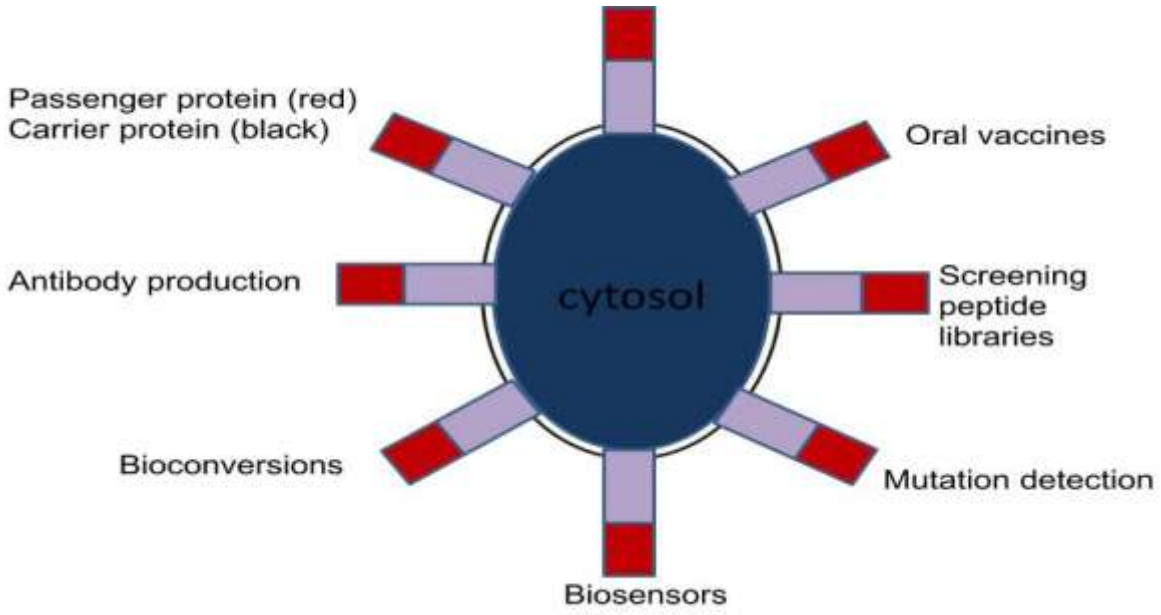
c) Development of novel recombinant *B. subtilis* strains with reduced nuclease and protease contents.

d) Better systematic understanding of the protein secretion method to elucidate the factors responsible for the secretion of intracellular proteins.

To get the soluble extracellular protein product from the gene, it can be linked to a DNA fragment coding for *B. subtilis* signal peptide for extra-cellular secretion. The signal

sequence may be preceded by a efficient translation initiator sequence or ribosome binding site (RBS) which is followed by mRNA stability-enhancing sequences (SES) at 5' end of mRNA. The 'strong' promoter for this gene consist a cluster of other efficient promoters which would be regulated temporally by the growth condition or growth medium components. Thus genes possess suitable properties not only for efficient transcription, translation but would be under temporal control avoiding other exogenous induction. Thus generated mRNA would be stabilized by 3'SES which protects mRNA from degradation by 3' exonucleases. The protein product consists of a typical *B. subtilis* signal peptide linked to N-terminus and have to be further processed with proteases to recover only the desired portion.

Microbial Cell-Surface Display



Different applications of various bio macro molecules generated in microbes

Cell-surface display allows proteins, peptides and other bio macro molecules to be displayed on the surface of microbial cells by fusing them with the anchoring motifs. The protein to be displayed -passenger protein - can be fused to an anchoring motif - the carrier protein - by N-terminal fusion, C-terminal fusion or sandwich fusion. The specific features of carrier protein, passenger protein and host cell, and various fusion methods affect the efficiency of surface display of bio-macromolecules. Microbial cell-surface display has many potential applications, including live vaccine development, peptide library screening, bioconversion using whole cell biocatalyst and bio-adsorption.

Potential applications of genetic manipulation of micro-organisms

One of the potential applications of microbial biotechnology is the production of pharmaceuticals, neutraceuticals by bacteria or other micro-organisms that produce economically, clinically important products like human insulin for diabetics or human growth hormone for dwarf individuals. Techniques are being perfected to transfer human genes into cows, sheep, and goats to obtain medically significant products from the milk of these animals.

Development of diagnostics is to detect disease-causing organisms and monitor the safety of food and water quality. Investigators are developing systems for identifying pathogens that may be used as biological weapons by rogue nations or even terrorist groups in future.

Bacteria can be genetically altered to emit a green fluorescent protein visible in ultraviolet light when they metabolize the explosive TNT leaking from land mines. Researchers envision a day when bacteria can be applied to a tract of land with a crop duster and then be analyzed from a helicopter. Genetically modified microorganisms can be used a living sensor to detect any particular chemicals in soil, air or other inorganic or biological specimens.

In Microbial Genome Program, alterations in the genome of the bacterium *Deinococcus radiodurans* are performed to increase its potential in cleaning up toxic-waste sites. The microbe's extraordinary DNA-repair processes enable it to thrive in high-radiation exposed environments.

Using various biotechnological processes, genes can be added from other organisms that will confer the ability to degrade toxinogenic chemicals such as toluene, commonly found in chemical and radiation waste sites.

Microbial product purification

Filtration

Water filtration is a mechanical or physical process of separating suspended and colloidal particles from fluids (liquids or gases) by interposing a medium through which only the fluid can pass. Medium used is

generally a granular material through which water is passed. In the conventional water treatment process, filtration usually follows coagulation, flocculation, and sedimentation.

Filtration process

- During filtration in a conventional down-flow depth filter, wastewater containing suspended matter is applied to the top of the filter bed.
- As the water passes through the filter bed, the suspended matter in the wastewater is removed by a variety of removal mechanisms.
- With passage of time, as material accumulates within the interstices of the granular medium, the head-loss through the filter starts to build up beyond the initial value.
- After some period of time, the operating head-loss or effluent turbidity reaches a predetermined head loss or turbidity value, and the filter must be cleaned (backwashed) to remove the material (suspended solids) that has accumulated within the granular filter bed. Backwashing is accomplished by reversing the flow through the filter.
- A sufficient flow of wash water is applied until the granular filtering medium is fluidized (expanded), causing the particles of the filtering medium to abrade against each other.

Filtration is classified into following three types

1) Depth filtration
    a) Slow sand filtration
    b) Rapid porous and compressible medium filtration
    c) Intermittent porous medium filtration
    d) Recirculating porous medium filtration

2) Surface filtration
    a) Laboratory filters used for TSS test
    b) Diatomaceous earth filtration
    c) Cloth or screen filtration

2) Membrane flirtation

DEPTH FILTRATION

In this method, the removal of suspended particulate material from liquid slurry is done by passing the liquid through a filter bed composed of granular or compressible filter medium.

- Depth filtration is the solid/liquid separation process in which a dilute suspension or• wastewater is passed through a packed bed of sand, anthracite, or other granular media.
- Solids (particles) get attached to the media or to the previously retained particles and are removed from the fluid [2].
- This method is virtually used everywhere in the treatment of surface waters for potable water supply.
- Depth filtration is also often successfully used as a tertiary treatment for wastewater.
- Failure of depth filtration affects the other downstream processes significantly and most of the times results in overall plant failure.
- Performance of a filter is quantified by particle removal efficiency and head loss across the packed bed.
- The duration of a filter run is limited by numerous constraints: available head, effluent quality or flow requirement.
- The head loss and removal efficiency of a filter are complicated functions of suspensionqualities (particle size distribution and concentration, particle surface chemistry, and solution chemistry), filter design parameters (media size, ty RSF is used today as an effective pretreatment procedure to enhance water quality prior to reverse osmosis (RO) membranes in desalination plants .

SURFACE FILTRATION Surface filtration involves removal of suspended material in a liquid by mechanical sieving. In this method, the liquid is passed through a thin septum (i.e., filter material). Materials that have been used as filter septum include woven metal fabrics, cloth fabrics of different weaves, and a variety of synthetic materials .

MEMBRANE FILTRATION Membrane filtration can be broadly defined as a separation process that uses semipermeable membrane to divide the feed stream into two portions: a permeate that contains the material passing through the membranes, and a retentate consisting of the species being left behind [5]. Membrane filtration can be further classified in terms of the size range of permeating species, the mechanisms of rejection, the driving forces employed, the chemical structure and composition of membranes, and the geometry of construction. The most important types of membrane filtration are pressure driven processes including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO).

MECHANISMS INVOLVED IN THE FILTRATION PROCESSES The process of filtration involves several mechanisms listed in the table. Straining has been identified as the principal mechanism that is operative in the removal of suspended solids during the filtration of settled secondary effluent from biological treatment processes. Other mechanisms including impaction, interception, and adhesion are also operative even though their effects are small and, for the most part, masked by the straining action.

Particles smaller than the pore space are trapped within the filter by chance contact Sedimentation Particles settle on the filtering medium within the filter Impaction Heavy particles do not follow the flow streamlines Interception Particles get removed during contact with the surface of the filtering medium Adhesion Particles become attached to the surface of the filtering medium as they pass through. Flocculation It can occur within the interstices of the filter medium. Chemical adsorption a) Bonding b) Chemical interaction Once a particle has been brought in contact with the surface of the filtering medium or with other particles, either one of these mechanisms, chemical or physical adsorption or both, may occur. Physical adsorption a) Electrostatic forces b) Electrokinetic forces c) Van der Waals forces Biological growth Biological growth within the filter reduces the pore volume and enhances the removal of particles with any of the above removal mechanisms.

FILTER-MEDIUM CHARACTERISTICS Grain size is the principle filter-medium characteristic that affects the filtration operation. Grain size affects both the clear-water head loss and the

buildup of head loss during the filter run. If too small a filtering medium is selected, much of the driving force will be wasted in overcoming the frictional resistance of the filter bed. On the other hand, if the size of the medium is too large, many of the small particles in the influent will pass directly through the bed. The size distribution of the filter material is usually determined by sieve analysis using a series of decreasing sieve sizes.

CLASSIFICATION OF FILTERS Filters that must be taken off-line periodically to be backwashed are classified operationally as semi-continuous. Filters in which is filtration and backwash operations occur simultaneously are classified as continuous. Within each of these two classifications, there are a number of different types of filters depending on bed depth (e.g., shallow, conventional, and deep bed), the type filtering medium used (mono-, dual-, and multi-medium), whether the filtering medium is stratified or unstratified, the type of operation (down-flow or upflow), and the method used for the management of solids (surface or internal storage). For the mono- and dual-medium semi-continuous filters, a further classification can be made based on the driving force (e.g., gravity or pressure)

TYPES OF DEPTH FILTERS The five types of depth filters used most commonly for wastewater filtration are (a) Conventional down-flow filters: Single-, dual-, or multimedium filter materials are utilized in conventional down-flow depth filters. Typically sand or anthracite is used as the filtering material in single-medium filters. Dual-medium filters usually consist of a layer anthracite over a layer of sand. Dual- and multimedium and deep-bed mono-medium depth filters were developed to allow the suspended solids in the liquid to be filtered to penetrate farther into the filter bed, and thus use more of the solids-storage capacity available within the filter bed. (b) Deep-bed down-flow filters: The deep-bed down-flow filter is similar to the conventional down-flow filter with the exception that the depth of the filter bed and the size of the filter medium are greater than corresponding values an conventional filter. Because of the greater depth and larger medium size, more solids can be stored within the filter bed and the run length can be extended. (c) Deep-bed upflow continuous-backwash filters: In this filter the wastewater to be filtered is introduced into the bottom of the filter where it flows upward through a series of riser tubes and is

distributed evenly into the sand bed through the open bottom of an inlet distribution hood. The water then flows upward through the downward-moving sand. The clean filtrate exits from the sand bed, overflows a weir, and is discharged from the filter. Because the sand has higher settling velocity than the removed solids, the sand is not carried out of the filter. (d) Pulsed-bed filter: The pulsed-bed filter is a proprietary down-flow gravity filter with an unstratified shallow layer of fine sand as the filtering medium. The shallow bed is used for solids storage, as opposed to other shallow-bed filters where solids are principally stored on the sand surface. An unusual feature of this filter is the use of an air pulse to disrupt the sand surface and thus allow penetration of suspended solids into the bed. (e) Travelling-bridge filters: The travelling-bridge filter is a proprietary continuous down-flow, automatic backwash, low-head, granular medium depth filter. The bed of the filter is divided horizontally into long independent filter cells. Each filter cell contains approximately 280 mm of medium. Treated wastewater flows through the medium by gravity.

Ion Exchange Chromatography

Ion exchange chromatography is a fast, economical and versatile technique for effective separation of ions, amino acids, peptides, nucleotide and nucleic acids etc. This technique is widely used in the pre-fractionation or purification of a target protein from crude biological samples. Before we go in details of ion-exchange chromatography of proteins, let us discuss, ionization and charge on proteins with respect to Ph.

Principle of Ion Exchange Chromatography:

Ion exchange chromatography separates proteins or other molecules based on differences in their accessible surface charges. In ion exchange chromatography the analyte molecules are retained on the column based on coulombic (ionic) interactions. The stationary phase surface contains ionic functional groups of opposite charge that interact with analyte ions. The elution is done by increasing salt gradient. Most commonly used salt is NaCl, exists in equilibrium with Na+

(cation) and Cl- (anion) in aqueous solution. As the concentration of salt increases concentration of Na+ (cation) and Cl- (anion) also increases.

The basic principle of ion exchange chromatography is the reversible exchange of analyte ions bound to solid support with similar ions generated from salt in liquid phase. Many biological molecules such as proteins, amino acids, nucleotides and other ions have ionisable groups which carries a net charge (positive or negative) dependent on their pKa and on the pH of the solution, which can be utilised in separating mixture of such molecules as explained in box.

Ion exchange chromatography experiments are carried out mainly in columns packed with ion exchangers. On the basis of type of exchanger used for separation this chromatography is further subdivided into cation exchange chromatography and anion exchange chromatography. Many biological molecules, especially proteins, are stable within a narrow pH range so the type of exchanger selected must operate within this range. Suppose if protein is most stable below its isolecteic point (pI), there will be net positive charge on the protein surface, so for separation of this protein cation exchanger should be used (experimental pH value should be between lowest pH wehere protein is stable and pI).

If protein is most stable above its pI, there will be net negative charge on the protein surface and anion exchanger should be used (experimental pH value should be between highest pH where protein is stable and pI value). If protein is stable over a wide range of pH, it can be separated by either type of ion exchanger (experimental pH value may be decided considering lowest and highest pH value stability of the protein). Weak electrolyte requires very high or very low pH for ionisation so it can only be separated on strong exchanger, as they only operate over a wide pH range, whereas in case of strong electrolytes, weak exchangers are preferred.

Affinity chromatography

Affinity chromatography was introduced almost 50 years back as a powerful tool for purification of biologically active molecules like proteins. This technique has revolutionary impact on modern

biological sciences such as molecular biology, biochemistry, medicine and biotechnology. This technique exploits molecular recognition principle of a biological compound to be separated by the specific ligand to purify it from a mixture of compounds.

The affinity chromatography is a type of liquid chromatography for the separation and specific analysis of sample components. This type of chromatography makes use of a reversible "biological interaction" (molecular recognition) for the separation and analysis of specific analytes within a sample e.g. enzyme with an inhibitor and antigen with an antibody. One of the components, the ligand is immobilized onto a solid matrix, which is then used to selectively purify the target protein. Including a competing ligand in mobile phase or changing pH that elutes the target protein out. For example, Ni-Affinity chromatography is applied for the purification of 6xHis tagged proteins in which Ni is the chelating metal which is attached on NTA matrix.

Theoretically affinity chromatography is capable of giving absolute purification in a single step. The technique was developed for purification of enzymes but now affinity chromatography is used for various other purposes like purification of nucleotides, nucleic acid, immunoglobulin, membrane receptors etc.

The principle of affinity chromatography is that the stationary phase consists of a support medium (e.g. cellulose beads) on which the substrate (or sometimes a coenzyme) has been bound covalently, in such a way that the reactive groups that are essential for enzyme binding are exposed. As the crude mixture of proteins is passed through the chromatography column, proteins with binding site for the immobilized substrate will bind to the stationary phase, while all other proteins will be eluted in the void volume of the column. Once the other proteins have all been eluted, the bound enzyme(s) can be eluted in various ways. We will discuss elution methods in during coming lectures.

IMMOBILIZATION

Traditionally, enzymes in free solutions (i.e. in soluble or free form) react with substrates to result in products. Such use of enzymes is wasteful, particularly for industrial purposes, since enzymes are not stable, and they cannot be recovered for reuse.

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.

Applications of Immobilization

1) Immobilized Enzyme-aminoacylase used for the first time by the immobilization method for the production of L-amino acids
2) In food industry, fructose syrup is produce from glucose by use of immobilized enzyme glucose-isomerase
3) Immobilized enzyme used in biosensor
4) Immobilized enzymes used in various analytical techniques where one can diagnose clinical problems
5) Accurate analysis of sample done with the help of specific immobilized enzyme and sensitive chemical analytical techniques uses immobilized enzyme
6) Immobilized Enzyme or Cells used in industry for the production of various industrial products

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.

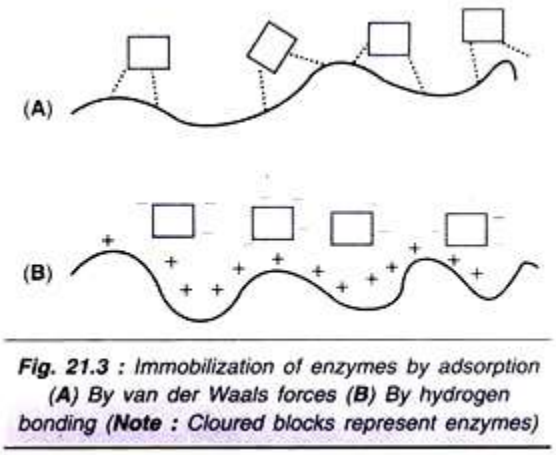


***Fig. 21.3** : Immobilization of enzymes by adsorption (**A**) By van der Waals forces (**B**) By hydrogen bonding (**Note** : Cloured blocks represent enzymes)*

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.

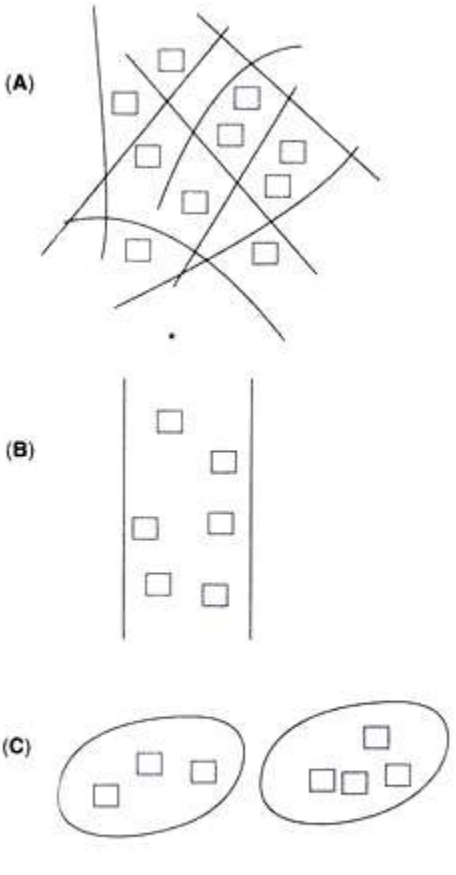


*Fig. 21.4 : Immobilization of enzymes by entrapment (A) Inclusion in gels (B) Inclusion in fibres (C) Inclusion in microcapsules (**Note** : Coloured blocks represent enzymes)*

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.

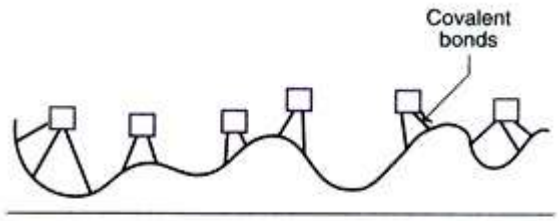


Fig. 21.5 : A general representation of immobilization of enzymes by covalent binding (Note : coloured blocks represent enzymes).

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 $NaNO_2$ and HCI. They, in turn, bind covalently to tyrosyl or histidyl groups of enzymes (Fig. 21.6B).

3. Peptide bond formation:

Enzyme immobilization 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 Fig. 21.6D.

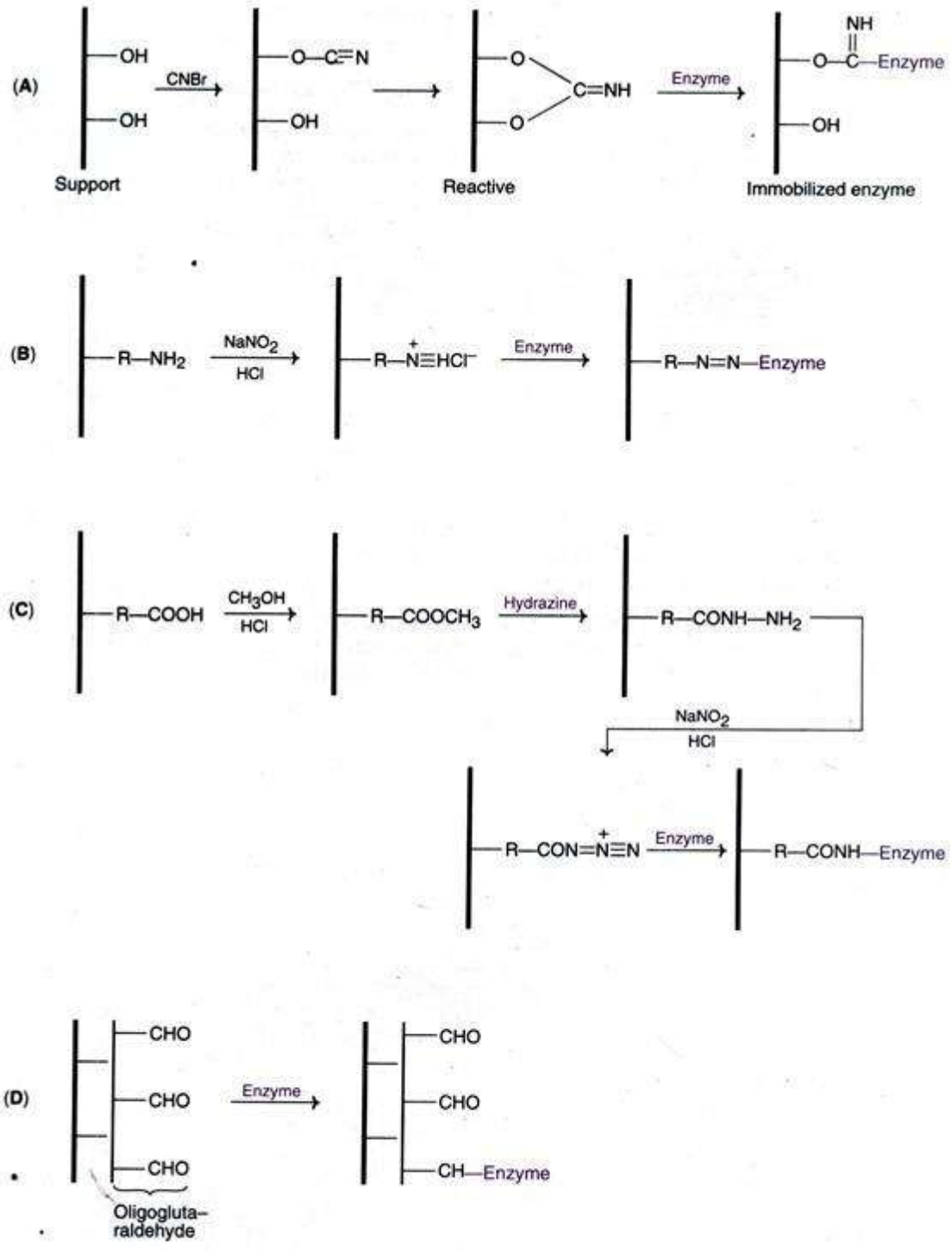


***Fig. 21.6** : Immobilization of enzymes by covalent binding (A) Cyanogen bromide activation, (B) Diazotation, (C) Peptide bond formation, (D) Activation by bifunctional agent.*

Cross-Linking:

The absence of a solid support is a characteristic feature of immobilization of enzymes by cross-linking. The enzyme molecules are immobilized by creating cross-links between them, through the involvement of poly-functional reagents. These reagents in fact react with the enzyme molecules and create bridges which form the backbone to hold enzyme molecules (Fig. 21.7). There are several reagents in use for cross-linking. These include glutaraldehyde, diazobenzidine, hexamethylene diisocyanate and toluene di- isothiocyanate.

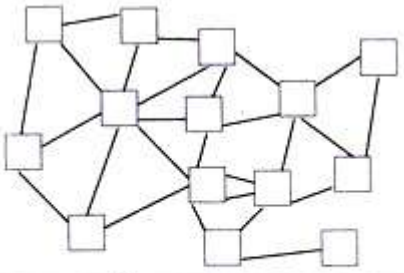


Fig. 21.7 : Immobilization of enzyme molecules by cross linking.

Glutaraldehyde is the most extensively used cross-linking reagent. It reacts with lysyl residues of the enzymes and forms a Schiff's base. The cross links formed between the enzyme and glutaraldehyde are irreversible and can withstand extreme pH and temperature. Glutaraldehyde cross- linking has been successfully used to immobilize several industrial enzymes e.g. glucose isomerase, penicillin amidase. The technique of cross-linking is quite simple and cost-effective. But the disadvantage is that it involves the risk of denaturation of the enzyme by the poly-functional reagent.

Choice of Immobilization Technique:

The selection of a particular method for immobilization of enzymes is based on a trial and error approach to choose the ideal one. Among the factors that decide a technique, the enzyme catalytic activity, stability, regenerability and cost factor are important.

Immobilization of L-amino acid acylase:

L-Amino acid acylase was the first enzyme to be immobilized by a group of Japanese workers (Chibata and Tosa, 1969). More than 40 different immobilization methods were attempted by this group. Only three of them were found be useful. They were covalent binding to iodoacetyl cellulose, ionic binding to DEAE-Sephadex and entrapment within polyacrylamide.

Stabilization of Soluble Enzymes:

Some of the enzymes cannot be immobilized and they have to be used in soluble form e.g. enzymes used in liquid detergents, some diagnostic reagents and food additives. Such enzymes can be stabilized by using certain additives or by chemical modifications. The stabilized enzymes have longer half-lives, although they cannot be recycled. Some important methods of enzyme stabilization are briefly described.

Solvent Stabilization:

Certain solvents at low concentrations stabilize the enzymes, while at high concentrations the enzymes get denatured e.g. acetone (5%) and ethanol (5%) can stabilize benzyl alcohol dehydro-genase.

Substrate Stabilization:

The active site of an enzyme can be stabilized by adding substrates e.g. starch stabilizes a-amylase; glucose stabilizes glucose isomerase.

Stabilization by Polymers:

Enzymes can be stabilized, particularly against increased temperature, by addition of polymers such as gelatin, albumin and polyethylene glycol.

Stabilization by Salts:

Stability of metalloenzymes can be achieved by adding salts such as Ca, Fe, Mn, Cu and Zn e.g. proteases can be stabilized by adding calcium.

Stabilization by Chemical Modifications:

Enzymes can be stabilized by suitable chemical modifications without loss of biological activity. There are several types of chemical modifications.

a. Addition of poly-amino side chains e.g. polytyrosine, polyglycine.

b. Acylation of enzymes by adding groups such as acetyl, propionyl and succinyl.

Stabilization by Rebuilding:

Theoretically, the stability of the enzymes is due to hydrophobic interactions in the core of the enzyme. It is therefore, proposed that enzymes can be stabilized by enhancing hydrophobic interactions. For this purpose, the enzyme is first unfold and then rebuilt in one of the following ways (Fig. 21.8).

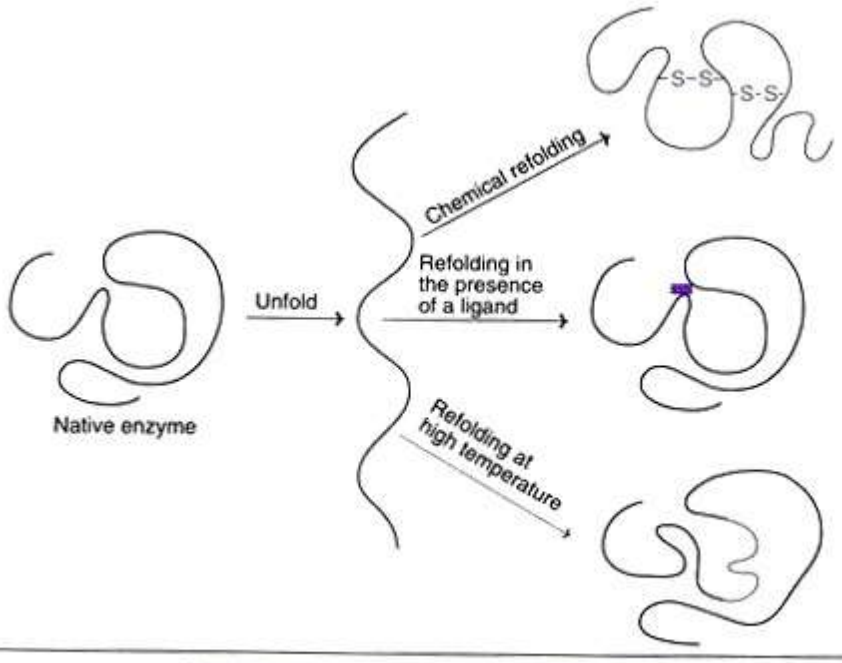


Fig. 21.8 : Stabilization of an enzyme by refolding.

1. The enzyme can be chemically treated (e.g. urea and a disulfide) and then refolded.

2. The refolding can be done in the presence of low molecular weight ligands.

3. For certain enzymes, refolding at higher temperatures (around 50°C) stabilize them.

Stabilization by Site-Directed Mutagenesis:

Site-directed mutagenesis has been successfully used to produce more stable and functionally more efficient enzymes e.g. subtilisin E.

Immobilization of Cells:

Immobilized individual enzymes can be successfully used for single-step reactions. They are, however, not suitable for multi-enzyme reactions and for the reactions requiring cofactors. The whole cells or cellular organelles can be immobilized to serve as multi-enzyme systems. In addition, immobilized cells rather than enzymes are sometimes preferred even for single reactions, due to cost factor in isolating enzymes. For the enzymes which depend on the special arrangement of the membrane, cell immobilization is preferred.

Immobilized cells have been traditionally used for the treatment of sewage. The techniques employed for immobilization of cells are almost the same as that used for immobilization of enzymes with appropriate modifications. Entrapment and surface attachment techniques are commonly used. Gels, and to some extent membranes, are also employed.

Immobilized Viable Cells:

The viability of the cells can be preserved by mild immobilization. Such immobilized cells are particularly useful for fermentations. Sometimes mammalian cell cultures are made to function as immobilized viable cells.

Immobilized Non-viable Cells:

In many instances, immobilized non-viable cells are preferred over the enzymes or even the viable cells. This is mainly because of the costly isolation and purification processes. The best example is the immobilization of cells containing glucose isomerase for the industrial production of high fructose syrup. Other important examples of microbial biocatalysts and their applications are given in Table 21.5.

TABLE 21.5 Selected examples of immobilized cells (to bring out one or two enzyme reactions) in industrial applications

Immobilized microorganism (microbial biocatalyst)	*Application(s)*
Escherichia coli	For the synthesis of L-aspartic acid from fumaric acid and NH_3
Escherichia coli	For the production of L-tryptophan from indole and serine
Pseudomonas sp	Production of L-serine from glycine and methanol
Saccharomyces cerevisiae	Hydrolysis of sucrose
Saccharomyces sp	Large scale production of alcohol
Zymomonas mobilis	Synthesis of sorbitol and gluconic acid from glucose and fructose
Anthrobacter simplex	Synthesis of prednisolone from hydrocortisone
Pseudomonas chlororaphis	Production of acrylamide from acrylonitrile
Humicola sp	For the conversion of rifamycin B to rifamycin S
Bacteria and yeasts (several sp)	In biosensors

Limitations of Immobilizing Eukaryotic Cells:

Prokaryotic cells (particularly bacterial) are mainly used for immobilization. It is also possible to immobilize eukaryotic plant and animal cells. Due to the presence of cellular organelles, the metabolism of eukaryotic cells is slow. Thus, for the industrial production of biochemical, prokaryotic cells are preferred. However, for the production of complex proteins (e.g. immunoglobulin's) and for the proteins that undergo post- translational modifications, eukaryotic cells may be used.

Effect of Immobilization on Enzyme Properties:

Enzyme immobilization is frequently associated with alterations in enzyme properties, particularly the kinetic properties of enzymes.

Some of them are listed below:

1. There is a substantial decrease in the enzyme specificity. This may be due to conformational changes that occur when the enzyme gets immobilized.

2. The kinetic constants K_m and V_{max} of an immobilized enzyme differ from that of the native enzyme. This is because the conformational change of the enzyme will affect the affinity between enzyme and substrate.

Immobilized Enzyme Reactors:

The immobilized enzymes cells are utilized in the industrial processes in the form of enzyme reactors. They are broadly of two types — batch reactors and continuous reactors. The frequently used enzyme reactors are shown in Fig. 21.9.

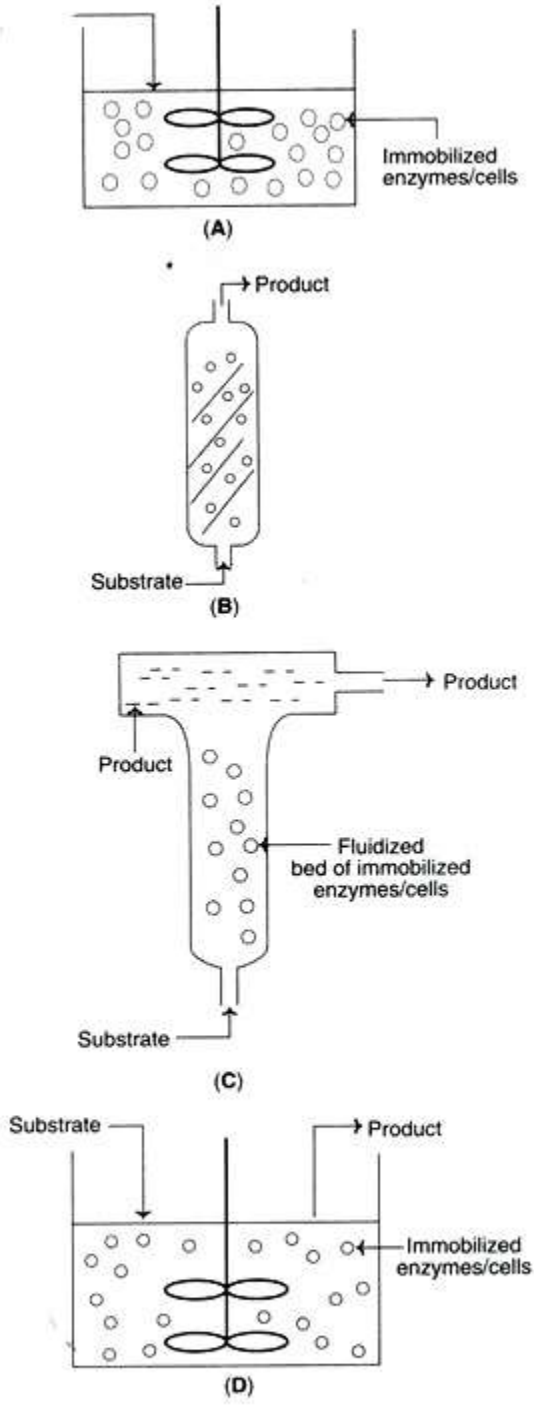


Fig. 21.9 : *Immobilized enzyme (cell) reactors (A) Batch stirred tank reactor, (B) Packed bed reactc (C) Fluidized bed reactor, (D) Continuous stirred tank reactor.*

Batch Reactors:

In batch reactors, the immobilized enzymes and substrates are placed, and the reaction is allowed to take place under constant stirring. As the reaction is completed, the product is separated from the enzyme (usually by denaturation).

Soluble enzymes are commonly used in batch reactors. It is rather difficult to separate the soluble enzymes from the products; hence there is a limitation of their reuse. However, special techniques have been developed for recovery of soluble enzymes, although this may result in loss of enzyme activity.

Stirred tank reactors:

The simplest form of batch reactor is the stirred tank reactor (Fig. 21.9A). It is composed of a reactor fitted with a stirrer that allows good mixing, and appropriate temperature and pH control. However, there may occur loss of some enzyme activity. A modification of stirred tank reactor is basket reactor. In this system, the enzyme is retained over the impeller blades. Both stirred tank reactor and basket reactor have a well-mixed flow pattern.

Plug flow type reactors:

These reactors are alternatives to flow pattern type of reactors. The flow rate of fluids controlled by a plug system. The plug flow type reactors may be in the form of packed bed or fluidized bed (Fig. 21.9B and 21.9C). These reactors are particularly useful when there occurs inadequate product formation in flow type reactors. Further, plug flow reactors are also useful for obtaining kinetic data on the reaction systems.

Continuous Reactors:

In continuous enzyme reactors, the substrate is added continuously while the product is removed simultaneously. Immobilized enzymes can also be used for continuous operation. Continuous reactors have certain advantages over batch reactors. These include control over the product formation, convenient operation of the system and easy automation of the entire process. There are mainly two types of continuous reactors-continuous stirred tank reactor (CSTR) and plug

reactor (PR). A diagrammatic representation of CSTR is depicted in Fig. 21.9D. CSTR is ideal for good product formation.

Membrane Reactors:

Several membranes with a variety of chemical compositions can be used. The commonly used membrane materials include polysulfone, polyamide and cellulose acetate. The biocatalysts (enzymes or cells) are normally retained on the membranes of the reactor. The substrate is introduced into reactor while the product passes out. Good mixing in the reactor can be achieved by using stirrer (Fig. 21.10A). In a continuous membrane reactor, the biocatalysts are held over membrane layers on to which substrate molecules are passed (Fig. 21.10B).

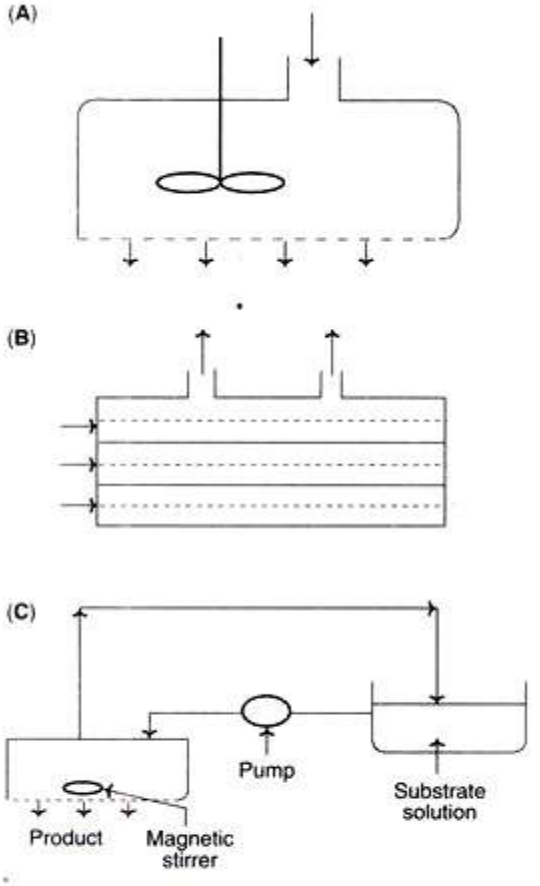


Fig. 21.10 : Membrane reactors (A) Batch membrane reactor, (B) Continuous membrane reactor, (C) Recycle membrane reactor (Coloured lines indicate membranes).

In a recycle model membrane reactor, the contents (i.e. the solution containing enzymes, cofactors, and substrates along with freshly released product are recycled by using a pump (Fig. 21.10C). The product passes out which can be recovered.

Applications of Immobilized Enzymes and Cells:

Immobilized enzymes and cells are very widely used for industrial, analytical and therapeutic purpose, besides their involvement in food production and exploring the knowledge of biochemistry, microbiology and other allied specialties. A brief account of the industrial applications of immobilized cells is given in Table 21.5.

Manufacture of Commercial Products:

A selected list of important immobilized enzymes and their industrial applications is given in Table 21.6. Some details on the manufacture of L-amino acids and high fructose syrup are given hereunder.

TABLE 21.6 A selected list of important immobilized enzymes and their industrial applications

Immobilized enzyme	*Application(s)*
Aminoacylase	Production of L-amino acids from D, L-acyl amino acids
Glucose isomerase	Production of high fructose syrup from glucose (or starch)
Amylase	Production of glucose from starch
Invertase	Splitting of sucrose to glucose and fructose
β-Galactosidase	Splitting of lactose to glucose and galactose
Penicillin acylase	Commercial production of semi-synthetic penicillins
Aspartase	Production of aspartic acid from fumaric acid
Fumarase	Synthesis of malic acid from fumaric acid
Histidine ammonia lyase	Production of urocanic acid from histidine
Ribonuclease	Synthesis of nucleotides from RNA
Nitrilase	Production of acrylamide from acrylonitrile

Production of L-Amino Acids:

L-Amino acids (and not D-amino acids) are very important for use in food and feed supplements and medical purposes. The chemical methods employed for their production result in a racemic mixture of D- and L-amino acids. They can be acylated to form D, L-acyl amino acids. The immobilized enzyme aminoacylase (frequently immobilized on DEAE sephadex) can selectively hydrolyse D, L-acyl amino acids to produce L-amino acids.

$$\text{D, L-Acyl amino acids} \xrightarrow{\text{Aminoacylase}} \text{L-Amino acids} + \text{D, L-Acyl amino acids}$$

The free L-amino acids can separated from the un-hydrolysed D-acyl amino acids. The latter can be recemized to D, L-acyl amino acids and recycled through the enzyme reactor containing immobilized aminoacylase. Huge quantities of L-methionine, L-phenylalanine L-tryptophan and L-valine are produced worldwide by this approach.

Production of High Fructose Syrup:

Fructose is the sweetest among the monosaccharide's, and has twice the sweetening strength of sucrose. Glucose is about 75% as sweet as sucrose. Therefore, glucose (the most abundant monosaccharide) cannot be a good substitute for sucrose for sweetening. Thus, there is a great demand for fructose which is very sweet, but has the same calorific value as that of glucose or sucrose.

High fructose syrup (HFS) contains approximately equivalent amounts of glucose and fructose. HFS is almost similar to sucrose from nutritional point of view. HFS is a good substitute for sugar in the preparation of soft drinks, processed foods and baking.

High fructose syrup can be produced from glucose by employing an immobilized enzyme glucose isomerase. The starch containing raw materials (wheat, potato, corn) are subjected to hydrolysis to produce glucose. Glucose isomerase then isomerizes glucose to fructose (Fig. 21.11). The product formed is HFS containing about 50% fructose. (Note: Some authors use the term high fructose corn syrup i.e. HFCS in place of HFS).

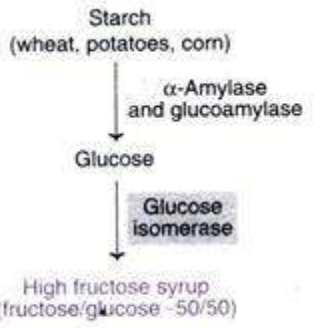


Fig. 21.11 : Production of high fructose syrup from starch (glucose isomerase is the immobilized enzyme).

Glucose isomerase:

This is an intracellular enzyme produced by a number of microorganisms. The species of Arthrobacter, Bacillus and Streptomyces are the preferred sources. Being an intracellular

enzyme, the isolation of glucose isomerase without loss of biological activity requires special and costly techniques. Many a times, whole cells or partly broken cells are immobilized and used.

Immobilized Enzymes and Cells- Analytical Applications:

In Biochemical Analysis:

Immobilized enzymes (or cells) can be used for the development of precise and specific analytical techniques for the estimation of several biochemical compounds. The principle of analytical assay primarily involves the action of the immobilized enzyme on the substrate.

A decrease in the substrate concentration or an increase in the product level or an alteration in the cofactor concentration can be used for the assay. A selected list of examples of immobilized enzymes used in the assay of some substances is given in Table 21.7. Two types of detector systems are commonly employed.

TABLE 21.7 Selected examples of immobilized enzymes used in analytical biochemistry

Immobilized enzyme	*Substance assayed*
Glucose oxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Lactate dehydrogenase	Lactate
Alcohol oxidase	Alcohol
Hexokinase	ATP
Galactose oxidase	Galactose
Penicillinase	Penicillin
Ascorbic acid oxidase	Ascorbic acid
L-Amino acid oxidase	L-Amino acids
Cephalosporinase	Cephalosporin
Monoamine oxidase	Monoamine

Thermistors are heat measuring devices which can record the heat generated in an enzyme catalysed reaction. Electrode devices are used for measuring potential differences in the reaction

system. In the Fig. 21.12, an enzyme thermistor and an enzyme electrode, along with a specific urease electrode are depicted.

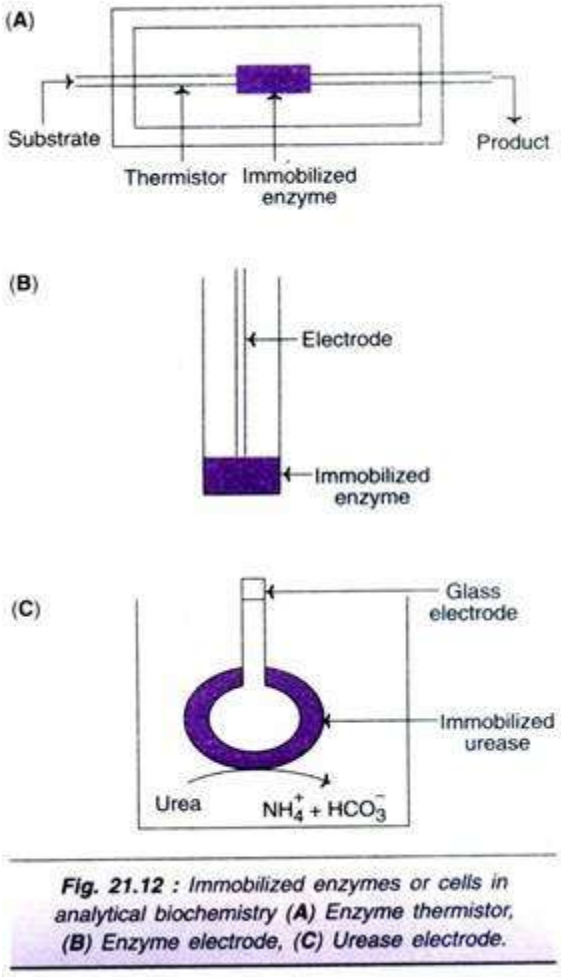


Fig. 21.12 : *Immobilized enzymes or cells in analytical biochemistry (**A**) Enzyme thermistor, (**B**) Enzyme electrode, (**C**) Urease electrode.*

1	Scienticfic theories, mathematical methods and therapeutic treatments are	Easily patentable	Non patentable	Un copyrightable	long time processing	Non patentable
2	The legal characterization and treatment of trade related biotechnological process and products popularly described as ----------	FDA	IPR	EPO	WISO	IPR
3	Development of crop varities are protected through ----------	Plant Breeder's Right	Plant biotech rights	Plant biotech regulations	Prevent breeders right	Plant Breeder's Right
4	According to USA ---------- means grant of right to exclude others from making using or selling an invention for a 17 years period	IPR	PBR	Patents	FDA	Patent
5	The Indian patent Act include ----------but not ----------	Product and invention	process patents and product patents	inventions and discoveries	FDA but not IPR	process patents and product patents
6	The duration of indian patent is ----------	5 years	6 months	1 years	10 years	5 years
7	Before the release of genetically engineered microbial pesticide it	FDA	TRIP	GATT	EPA	EPA

	should be approved by ----------					
8	Grant specification and clims are the parts of	Copy right	IPR	Patent	intelectual right	Patent
9	Which is the famous convention of London	European	paris	london	Budapest treaty	paris
10	Which are patentable	Inventions	Discoveries	Scienticfic throries	Treatments	Inventions
11	The first patent for living organism was awared in	1978	1988	1999	1965	1988
12	The Indian patent Act was formed in	1976	1878	1960	1970	1970
13	EMR stands for	Exclusive Market Rights	Exclusive Medical Rights	Exclusive Market Rate	External Market Rights	Exclusive Market Rights
14	A patent should contain	Name of the inventor	Name of the Patntence	Description of patent	All the three in needed	All the three in needed
15	Legal documents are	Pantents	Cliams	Description	Inventions	Cliams
16	Pseudomonas was patented by	TJ.C.Bose	Khorana	Dubey	Anand chakraboty	Anand chakraboty
17	Requirements of patents are	A New useful invention	Invention	A useful product	Previously known product	A New useful invention

18	When the Paris convention for protection of industrial property signed	1884	1890	1883	1863	1883
19	Paris convention is for the protection of	industrial property	personal property	institutes property	eqiupments	industrial property
20	How many years USPTO can issue a patent	4	2	1	3	3
21	IPR clasified in to	4 catagories	5 catagories	2 catagories	3 catagories	2 catagories
22	How many types of patents avilable	2	3	4	5	3
23	Duration of patent	5years	10years	20years	1years	20years
	Which company has the patent for tissue plasmogen activator	Sigma	biocon	Genetech	Genetech	Genetech
24	PCT came effective in India from	1999	1998	1997	1995	1998
25	To patent a product or process it must satisfy	3 Fundamental requirements	2 Fundamental requirements	5 Fundamental requirements	6Fundamental requirements	3 Fundamental requirements
26	How many claims present in the original patent application	10	20	30	5	20
27	In which country Genetech applied to get patent	france	Japan	USA	UK	UK

28	The colonical theory was proposed by	John	Haeckel	J.C.Bose	Anand chakrab oty	Haeckel
29	The colonical theory was proposed in the year	1874	1875	1876	1884	1874
30	The patenting of multicellular organisms constitute to raise	Ethical and social concern	Ethical concern	social concern	trede concern	Ethical and social concern
31	Indian patent Act allow to patent	products	process	preparation		process
32	In USA the maximum limit of monoply is for	10years	5 years	17 years	2 years	17 years
33	The word Patent derived from	Latin word patere	paten	pantor	patentor	Latin word patere
34	PCT is	Patent Cooperation Treaty	Patent Control Term	Public Cooperation Team	Private Cooperat ion Team	Patent Cooperatio n Treaty
35	PCT is an agreement for cooperation on patenting	National	Local	International	State	Internation al
36	What is the mode of revocation of patent	State government	Central government	Union territories	UN	Central governmen t
37	Who got the patent for Psuedomonas	Robert Koch	Louis Pastuer	Dr. Chakrabarty	Edward Jenner	Dr. Chakrabart y
38	The process of collecting biological samples for medical and scientific research is	Bioprospecting	Bioprojects	Biogenesis	Bioproce ssing	Bioprospec ting

39	refers to the illegal commercial development of naturally occuring biological material	Bio patenting	Bio piracy	Bio projects	Piracy	Bio piracy
40	is the form of protection of plant related inventions	Plant Variety Protection	Plant Protection Act	Plant Patent Act	Plant Utility Act	Plant Patent Act
41	investigation about cell bared therapies to treat disease	Plant cell research	Stem cell research	Animal tissue research	Plant enzymes	Stem cell research
42	is done to produce pure antibodies by fusing cells	Monoclonal antibody technique	Immunoglobulins	Antigens	Antigen antibody complex	Monoclonal antibody technique
43	Plant patents have been granted by	American Patent Office	England Patent Office	Japan Patent Office	European Patent Office	European Patent Office
44	The patentable product involving R-DNA technology	Genes	Vaccines	Transposons	Base pairs	Vaccines
45	is the patentable process involving R-DNA technology	Modifying genomic sequences	Enzyme sequences	Gene sequences	Plant genes	Modifying genomic sequences
46	The product is kept as a safety guarded and termed as	Tread mark	Trade secrete	patent	IPR	Trade secrete
47	protection is only form of expression of ideas	Trade mark	Patend	Copyright	Trade secrete	Copyright
48	In which year copyright was amended	1944	1994	1942	1960	1994
49	In which year copyright was brought enforced	1940	1990	1999	2002	1999
50	.Indian copy right Act was published in	1967	1957	1937	1977	1957

51	In which year Copy right was amended	1994	1944	1984	1997	1994
52	Which symbol was used to distiguish one treade to another?	Copy right	Patend	Treade mark	Treade secrete	Treade mark
53	In which year industrial design bill was passed	1940	1980	1999	1950	1999
54	In which year Treade mark bill was passed in India	1948	1978	1990	1958	1958

UNIT-V

SYLLABUS

Bio-ethanol and bio-diesel production: commercial production from lignocellulosic waste and algal biomass, Biogas production: Methane and hydrogen production using microbial culture. Microorganisms in bioremediation: Degradation of xenobiotics, mineral recovery, removal of heavy metals from aqueous effluents. Patents, patenting fundamental requirements- patent multicellular organisms, IPR, Copyrights, Trademarks

Introduction to biofuels.

Biofuels are a type of fuel derived from organic matter (broadly described as biomass) produced by living organisms i.e. plants and animals. Biofuels can also be referred to as substitutes for fossil fuel sourced mainly from a range of agricultural and energy crops, forests and waste streams . Examples of sources include energy crops such as Jatropha and Camelina, short rotation coppice (SRC) willow and timber, waste oils and kitchen/food waste, agricultural and forestry residues, industrial bio-wastes and more novel feedstocks such as algae.

The uses of biofuels are varied; unprocessed biomass can be used to generate electricity via steam turbines and gasifiers, or heat by directly combusting the raw material. Biomass can also be converted to bioliquids and used as fuels for transport, as is the case with bioethanol and biodiesel. Finally, biomass can be converted to an energy-rich gas (biogas or bio-SNG) that can be used in boilers and gas turbines to generate heat and electricity, used in gas-fuelled transport as compressed biomethane (CBM) or supplied to the gas grid.

Although biofuels have the potential to be a renewable alternative to conventional fossil fuels, there are various social, economic, environmental and technical issues surrounding their production and

final end-use. Currently, many governments around the world have implemented goals to replace a certain percentage of transportation fuel and natural gas demand with biofuels and this trend looks likely to continue.

Biofuels can be categorised into two major types: 1st generation biofuels and 2nd generation biofuels. 1st generation biofuels are biofuels currently on the market today produced largely from food crops e.g. corn (see Figure 2 above) and 2nd generation biofuels are those fuels produced by utilising the whole plant rather than just the sugar/oil component of the food crops (these are usually referred to as lignocellulosic feedstocks). Novel sources such as algae are also referred to as 2nd generation. The main reason why 2nd generation biofuels are being considered is to avoid the "food vs. fuel" controversy around the use of 1st generation biofuels.

Different sources of biofuel

Here are 4 biofuel sources, with some of their application in developmental stages, some ac- tually implemented:

Algae

Algae come from stagnant ponds in the natural world, and more recently in algae farms, which produce the plant for the specific purpose of creating biofuel. Advantage of algae focude on the followings: No CO2 back into the air, self-generating biomass, Algae can produce up to 300 times more oil per acre than conventional crops. Among other uses, algae have been used experimentally as a new form of green jet fuel designed for commercial travel. At the moment, the upfront costs of producing biofuel from algae on a mass scale are in process, but are not yet commercially viable.

Carbohydrate (sugars) rich biomaterial It comes from the fermentation of starches derived from agricultural products like corn, sugar cane, wheat, beets, and other existing food crops, or from inedible cellulose from the same. Produced from existing crops, can be used in an existing gasoline engine, making it a logical transition from petroleum. It used in Auto industry, heating buildings

("flueless fireplaces") At present, the transportation costs required to transport grains from harvesting to process- ing, and then out to vendors results in a very small net gain in the sustainability stakes. 2.3. Oils rich biomaterial It comes from existing food crops like rapeseed (aka Canola), sunflower, corn, and others, af- ter it has been used for other purposes, i.e food preparation ("waste vegetable oil", or WVO), or even in first use form ("straight vegetable oil", or SVO). Not susceptible to micro- bial degradation, high availability, re-used material. It is used in the creation of biodiesel fuel for automobiles, home heating, and experimentally as a pure fuel itself. At present, WVO or SVO is not recognized as a mainstream fuel for automobiles. Also, WVO and SVO are susceptible to low temperatures, making them unusable in colder climates.

MAJOR COMPONENTS OF PLANT BIOMASS

In the cell walls of the vascular tissues of higher land plants, cellulose fibrils are embedded in an amorphous matrix of lignin and hemicelluloses. These three kinds of polymers bind strongly to each other by noncovalent forces as well as by covalent cross-links, making a composite material that is known as *lignocellulose*. It represents over 90% of the dry weight of a plant cell. The quantity of each of the polymers varies with the species and age of a plant and from one part of the plant to another. Usually, softwoods have a higher content of lignin than do hardwoods. Hemicellulose content is highest in the grasses. In trees, on average, lignocellulose consists of 45%cellulose, 30% hemicelluloses,and 25%lignin.Theearth's estimated annual production of lignocellulose ranges from 2 to 5×10^{12} metric tons.

CELLULOSE

Cellulose is the most abundant organic compound on Earth. Every year, plants make more than 1011 metric tons of cellulose. *In situ*, a cellulose polymer is a linear chain of thousands of glucose molecules linked by *β*- (1:4)-glycosidic bonds. The basic repeating unit is *cellobiose.* Consecutive glucose units in cellulose are rotated through 180◦ with respect to their neighbors along the axis of the

chain, and the terminal cellobiose can thus appear inoneof two stereochemically different forms.Thecellulose polymer chain has a flat, ribbonlike structure stabilized by internal hydrogen bonds. Other hydrogen bonds between adjacent chains cause them to interact strongly with one another in parallel arrays of many chains that all have the same polarity. The resulting very long, largely crystalline aggregates
are called *microfibrils*.

HEMICELLULOSES

The components of hemicelluloses are complex polysaccharides that are structurally homologousto cellulose because they have a back bone made up of 1,4-linked*β*-d-pyranosylunits.Whereascellulose isalinearhomopolymer
with little variation in structure fromone species to another, hemicelluloses are highly branched, generally noncrystalline heteropolysaccharides. The sugar residues found in the hemicelluloses include pentoses (d-xylose, larabinose), hexoses (d-galactose, l-galactose, d-mannose, l-rhamnose, lfucose), and uronic acids (d-glucuronic acid). These residues are variously modified by acetylation or methylation. Hemicelluloses show a much lower
degree of polymerization (<200 sugar residues) than cellulose.

LIGNIN

Lignin is found in the cell walls of higher plants (gymnosperms and angiosperms), ferns, and club mosses, predominantly in the vascular tissues specialized for liquid transport. It is not found in mosses, lichens, and algae

that have no tracheids (long tubelike cells peculiar to xylem). The increased mechanical strength conferred on woody tissues by *lignification* allows huge trees several hundred feet tall to remain upright. Lignification is the process
whereby growing lignin molecules fill up the spaces between the preformed cellulose fibrils and hemicellulose chains of the cell wall.

Bioethanol

Bioethanol extraction Bioethanol is one of the most important renewable fuels due to the economic and environ- mental benefits of its use. The use of bioethanol as an alternative motor fuel has been steadi- ly increasing around the world for the number of reasons. 1) Fossil fuel resources are declining, but biomass has been recognized as a major reasons World renewable energy source. 2) Greenhouse gas emissions is one of the most important challenges in this century because of fossil fuel consumption, biofuels can be a good solution for this problem. 3) Price of petroleum in global market has raising trend. 4) Petroleum reserves are limited and it is monopoly of some oil-importing countries and rest of the world depends on them. 5) Also known petroleum reserves are estimated to be depleted in less than 50 years at the present rate of consumption. At present, in compare to fossil fuels, bioethanol is not produced eco- nomically, but according to scientific predictions, it will be economical about 2030.

Biomass commonly gathers from agricultural, industrial and urban residues. The wastes used for bioethanol production are classified in three groups according to pretreatment process in sugary, starchy and lignocellulosic biomasses. Lignocellulosic biomass, including forestry residue, agricultural residue, yard waste, wood products, animal and human wastes, etc., is a renewable resource that stores energy from sunlight in its chemical bonds. Lignocellulosic biomass typically contains 50%-80% (dry basis) carbohydrates that are poly- mers of 5C and 6C sugar units. Lignocellulosic biomasses such as waste wood are the most promising feedstock for producing

bioethanol. Bioconversion of lignocellulosic biomass to ethanol is significantly hindered by the structur- al and chemical complexity of biomass, which makes these materials a challenge to be used as feedstock for cellulosic ethanol production. Cellulose and hemicellulose, when hydro- lyzed into their component sugars, can be converted into ethanol through well-established fermentation technologies. However, sugars necessary for fermentation are trapped inside the crosslinking structure of the lignocellulose. Conventional methods for bioethanol production from lignocellulosic biomasses take three steps: pretreatment (commonly acid or enzyme hydrolyses), fermentation, distillation. Pretreat- ment is the chemical reaction that converts the complex polysaccharides to simple sugar. pretreatment of biomass is always necessary to remove and/or modify the surrounding ma- trix of lignin and hemicellulose prior to the enzymatic hydrolysis of the polysaccharides (cellulose and hemicellulose) in the biomass. Pretreatment refers to a process that converts lignocellulosic biomass from its native form. In general, pretreatment methods can be classi- fied into three categories, including physical, chemical, and biological pretreatment. In this step, biomass structure is broken to fermentable sugars. This project focused on chemically and biologically pretreatment. For example: this project shows the effect of sulfuric acid, hy- drochloric acid and acetic acid with different concentration by different conditions also shows the effect of cellulase enzyme by different techniques. Then fermentation step in which there are a series of chemical or enzymatic reactions that converted sugar into etha- nol. The fermentation reaction is caused by yeast or bacteria, which feed on the sugar such as Saccharomyces cerevisae. After that, distillation step in which the pure ethanol is separated from the mixture using distiller which boil the mixture by heater and evaporate the mixture to be condensate at the top of the apparatus to produce the ethanol from joined tube.

How to produce bio-ethanol:

Materials

Sugarcane stems 5kg

Dry yeast, 15g

• Items

Brix meter, 5L flask, Dimroth condenser, Liebig condenser, Stick, Beaker Cloth filter

1. Fermentation method

2. Mill juice out of Sugarcane stems. (about 3L of juice)

3. The juice is filtered out impurities.

4. Measurement Brix of juice.

5. Dry yeast is added to juice, the rate of 6g/L.

6. It keeps in the flask which sealed except the vent.

7. A cover is opened one day and once, then juice and dry yeast mixes so that air may en- ter with stick. 8. It continues until Brix becomes fixed.

9. Distillation method

10. Fermented juice is filtered out sediment.

11. It heats to boiling point in distiller.

12. Dimroth condenser is kept warm (about 70 degree) with hot water which is made to cir- culate by a pump.

13. Allihn condenser cools with tap water (about 20 degree).

14. Bio-ethanol which falls from the point of a allihn condenser is caught with beaker on ice.

BIOGAS

Biogas is the gas produced from the breakdown of organic matter in the absence of oxygen. The raw gas is typically composed of 60% methane (CH4) and 40% carbon dioxide, however, depending on the source, other components can exist which include oxygen (O2), hydrogen (H2), hydrogen sulphide (H2S), siloxanes, ammonia (NH3) and water vapour (moisture).

Compound	Chemical formula	%
Methane	CH_4	50-85
Carbon dioxide	CO_2	5-50
Hydrogen	H_2	0-1
Hydrogen sulfide	H_2S	0-3
Nitrogen	N_2	0-5
Oxygen	O_2	0-2

For most purposes, biogas can be divided into two categories: land-fill type and anaerobic digestion type. Land-fill (LF) type biogas is produced by allowing natural decay to occur within a land-fill producing a gas that is captured, while anaerobic digestion biogas is produced in purpose-designed above-ground plants to optimise the gasproducing decay process for greater efficiencies. There is a major environmental driver to capture the gas produced from the breakdown of organic matter. Naturally decayed waste, both household waste which is usually land-filled and farm waste, produce a lot of CH4 which is 20 times more potent as a GHG than CO2. As a result, from a policy point of view, there is a huge amount CO2 reduction achieved when waste is enclosed in a sealed tank and the captured methane is burned or flared to produce CO2 as the main byproduct.

Biogas can be produced from a range of feedstock including some biomass sources and waste streams. Waste sources including those from food waste, energy crops, crop residues, slurry or sewage waste, landfill gas and manure from animals can all be processed to biogas via AD. The type of feedstock processed is critical to the performance and overall efficiency of the AD process. The faster the feedstock breaks down, the better the overall efficiency and gas yields obtained per unit of raw material.

Production of biogas from anaerobic digesters

The anaerobic digestion (AD) process for biogas production can be classified according to the following categories:

- The operating temperature of the digester:
  Mesophilic (25-45°C) or Thermophilic (50-60°C)
- The state of the organic matter in the digester:

Wet feed (5-15% dry matter) or dry (over 15% dry matter)

- The mode of operation:

Continuous or batch process

- Single or multistage digesters

Thermophilic systems are known to provide much faster biogas production rates per unit of feedstock and cubic metre of digester than mesophilic systems. The degree of wetness (or dryness) of the AD system is also a critical operating factor. Dry AD operations tend to be cheaper to run because there is less water to evaporate but have high set-up costs per unit of feedstock. Wet AD processes, on the other hand, have lower set-up costs but higher operating costs than dry AD processes. 52. Biogas digesters can also be operated in either batch or continuous mode. There are usually technical justifications behind operating the AD in either mode such as the need to overcome peaks or troughs in gas production which can be accomplished by operating multiple batch digesters in parallel. It is also possible to run continuous digesters provided there is a gas holder available on-site big enough to deal with the variations. 53. Anaerobic digestion is essentially a 3 stage biological process. The first stage is the breakdown of the complex organic molecules into simpler molecules, volatile fatty acids (VFAs), NH3, CO2 and H2S. The simpler molecules are then further digested to produce more CO2, hydrogen and acetic acid. The final stage involves further breakdown of the fatty acids into CH4, CO2 and water. Each of these 3 stages uses completely different bacteria that operate at different conditions. In a single stage digester, all the bacteria needed for the process work at a compromise

because none of them operate at their optimum efficiency. In a multistage digester, the 3 stages of the AD process can be optimised to get bigger gas yields per unit of feedstock. Multistage digesters, however, are more expensive to build and more complex to control.

BIOREMEDIATION STRATEGIES

The objective of various strategies is to maximize microbial growth and consequentlybioremediation. Different techniques are employed depending on the degree of saturationand aeration of an area

I n s i t u techniques are defined as those that are applied to soil and groundwaterat the site with minimal disturbance.

E x s i t u techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water).

I n s i t u

bioremediation

These techniques are generally the most desirable options due to lower cost and lessdisturbance since they provide the treatment in place avoiding excavation and transport ofcontaminants.

These techniques are effective from only a few centimeters to about 30 cminto the soil.The most important land treatments are:

B i o v e n t i n g - is the most commonin situ treatment and involves supplying air and nutrientsthrough wells to contaminated soil to stimulate the indigenous bacteria. Bioventingemploys low air flow ratesand provides only the amount of oxygen necessary for thebiodegradation. It works for simple hydrocarbons and can be used where thecontamination is deep under the surface.

In situ biodegradation-

involves supplying oxygen and nutrients by circulating aqueoussolutions through contaminated soils to stimulate naturally occurring bacteria to degradeorganic contaminants.

Biosparging - Biosparging involves the injection of air under pressure below the watertable to increase groundwater oxygen concentrations and enhance the rate of biologicaldegradation of contaminants by naturally occurring bacteria.

Bioaugmentation

It involve the addition of microorganisms with the ability to degradepollutants. Bioremediation frequently involves the addition of microorganisms indigenousor exogenous to the contaminated sites.

Ex situ **bioremediation**

These techniques involve the excavation or removal of contaminated soil from ground.

Landfarming - is a simple technique in which contaminated soil is excavated and spreadover a prepared bed and periodically tilled until pollutants are degraded. The goal is tostimulate indigenous biodegradative microorganisms and facilitate their aerobicdegradation of contaminants. In general, the practice is limited to the treatment ofsuperficial 10- 35 cm of soil.

Composting - is a technique that involves combining contaminated soil withnonhazardous organic amendants such as manure or agricultural wastes. The presenceof these organic materials supports the development of a rich microbial population.

Biopiles are a hybrid of landfarming and composting. Essentially, engineered cells areconstructed as aerated composted piles. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms.

Bioreactors-Slurry reactors or aqueous reactors are used for
ex situ treatment of contaminated soil and water. Bioremediation in reactors involves the processing ofcontaminated solid material (soil, sediment, sludge) or water through an engineeredcontainment system. A slurry bioreactor may be defined as a containment vessel andapparatus used to create a three-phase (solid, liquid, and gas) mixing condition toincrease the bioremediation rate of soil bound and water-soluble pollutants as a waterslurry of the contaminated soil and biomass (usually indigenous microorganisms) capableof degrading target contaminants. In general, the rate and extent of biodegradation aregreater in a bioreactor system than in situ or in solid-phase systems because thecontained environment is more manageable and hence more controllable and predictable.

DEGRADATION OF XENOBIOTICS

The word, xenobiotic, is a combination of two different roots, "xeno" and "biotic." Xeno is from the Greek and means strange, unnatural, or different. Biotic is a word that implies life. Xenobiotic, therefore, refers to an organic compound that mimics natural biochemicals that are essential for life, but which have characteristics about them that are strange and unnatural. Xenobiotics are often toxic to life. Also, they may not be recognized by biochemical processes in plants and microorganisms and are thus resistant to degradation in the environment. Xenobiotics include many compounds that are involved in both industrial and agricultural activities. The fate of industrial solvents and other industrial chemicals in the soil environment is an important domain of soil biochemistry. Research is desperately needed to determine the biochemical reactions in soil that transform these xenobiotic compounds to their mineral components. Synthetic organic pesticides are a class of xenobiotics that are commonly used in agriculture and are added to the soil in large amounts each year. Chemicals with pesticide activity were designed primarily to control insect, weed, fungal or nematode pests. In

1939, DDT (dichloro diphenyl trichloroethane) was discovered by a chemist named Paul Herman Mueller. Mueller was awarded a Nobel Prize for Physiology and Medicine in 1948 for his discovery. Increased use of pesticides began during the 1950s and has continued to the present time. Since that time, more than 600 different chemical pesticides have been tested and registered for use in the United States. The fates of xenobiotics in soil include (i) complete mineralization or (ii) stabilization of the parent compound, or some metabolite of the compound, in soil. If the xenobiotics are toxic and the rate of degradation is very slow, adverse effects on human and on ecological health are possible. Therefore it is desirable to maintain xenobiotic concentrations in soil at as low a level as possible. Because of these concerns, considerable research effort has been spent in trying to understand the metabolic degradation pathways of xenobiotics in soil. The remainder of the introductory remarks to this chapter will pertain to pesticide reactions in soil, although many of the comments would also apply to other classes of xenobiotics. Effect of Pesticides on Soil Processes. Evaluations of the effects of pesticides on soil processes have often yielded conflicting results. This is because the toxicity of pesticides varies and other soil factors interact with the pesticide to influence its activity. Tests conducted in the laboratory with pure microbial cultures and growth media provides data that, generally, cannot be ecologically interpreted. Contrary to model laboratory systems, pesticides in soil may be sorbed onto colloids, degraded rapidly, or exist in widely varying concentrations at different microsites within the soil. Also a wide diversity of different microorganisms in soil and the interaction between them cannot be predicted when pure cultures are used for study. A common technique of evaluating the fate of a pesticide in soil An understanding of the soil, biochemical, and microbial factors and the interactions involved that bring about enhanced biodegradation is lacking. Application to an agricultural field of a pesticide that fails to perform as expected can result in large financial losses. On the other hand, if the process of enhanced biodegradation were clearly understood, it could be applied to cases where compounds must be rapidly reduced in their detrimental effect. The persistence of a pesticide in soil causes different types of problems. If the pesticide remains in soil for a long period of time it may

(i) be assimilated by plants and accumulate in edible portions,

(ii) be transported with eroding soil particles or with surface and subsurface waters to locations where its presence may cause harm or

(iii)it may accumulate in the animal food chain and change the ecological balance of nature. Compounds that are short-lived in the environment are less likely to achieve these fates. Metabolism of Pesticides in Soil. Metabolism of pesticides in soil may be classified into two main categories. The first category is defined by the ability of the chemical to support microbial growth by supplying nutrients such as carbon and nitrogen, as well as supplying a source of energy. The presence of the pesticide will cause a rise in numbers of the microbial subpopulation that can utilize the chemical while at the same time bringing about the disappearance of the pesticide from the soil. Microorganisms of this type can be isolated from soil through enrichment techniques. The second category of metabolic processes is called cometabolism. The pesticide is degraded but does not serve as a source of energy or nutrients. Heterotrophs of this sort cannot be isolated by enrichment culture techniques. Martin Alexander has outlined six different classes of reactions that result in partial or total transformation of a pesticide molecule. Detoxification is the conversion of an inhibitory molecule to a nontoxic product. Complete mineralization is the extreme case of detoxification. However, more common is the removal of a functional group from the pesticide molecule. Degradation is the transformation of a complex substrate molecule to simple products and is often considered synonymous with mineralization. A reaction that results in making the pesticide more complex by combining with itself or other soil compounds is called conjugation, complex formation, or addition reactions. Oxidative coupling is an example of this class of reactions. Activation is the conversion of a nontoxic substrate or a pesticide precursor to a toxic molecule. For example the insecticide phorate is transformed in soil to give metabolites that are also toxic to insects. A changing of the spectrum of toxicity is another reaction in which pesticides may participate. Some pesticides may be toxic to one group of organisms but may be metabolized to become toxic to completely different group of, and possibly nontarget, organisms. Metabolic

pathways of pesticide degradation have been established in the laboratory in model systems involving pure cultures for a large number of pesticides. These "type reactions" are very useful in making educated guesses about the types of compounds that may be formed in soil during the degradation of a specific pesticide molecule. However, the type reactions do not allow us to make predictions about which step is the rate-limiting step in the overall degradation process, and thus, which metabolic products may accumulate in the soil. Yet in view of the large number of chemicals that eventually reach the soil and the inability to test each one rapidly and thoroughly, the ability to predict the metabolic fates and products by applying type reactions to the chemical of interest has utility. The rate at which a pesticide degrades in soil is subject to a number of variables. Five conditions have been outlined which must be met before a pesticide molecule may be degraded. These conditions include: 1. an organism effective in metabolizing a pesticide molecule must live or be capable of living in the soil, 2. the compound must be in a chemical and physical form suitable for degradation, 3. the chemical must be located in the same environment as the active agent, e.g. a microbial cell or an extracellular enzyme, of degradation, 4. the compound must be capable of inducing formation of the enzymes or enzymes appropriate for detoxification - low solubility or low concentration of the pesticide and low uptake into the microbial cell may result in lack of induction, and 5. environmental conditions such as pH, temperature, and organic matter must be suitable for the degrading microorganisms to proliferate and the enzyme to operate. If any of these conditions are not met, the pesticide will not readily be decomposed and may persist in the soil environment for long periods of time. The factors that control the rate of degradation of pesticides in soil are characteristic of the soil, microorganism, and the pesticide molecule. An understanding of the direct and indirect effect of all three factors must exist before accurate assessments of the ecological impact of pesticides in the soil can be made.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB COURSE NAME: Microbial Biotechnology

COURSE CODE: 16MBU504A MCQ – UNIT V BATCH-2016-2019

	Questions	Option A	Option B	Option C	Option D	Answer
	---------- include the rights arising from conserving, improving and making	Agricultural act	Plant breeder's act	Farmer's right	Copyright	Farmer's right
2	IPR are usually limited to _____	Non-rival goods	Rival goods	Imported goods	Food products	Non-rival goods
3	Modern usage of the term IPR began in _____	1987	1977	1967	1955	1967
4	In 1994 which act was amended _____	Tread mark	trade secrete	patent	copy right	copy right
5	In 1957 which agreement was approved for copy right act	TRIPS	PPVFR	IPR	PCT	TRIPS
6	The maximum limit of 17 years for monoply was given by	Paris	UK	USA	India	USA
7	The meaning for patere	property	To lay open	Protection	To open	To lay open

8	Haeckel proposed the theory of _____	Colonical	gem theory	Theory of koch	Theory of Pasteur	Colonical
9	There is a 20 claims present in _____	original patent application	New developed patent application	patent application	IPR application	original patent application
10	In 1988 the first patent was given to	Living organism	hybride plant	plant	Fungi	Living organism
11	In 1883 which convention for production of industrial property was signed	Paris	US	UK	USSR	Paris
12	Three years of patent was issued by	TRIPS	PPVFR	IPR	USPTO	USPTO
13	According to PPVFR Act How many rights have been given to farmers	6	7	10	9	9
14	_____is classified into two categories	PBR	IPR	FDA	USR	IPR

15	Trademark uses symbols in _____	Intellectual committee	Intellectual property	Indian committee	Indian property	Intellectual property
16	Geographical indications protects the quality,reputation of products originated from _____	Histarical area	Seasonal area	developmental area	Geographical area	Geographical area
17	Tade secrets protects _____ of industries	Trade information	confidential information	Machinery information	Tribunal information	confidential information
18	Copyrights prevents copying and _____	Reproduction	Construction	Development	Tradition	Reproduction
19	IPR develops and protects ____resources.	Physical	chemical	biological	academic	biological
20	For breeder's right, _____ Act is in practice.	PPR	PPVFR	PPVR	PPFR	PPVFR
21	In which year patent Act was published	1945	1990	1999	2000	1999

	in India					
22	In which year patent bill was introduced to upper part of india parliament	1970	1980	1960	1990	1970
23	When copyright Act got TRIPS agreement	1950	1960	1957	1980	1957
24	When did geographical indication good bill got published	1920	1960	1999	2004	1999
25	Which year Act was replaced in 1999	1920	1999	1990	1911	1911
26	Stem cell research investigation about--- therapies to treat disease	cell bared therapies	cell therapies	Plant enzymes	Plant cell research	cell bared therapies
27	Trade secrecy applicable rather than patents in ----------	Fermentation	drugs	chemicals	invitro fertilization	Fermentation

28	Which can be protected using patents	micropropagation	tissue	organ culture techniques	method for reducing pathogenecity	method for reducing pathogenecity
29	Not possible to get patents for ----------	Plastic surgery	modified plants	DNA sequences	modified Microorganisms	Plastic surgery
30	The existing patent law does not allow ----------	Process patent	. Product patent	Live forms	Inventions	. Product patent
31	In India the patent law is based on ----------	Indian Patent Act of 1970	Indian Patent Act of 1911	. Indian Patent Act of 1991	. Indian Patent Act of 1811	Indian Patent Act of 1970
32	.The main instrument of international collaboration for intellectual property is the ----------	GATT	WIPO	OECD	Court	WIPO

33	Patents are granted by ----------	GATT	WIPO	OECD	Court.	Court.
34	The provision of _____ enables the developing countries to paten GM crops.	TRIPS-GATT	WHO	. UN	UNESCO	TRIPS-GATT
35	. The TRIPS final negotiation schedule was proposed in	Jan-00	Jan-02	Jan-01	Jan-99	######
36	Monopoly rights were granted in -------	.USA	Japan	Astralia	Europe.	Europe.
37	In USA period of patents ----------	25 years	12years	10 years	17 years	17 years

38	In TRIPS, appellations of origin are covered in	Patents	Trade marks	Geographical indications	Copy rights	Geographical indications
39	. Trade Related Aspects of Intellectual Property Rights (TRIPS) is administered by	WHO	WTO	. FAO	UNDP	WTO
40	_____are the rights given to people over the creations of their minds.	Intellectual property rights	Human rights	Right to speech	Right to speech	Intellectual property rights
41	International patent protection for Biotechnology was published by	WIPO	EC	.OECD	EPC.	OECD

4 2	TRIP Strands for ----------	Trade related intellectual property.	Trademark related intellectual property.	Trend related intellectual property.	Trade related international property.	Trade related intellectual property.
4 3	WIPO Strands for ----------	Word intellectual property organization.	World intellectual property organization	Word intellectual property origin	World intellectual property origin	World intellectual property organization

4	4. Comprise of private information about specific technical procedures and formulations.	copy right	trade secret	trade mark	patent	trade secret
5	4. is a milk clotting proteolytic enzyme that hydrolyses the K-casein protein of milk.	chymosin	.renin	both	Casein	chymosin
6	4 ---------- --- is the key component of the rennet	Chymosin	Lipin	Casein	Rennin	Chymosin
7	4 Milk clotting activity found in ----------	Chymosin	Lipin	Casein	Ptotein	.Chymosin

48	Mastitis is the ----------	viral infection of milk gland	Parasitic infection of milk gland	Fungal infection of milk gland	Bacterial infection of milk gland	Bacterial infection of milk gland
49	EBT contaminate the ----------	Methonine	Lipin	Alanin	Tryptophan	Tryptophan
50	. _____ is an important example for gene piracy.	. GM *Pseudomonas*	Pentaclipandra brazzeana	*Clostridium*	*Bacillus*	*Pentacliplandra brazzeana*
51	In India, _____ is an	Dalbergia	Neem	Ginger	Onion	Neem

	important example biopiracy					
52	---------- Serve as a source of transplanted organs for humans.	Transgenic pigs	Transgenic rabbits	Transgenic goats	Trans genic sheep	Transgenic pigs
53	Trade secrete comprise the information about ----------	Specific technical procedures	symbol	particu lar product	docu ment.	Spec ific technical procedures
54	Recombinant DNA technology,were known by several phasses such as	playing god	manipu lation of life	man made evolution	all the above	all the above
55	The original guide line providede by NIH was modified in need by……committee	NIH-RAC	GEO's	FDA	none of the above	NIH-RAC

56	3.earlier, most prominently used strain/host organism in RD	*klebsiella.spp*	E.coli k-12	*E.coli*	*Pseudo monas aeruginosa*	*E.coli k-12*
57	……is responsible for the regulating the introduction of foods,drugs,pharmaceutical and medical devices into the market place	FDA	WHO	NIH	none of the above	FDA
58	…..is an enzyme approved by FDA in making cheese	chymosin	tryptophan	streptomycin	Penicillin	chymosin
59	milk clotting activity for cheese making is derived from the fourth stomach of calves and consist of mixture of substances called…..	K-casein	rennet	chymosin	proteolytic enzyme	rennet
60	for cheaper industrial supply of chymosin,genes were cloned and the product	*E.coli k-12*	Pseudo monas aeruginosa	*klebsiella .spp*	B.thuringiensis	*E.coli k-12*

	was harvested from….					
61	a consistent feature among the occurrence of eosinophilia myalgia syndrome(EMS),due to the consumption of large doses of aminoacid……in food supplement	Tryptophan	leucine	isoleucine	lysine	tryptophan
62	recombinant bovine somatotropin (BST) which also known as ……	bovine growth hormone	bovine releasing hormone	bovine serum	none of the above	bovine growth hormone
63	milk production in dairy cows was increased by……% after the injection of recombinant form of BST	20-25%	30-35%	.40-45%	20-45%	20-25%
6	A protected	old	New	Not	Moderat	New

4	hybrid crop varieties should be			uniform	e	
65	protects confidential information of the industry with commercial value	Patent rights	Patent Control Term	Trade marks	Trade secrets	Trade secrets
66	PPVFR refers to	Protection of plant varities and Farmers Rights Act	Production of plant varities and Farmers Rights Act	Preparation of plant varities and Farmers Rights Act	Proper plant varities and Farmers Rights Act	Protection of plant varities and Farmers Rights Act

Reg. No. : ----------
[16MBU504A]

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Under Section 3 of UGC Act 1956)
B.Sc. DEGREE EXAMINATION, July2018
DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST - FIFTH SEMESTER

MICROBIAL BIOTECHNOLOGY

Time: 2 hours **Maximum: 50 marks**
Date / Session: .07.2018 ( ) **Class : III BSc**

PART-A

Multiple Choice Questions No.1 - 20 **20 x 1 = 20 marks**

1. Bioreactor is _____
   a.Specially designed vessel to carry out microbial reaction.
   b.Vessel to produce drugs
   c.Container for Compound Separation
   d.Vessel Used for culture maintenance
2. The Recombinant technology makes it possible to
   a. Transfer gene b. Preserve the culture
   c. Identify the strain d. Sequencing the culture.
3. Gene therapy is _____
   a.Correcting the faulty genes b.Isolation of gene
   c.removal of functional gene d.Addition of non coding genes
4. GMO strands for _____
   a. Gene manage organization b.Genetically modified organism
   c. Genetically modified operan d.Genetically managing organism
5. Genetic engineering for _____
   a. production of desired biological product b.Reduce the unwanted growth
   c.Preservation d.standardization of production process
6. Organism altered at molecular level is called as
   a. Gene therapy b. Molecular cutting
   c.Genetic engineering d.Gene replacement
7. The steps taken to kill insects in plant roots _____
   a. to inject Bt toxin into roots b. to introduce Bt gene into cells of root
   c. Spray Bt toxin into roots d. Use of chemicals

8. The *B. thuringiensis* subsp ---------- insecticidal protein is highly toxic when injested by mosquito larvae.
   a. israelensis b. kurstaki c. tenebrionis d. haizawa

9. ---------- produce parasporal crystal which kills insects
   a. B.thuringinsis b. *A.rhizogenes* c. *Heliothis virescens* d. *Autographa californica*

10. Hairy root disease in higher plants is caused by
    a *A.tumefaciens* b. *Bacillus* *c A.rhizogenes* d. *E.coli*

11. The Cry I proteins are toxic to ----------
    a.Diptera b. Coleoptera c. Lepidoptera d. epidoptera

12. _____ is involved in Bt toxin
    a. Staphylococcus b.Bacillus c) A.rhizogenes d) *E.coli*

13. Golden rice for _____
a. beta-carotene b.Antibiotic c. Streptokinase d.Somatotropin

14. Baculo virus particle consist of cylindrical ----------that surrounds viral DNA
a. Nucleocapsid b. Nucleus c. Nucleosome d. Proteosome

15. Recombinant protein _____-
a. Protein produced by expression of cloned gene
b.Protein produced by microbes
c.Produced during biological process
d.Produced by natural process

16. Secondary metabolites _____
a.Required for own growth b. Used for own cellular function
c. is not Required for own growth d. is Used for own cellular function

17.Enzyme used for cutting DNA_____
a.Ligase b.Permease c.Polymerase d. Restriction Endonuclease

18. Invivo _____
a. Natural situation - inside the cell b. Out side the cell
c. Dead materials d. Artificial condition

19. Cloning is a _____
a. Different copy b. Identical copy c. Modified copy d. The best copy

20.Cloning vector ----------
a. Small piece of DNA b. Synthesized DNA c. Artificial Material d. Living cell

Part B

Answer all the questions **3x2 = 6 marks**

21. Define Genetic Engineering
22. What is recombinant protein. Give two examples
23. List the two transgenic plants and its application

Part C

Answer all the questions **3x8 = 24 marks**

24. A. Describe in detail about gene therapy (Or)
B. Detail account on Genetically engineered microbes for industrial application.

25. A. Justify -Genetic engineered plants as protein factories (Or)
B. Narrate the role of DNA technology In pharmaceutical products

26. A. Describe about the development of recombinant vaccine (Or)
B. Detail account on Biopesticide production

Reg. No. : ----------
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MICROBIAL BIOTECHNOLOGY

Time: 2 hours **Maximum: 50 marks**
Date / Session: .07.2018 ( ) **Class : III BSc**

PART-A

Multiple Choice Questions No.1 - 20 **20 x 1 = 20 marks**

1. Biopol produced by ----------
   a. Rhizobium
   b. Agrobacterium
   c. Ralstonia eutropha
   d. Baculo virus
2. Xanthan gum is used as
   a. viricide b. pesticide c. food additives d. bactericide
3. The ---------- is the Commercially available first polysacharide
   a. alginate b. Gellan c. Xanthan d. dextran
4. Streptokinase (SK) is ----------
   a. potent plasminogen activator b. potent plasmin activator
   c. activator d. plasminogen suppressor
5. Which of the following material is used as bioplastic
   a. Polystyrene b. Polypropylene
   c. Polyhydroxybutyrate d. Dextran
6. Downstream processing involves:
   a. Purification b. production C. optimization D. solid liquid separation
7. Streptokinase *prepared from* ----------
   a. E.coli. b. S.aureus c. Streptococcus pyogens d. Streptococcus equisimilis
8. many strains of beta-hemolytic Streptococci able to produce _____
   a. insulin b. antibiotic c. streptokinase . factor VIII
9. Depolymerization can be caused by the enzyme
   a. Polymerase b. *Depolymerase* c. *Ligase* d. restrictionenzyme
10. Biodegradable plastics must undergo---------- by microbes
    a. degradation b. emulsion c. Adsorption d. Mineralization
11. When X-gal added to the agar the cells of which synthesize B-galactosidase will be coloured
    a. Yellow b. Red c. Blue d. Black
12. Microbial transformation of steroids involves ----------
    a. oxidation b. isomerization c. polythene formation d. hydrostatic
13. Organism ----------involved in the development of the practical synthesis of steroids with useful biological activity
    a. Saccharomyces b. Aspergillus c. Rhizopus d. Penicillium
14. Steroid as ----------perform wide range of functions
    a. Therapeutic agent b. thrombolytic agent c. additives d. sweetner
15. Steroid as ----------perform wide range of functions
    a. lipid b. Hormone c. carbohydrate d. protein
16. Biotransformation of progestrone to hydroxy progesterone by
    a. Rhizopus nigricans. b. E.coli c. Yeast d. Bacillus
17. Enzyme used for cutting DNA_____
    a.Ligase b.Permease c.Polymerase d. Restriction Endonuclease
18. cortisone formed from cortisol can be subjected to biotransformation by ----------

a. Rhizopus nigricans. b. Corynebacteriium C. Yeast D. Bacillus

19. liquid sweetner ----------
    a. HF b. HFCS c. HDFS d. HCSF

20. which is used extensively in a wide variety of processed food
    a. SCP b. Saurkraut c. sugar syrup d. High fructose syrup

Part B

Answer all the questions **3x2 = 6 marks**

21. Define Genetic Engineering
22. What is recombinant protein. Give two examples
23. List the two transgenic plants and its application

Part C

Answer all the questions **3x8 = 24 marks**

24. A. Describe in detail about gene therapy (Or)
    B. Detail account on Genetically engineered microbes for industrial application.

25. A. Justify -Genetic engineered plants as protein factories (Or)
    B. Narrate the role of DNA technology In pharmaceutical products

26. A. Describe about the development of recombinant vaccine (Or)
    B. Detail account on Biopesticide production

Reg. No. : ----------
[16MBU504A]

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Under Section 3 of UGC Act 1956)
B.Sc. DEGREE EXAMINATION, July2018
DEPARTMENT OF MICROBIOLOGY

MODEL EXAMINATION - FIFTH SEMESTER

MICROBIAL BIOTECHNOLOGY

Time: 2 hours **Maximum: 50 marks**
Date / Session: 09.10.2018 (AN) **Class : III BSc**

PART-A

Multiple Choice Questions No.1 - 20 **20 x 1 = 20 marks**

1. Particles smaller than the pore space are trapped ----------

a. within the filter b. within the column c. out of the filter d. out of the column

2. The duration of indian patent is ----------

a. 5 years b. 15 years c.25 years d. 10 years

3. Which of the following bacteria responsible for oil degradation

a.*Pseudomonas* b.*Mycobacterium* c.*Vibrio* d.*Salmonella*

4.. Scienticfic theories, mathematical methods and therapeutic treatments are

a. Easily patentable b. Non patentable c. Un copyrightable d. long time processing

5. Trade secrete comprise the information about ----------

a. Specific technical procedures b. symbol c. particular product d. document.

6. Liposome entrapement is ----------

a.Immobilization b. Purification c. Upstream process d. Down stream process

7. The process of Anaerobic breakdown of organic material is

a. Preservation of food b.Production of food c. Purification d. Fermentation

8. Secondary metabolites ----------

a. Required for own growth b. is not Required for own growth

c . is Used for own cellular function d. Used for own cellular function

9. Recombinant protein _____-

a. Protein produced by expression of cloned gene b.Protein produced by microbes

c.Produced during biological process d.Produced by natural process

10. Xenobiotics are _____

a. do not easily undergo biodegradation b. easily undergo biodegradation

c. chemically unstable d. Biologically unstable

11. Anti sense RNA is for _____

a. Preservation of food b.Production of food
c. aid in Cheap cost d. Tolerance plant

12. What are the reasons for considering the yeast as a vector?

a. length b. can be grown on complex media c. short doubling time d. tough selection

13. _____ protects confidential information of the industry with commercial value
a. Patent rights b.Patent Control Term c. Trade marks d. Trade secrets

14. Penicillin G - acylase is immobilized in ----------by covalent bonding

a. Sephadex polymer b. Glutaraldehyde c. Polyacrylamide d. Agarose

15. ---------- completely surrounds the enzyme and forms a envelop in liposomal entrapment.

a. Sugars b. lipids c. proteins d. glycoproteins

16. RNA interference (RNAi) is a biological process in which RNA molecules ----------

a. induce gene expression b. regulate the gene expression

c. inhibit gene expression d. modify the gene expression

17. The term biomass most often refers to _____

a. Inorganic matter b. Chemicals c. organic matter d. Ammonium compounds

18. Which one of the following is an example of starch crops biomass feed stocks?

a. Sugar cane b. Wheat straw c. Corn stover d. Orchard prunings

19. The use of living microorganism to degrade environmental pollutants is called

a. Microremediation b. Nanoremediation c. bioremediation d. recovery

20. The process of extracting metals from ore bearing rocks is called

a. Bioextraction b. Biofiltration c. microbial extraction d. bioleaching

Part B

Answer all the questions **3x2 = 6 marks**

21. What is encapsulation?
22. Write the properties of Biodiesel
23. Write about superbug in bioremediation.

Part C

Answer all the questions **3x8 = 24 marks**

24 a. Comment on microorganism in bioremediation Or

b. Describe the method of cell immobilization and its application

25. a. Discuss in detail on patenting fundamental requirements Or

b. Write about methane production using microbes

26. a. Discuss in detail on patent, IPR and copyrights Or

b. Write about xenobiotic degradation