Semester – V

17MBU504A 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: 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

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

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. Phage typing, gene therapy.

Unit IV

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

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

(Deemed to be University)
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Coimbatore – 641 021.
(For the candidates admitted from 2017 onwards)

DEPARTMENT OF MICROBIOLOGY

SUBJECT NAME: MICROBIAL BIOTECHNOLOGY
SEMESTER: V
SUB.CODE:17MBU514A **CLASS: III B.Sc (MB)**

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

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	T3:9-13
04	Environmental technology	R1: 235-243 T2:679-684
05	Food Technology	T2:718-726
06	Use of prokaryotic and eukaryotic microorganisms in biotechnological applications	T1: 412-425
07	Genetically microbes for industrial applications application	T2:81-82 R1:153-165
08	Industrial applications of bacteria and 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.

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, Elements of 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

UNIT3

Duration	Topic	Reference
01	Microbial based transformation of steroids and sterols	T2:308-310
02	Biocatalytic processes and their industrial applications	W3
03	Production of High Fructose syrup	W3
04	Production of coca Butter substitutes	T2-296-297
05	Phage typing	T2-367-368
06	Safety of biotechnology Food product	R1-247-249
07	Gene therapy	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

UNIT4

Duration	Topic	Reference
01	Microbial product purification and filtration	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	RNAi application & silence gene	T1:51-519
06	Drug resistance	W5
07	Therapeutics and host pathogen interactions	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

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 requirements	R1-446-448 W6
08	Unit Revision	
09	Unit Revision &Old qp	
	Total hours: 09	

W6:www.mondaq.com

Introduction:

Biotechnology is defined as the 'application of scientific and engineering principles to the processing of material by biological agents to provide goods and services'. The Spinks Report (1980) defined biotechnology as 'the application of biological organisms, systems or processes to the manufacturing and service industries'. United States Congress's Office of Technology Assessment defined biotechnology as 'any technique that used living organisms to make or modify a product, to improve plants or animals or to develop microorganisms for specific uses'.

The document focuses on the development and application of modern biotechnology based on new enabling techniques of recombinant-DNA technology, often referred to as genetic engineering. The history of biotechnology begins with zymotechnology, which commenced with a focus on brewing techniques for beer. By World War I, however, zymotechnology would expand to tackle larger industrial issues, and the potential of industrial fermentation gave rise to biotechnology. The oldest biotechnological processes are found in microbial fermentations, as born out by a Babylonian tablet circa 6000 B.C. unearthed in 1881 and explaining the preparation of beer.

In about 4000 B.C. leavened bread was produced with the aid of yeast. The Sumerians were able to brew as many as twenty types of beer in the third millennium B.C. In the 14th century, first vinegar manufacturing industry was established in France near Orleans.

In 1680 Antony Van Leeuwenhoek first observed yeast cells with his newly designed microscope. In 1857, Louis Pasteur highlighted the lactic acid fermentation by microbe.

By the end of 19th century large number of industries and group of scientists were involved in the field of biotechnology and developed large scale sewage purification system employing microbes were established is Germany and France.

In 1914 to 1916, Delbruck, Heyduck and Hennerberg discovered the large-scale use of yeast in food industry. In the same period, acetone, butanol and glycerin were obtained from bacteria.

In 1920, Alexander Fleming discovered penicillin and large scale manufacturing of penicillin started in 1944. Chronological history of biotechnology.

Table 22.1. Historical development of biotechnology.

Year	Development
Before 6000 B.C.	Yeast employed to make wine and beer
4000 B.C.	Leavened bread produced with the aid of yeast
1521	Aztecs harvested algae from lakes as a source of food.
1670-1680	Copper mined with aid of microbes, Rio Tinto, Spain.
	Antoine van Leeuwenhoek first observed microbes with newly designed microscope.
1869	Johann Meischer isolated DNA from the nuclei of white blood cells.
1876	Louis Pasteur identifies extraneous microbes as a cause of failed beer fermentations
1890	Alcohol first used to fuel motors
1893	Fermentation process patented by Koch, Pasteur.
1897	Edurad Buchner discovered that enzymes extracted from yeast can convert sugar into alcohol.
1910	Thomas H. Morgan proved that genes are carried on chromosomes "Biotechnology" term coined.
	Large-scale sewage purification systems employing microbes are established.
1912-1914	Three important industrial chemicals- acetone, butanol and glycerol were obtained from bacteria.
1918	Germans use acetone produced by plants to make bombs.
	Yeast grown in large quantities for animal and glycerol.
1920	Alexander Fleming discovered penicillin.
	Plant hybridization.
1928	Yeast grown in large quantities for animal and glycerol.
1938	Proteins and DNA studied by X-ray crystallography.
1941	George Beadle proposed "one gene, one enzyme" hypothesis.
1943-1953	Linus Pauling described sickle cell anemia calling it a molecular disease. Cortisone made in large amounts.
	DNA identified as the genetic material.

1944	DNA as genetic material and transformation factor in bacteria (Avery, Macleod and McCarty).
1946	Bacterial recombination discovered (Lederberg and Tatum)
1951	Lambda bacteriophage discovered (Lederberg)
1953	Double helix structure of DNA revealed (Watson and Crick).
1956	Isolation of DNA polymerase I, enzymatic synthesis of DNA (Kornberg).
1958	Semiconservative replication of DNA (Messelson and Stahl).
1960	Isolation of mRNA.
1961	Nucleic acid hybridization (Marmur and Doty), Operon model (Jacob and Monod) and *in vitro* protein synthesis allows codon assignment (Nirenberg and Mathaei).
1962	Mining of uranium with the aid of microbes begin in Canada.
1973	Beginning of genetic engineering. Stanley Cohen produced first recombinant DNA organism.
	Brazilian government initiates major fuel programme to replace oil with alcohol.
1975	Hybridomas which make monoclonal antibodies were first created
1976	US National Institute of Health introduces guidelines on genetic engineering
1977	Human Growth Hormone produced by bacterial cells.
1978	Genetic engineering techniques used to produce human insulin in *E. coli* by Genentech Inc.
1979	Genentech Inc. produce human growth hormone and two kinds of interferon DNA from malignant cells transformed a strain of cultured mouse cells - new tool for analyzing cancer genes.
1980	Rank Hovis McDougall receives permission to market fungal food for human consumption in UK.
	The US Supreme Court rules in Diamond v. Chakrabarty that genetically engineered microorganisms can be patented.
1981	Monoclonal antibodies receive US approval for use in diagnosis.
1982	The Food and Drug Administration approves the first biotechnology therapy, a human insulin drug made by Genentech.
1983	Syntex Corporation received FDA approval for a monoclonal antibody-based diagnostic test for *Chlamydia trachomatis*.
1984	Chiron Corp. announced the first cloning and sequencing of the entire human immunodeficiency virus (HIV) genome.
	Stanford University received a product patent for prokaryote DNA.
	Charles Cantor and David Schwartz developed pulsed-field gel electrophoresis.
	Animal interferon's approved for protection against cattle disease.
1985	Genetically engineered plants resistant to insects, viruses, and bacteria were field tested for the first time.
	Axel Ullrich reported the sequencing of the human insulin receptor
	Plants can be patented.
1986	Orthoclone OKT3® (Muromonab-CD3) approved for reversal of acute kidney transplant rejection.
	The EPA approved the release of the first genetically engineered crop, gene-altered tobacco plants
1987	Genentech received FDA approval to market rt-PA (genetically engineered tissue plasminogen activator) to treat heart attacks.
	Recombivax-HB® (recombinant hepatitis B vaccine) approved.

1989	Epogen® (Epoetin alfa) a genetically engineered protein introduced, providing a means to help patients with kidney failure.
1990	GenPharm International, Inc. created the first transgenic dairy cow. The cow was used to produce human milk proteins for infant formula.
	The Human Genome Project, the international effort to map all of the genes in the human body, was launched.
	The first successful field trial of genetically engineered cotton plants was conducted by Calgene Inc. The plants had been engineered to withstand use of the herbicide Bromoxynil.
1993	Kary Mullis won the Nobel Prize in Chemistry for inventing the technology of polymerase chain reaction
1994	The first genetically engineered food product, the Flavr Savr tomato, gained FDA approval.
1996	Dolly the sheep is cloned.
1997	Researchers at Scotland's Roslin Institute report that they have cloned a sheep--named Dolly--from the cell of an adult ewe. Polly the first sheep cloned by nuclear transfer technology bearing a human gene appears later.
	A group of Oregon researchers claim to have cloned two Rhesus monkeys.
	Rituxan, the first antibody-based therapy for cancer (for patients with non-Hodgkin's lymphoma) was approved.
	A new DNA technique combines PCR, DNA chips, and computer programming providing a new tool in the search for disease-causing genes.
1998	Human embryonic stem cell lines are established.
	University of Hawaii scientists, clone three generations of mice from nuclei of adult ovarian cumulus cells.
2000	"Working draft" of the human genome's 3.15 billion letters is completed after a decade of research.
2003	Broad Institute is founded in Cambridge to give scientists access to the human genome project, and to understand the molecular basis of disease.
2007	Craig C. Mello, a University of Massachusetts researcher, shares the Nobel Prize with Andrew Fire of Stanford University for discovering a special kind of RNA that can shut down individual genes.

In last fifteen years progress have been made by microbiologists and genetic engineers, and we are hopeful to solve many fold problems of the present day, specially energy and food crisis to cater the need of growing population of the world. Mineral ore deposits are also becoming more scarce and expensive to recover from earth's crust.

Microorganisms can be used to enhance to recovery of metals from low-grade ores and from effluents containing undesirable quantities of heavy metals or other toxins. When these technologies are applied at industrial level, they constitute bio- industry.

Table 22.2. Principal products of bio-industry.

Technology	Examples of Products
Fermentation technology	Antibiotics, vitamins, enzymes, amino acids, citric acid, lactic acid, malic acid, ethanol, acetone, butanol, biogas, biopesticides nucleotides, steroids, alkaloids, diagnostic reagents etc.
Enzymatic engineering	Isoglucose, glucose syrup etc.
Genetic engineering and Cell cultures	Single cell protein, clones, Interferon's, vaccines, blood products, monoclonal antibodies.

2. Scope of Microbial Biotechnology:

Genetic engineering in biotechnology stimulated hopes for both therapeutic proteins, drugs and biological organisms themselves, such as seeds, pesticides, engineered yeasts, and modified human cells for treating genetic diseases. The field of genetic engineering remains a heated topic of discussion in today's society with the advent of gene therapy, stem cell research, cloning, and genetically-modified food.

Biotechnology is the applied science and has made advances in two major areas, viz., molecular biology and production of industrially important bio-chemical. The scientists are now diverting themselves toward biotechnological companies; this has caused the development of many biotechnological industries.

In USA alone more than 225 companies have been established and successfully working, like Biogen, Cetus, Geneatech, Hybritech, etc. In world, USA, Japan, and many countries of Europe are leaders in biotechnological researchers encouraged by industrialists.

These companies are working for human welfare and opted following areas for research and development:

(a) Automated bio-screening for therapeutic agents.

(b) Bio-processing alkenes to valuable oxides and glycols.

(c) Developing immobilized cell and enzyme systems for chemical process industries.

(d) Engineering of a series of organisms for specific industrial use.

(e) Genetical improvement of microorganisms for production of pharmaceutical products.

(f) Human gene therapy.

(g) Improved production of Vitamin B12.

(h) Large-scale production of fructose from inexpensive forms of glucose.

(i) Manufacturing ethanol by continuous fermentation.

(j) Microbiological based production of human insulin and interferon's.

(k) Microbiologically up-gradation of hydrocarbons.

(l) Production and development of vaccine to prevent calibacillosis.

(m) Production of bio-pesticide and bio-fertilizers.

(n) Production of diagnostic kits for toxoplasmosis identification.

(o) Production of monoclonal antibodies for organ transplant tissue typing.

(p) Production of photo-synthetically efficient plants.

(q) Production of transgenic plants and animals.

(r) Production of xanthan gum in oil fields for recovery of crude mineral oils.

The advances in recombinant DNA technology have occurred in parallel with the development of genetic processes and biological variations. The development of new technologies have resulted into production of large amount of biochemically-defined proteins of medical significance and created an enormous potential for pharmaceutical industries.

Biotechnology in itself is a vast subject and its scope is extended to various branches of biology. This includes plant tissue culture, production of transgenic in animal and plants, applications in medicine as tools and therapeutics, creation of new enzymes and their immobilization for industrial use, development of monoclonal antibodies and control of pollutions, etc.

3. Applications of Microbial Biotechnology:

Industrial Applications of Biotechnology:

The industrial application of molecular biotechnology is often subdivided, so that we speak of red, green, gray or white biotechnology. This distinction relates to the use of the technology in the medical field (in human and animal medicine), agriculture, the environment and industry.

Some companies also apply knowledge deriving from molecular biotechnology in areas that cut across these distinctions (e.g., in red and green biotechnology, sequencing services). According to an investigation by Ernst and Young relating to the German biotech industry, 92% of companies are currently (2004) working in the field of red biotechnology, 13% in green, and 13% in gray or white biotechnology.

Biotechnology in Medicine:

Biotechnology products for therapeutic use include a very diverse range of products, 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.

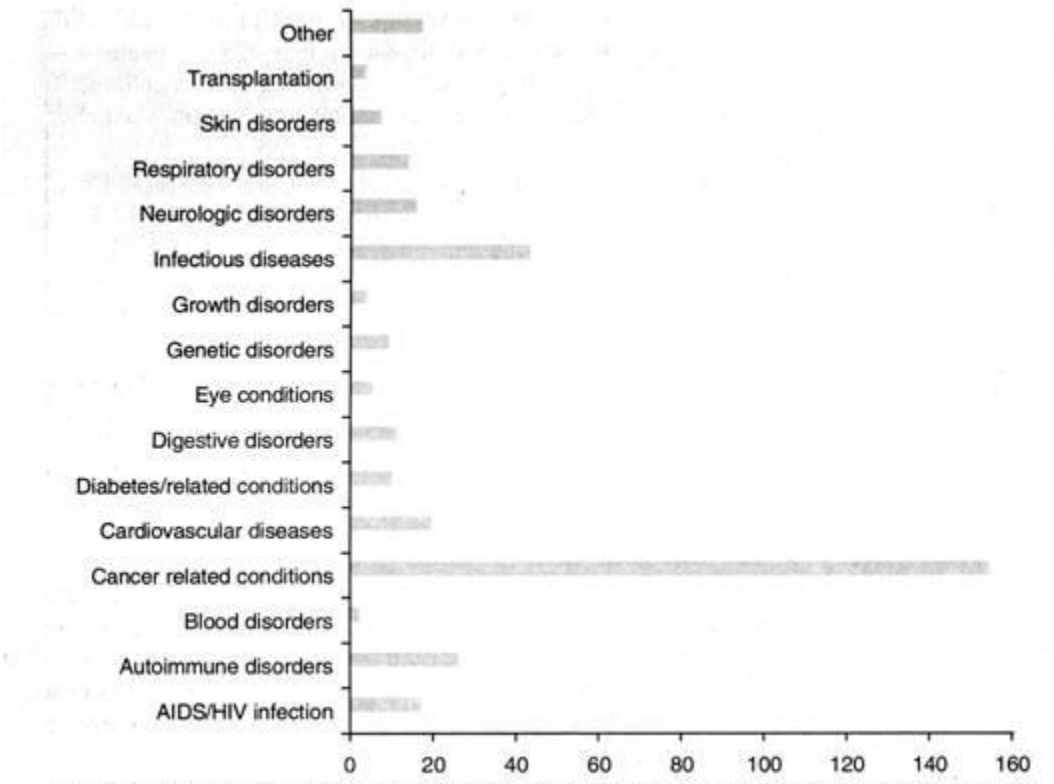


Fig. 22.1. Different areas of medicine in which efforts are being made with the help of biotechnology.

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

Red Biotechnology:

Within the field of red biotechnology, which deals with applications in human and animal medicine, there are various further distinctions that can be made: biopharmaceutical drug development, drug delivery cell and gene therapies, tissue engineering/regenerative medicine, pharmacogenomics (personalized medicine), system biology, and diagnosis using molecular medicine.

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

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

Drug Delivery:

Closed linked to the development of therapeutic agents are the means of achieving their targeted delivery to their site of action. These drug delivery systems are mainly used for drugs whose physical and chemical characteristics make them insufficiently stable in reaching their site of action intact. They can also be used to transport drugs in a targeted way to particular sites of

action (tissue specific targeting), or to overcome biological barriers such as the intestinal wall or the blood-brain barrier.

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.

Importance of Biofertilizers in Agriculture Biotechnology

Over the past few decades, many farmers have been involved in improving wild plants and animals by selection and breeding of their desirable characteristics. It has been estimated that by 2050, food supply has to be increased by 70 per cent to fulfill the demand of overgrowing population. Therefore, there is an urgent need to adapt biological sciences applications in agriculture field. Biotechnology is an amalgamation of variety of disciplines- molecular biology, bioinformatics, biochemistry, genetics and microbiology. The usage of combinations of these disciplines in agricultural field leads to generation of biotech crops with increased yield and enhanced quality. Agriculture biotechnology not only upgrades the quality but also utilizes the

resources and livestock for the well-being of animals and wild plants. Some of the application of agriculture biotechnology encompasses genetic engineering, plant and animal tissue culture technology, hybridization, bioprocess and fermentation technology, gene selection through mutagenesis and biosensors for biological monitoring. New information technologies such as electronic communication systems, data processing and automation are gaining tremendous attention in order to improve the quality and efficiency of the farm work. This study mainly focuses on the improvisation and development of new varieties of crop plants through biofertilizers included in agriculture biotechnology.

Biotechnology is the term which uses living organisms to improve plants, modify the product and develop organisms for further uses. Agriculture biotechnology is defined as that is used for livestock and crop improvement. Some of the following biotechnology tools which play vital role in agriculture biotechnology are-

• Genetic engineering and genetically modified crops

• Molecular breeding

• Conventional plant breeding

• Molecular diagnostic tools

• Tissue culture and micro propagation

Biofertilizers

There has been tremendous use of insecticides, fungicides and pesticides to increase the productivity but these products are responsible for depleting essential minerals from the soil thus affecting it in a negative way. This problem has leads to the production of biofertilizers which are the cultures of microorganisms packed in a carrier material. Biofertilizers contain live or latent cells of efficient strains of phosphate solubilizing, nitrogen fixing or cellulolytic microorganisms used for the application to seeds, soil or composting areas. The objective behind

using biofertilizers is to increase the number of such micro-organisms and accelerating those microbial processes which are helpful for the availability of nutrients that can be easily assimilated by plants. They play a very important role in improving soil fertility by fixing atmospheric nitrogen and also produce plant growth substances in the soil. They promote root growth by producing hormones and antimetabolites. They help in soil mineralization and decomposition of nutrients. They are cost-effective and can be used as a supplement to chemical fertilizers. Microorganisms like bacteria, fungi and blue-green algae are used as biofertilizers and to increase their shelf-life they are packed in carrier materials like peat and lignite powder. In this regard, biofertilizers have paramount significance in sustaining agricultural productivity and healthy environment. They can be characterized into various categories like:

• Nitrogen fixing biofertilizers

• Phosphate solubilizing biofertilizers

• Phosphate mobilizing biofertilizers

• Biofertilizers for micro-nutrients

• Plant Growth Promoting biofertilizers

Recent techniques include the encouragement to use pellets for direct soil application and methylcellulose for seed coating. There are various environmental factors responsible involved such as type of soil, inadequacy of organic matter, high temperature and soil water deficit. Plant growth and crop yield can be enhanced by mixing biofertilizers therefore; farmers should have knowledge about the benefits of synergistic effects of biofertilizers. Chemical fertilizers should be applied to the soil with the gap of 15-20 days for better nitrogen fixation. At district level, cold storage should be provided for timely availability of biofertilizers even after the expiry date. Biofertilizers will not only have a great impact on sustainable agriculture economic development but they will contribute the holistic wellbeing and sustainable ecosystem.

Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting Rhizobacteria (PGPR) shows an important role in the sustainable agriculture industry. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. In addition, PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere and beyond in bulk soil, which indirectly boosts plant growth rate. There are many bacteria species that act as PGPR, described in the literature as successful for improving plant growth.

Mycorrhiza as Biofertilizers

Mycorrhiza (fungus roots) is a distinct morphological structure which develops as a result of mutualistic symbiosis between some specific root - inhabiting fungi and plant roots. Plants which suffer from nutrient scarcity, especially P and N, develop mycorrhiza *i.e.* the plants belong to all groups *e.g.* herbs, shrubs, trees, aquatic, xerophytes, epiphytes, hydrophytes or terrestrial ones. In most of the cases plant seedling fails to grow if the soil does not contain inoculum of mycorrhizalfungi. In recent years, use of artificially produced inoculum of mycorrhizal fungi has increased its significance due to its multifarous role in plant growth and yield, and resistance against climatic and edaphic stresses, pathogens and pests.

Mechanism of Symbiosis

The mechanism of symbiosis is not fully understood. Biorkman (1949) postulated the carbohydrate theory and explained the development of mycorrhizas in soils deficient in available P and N, and high light intensity. Slankis (1961) found that at high light intensity, surplus carbohydrates are formed which are exuded from roots. This in turn induces the mycorrhizal fungi of soil to infect the roots. At low light intensity, carbohydrates are not produced in surplus, therefore, plant roots fail to develop mycorrhizas.Types of Mycorrhizas

By earlier mycologists the mycorrhizas were divided into the following three groups :

***(i)* Ectomycorrhiza.** It is found among gymnosperms and angiosperms. In short roots of higher plants generally root hairs are absent. Therefore, the roots are infected by mycorrhizal fungi which, in turn, replace the root hairs (if present) and form a mantle. The hyphae grow intercellularly and develop Hartig net in cortex. Thus, a bridge is established between the soil and root through the mycelia.

***(ii)* Endomycorrhiza.** The morphology of endomycorrhizal roots, after infection and establishment, remain unchanged. Root hairs develop in a normal way. The fungi are present on root surface individually. They also penetrate the cortical cells and get established intracellulary by secreting extracellular enzymes. Endomycorrhizas are found in all groups of plant kingdom.

(***iii)* Ectendomycorrhiza.** In the roots of some of the gymnosperms and angiosperms, ectotrophic fungal infection occur. Hyphae are established intracellularly in cortical cells. Thus, symbiotic relation develops similar to ecto- and endo-mycorrhizas.

Marks (1991) classified the mycorrhizas into seven types on the basis of types of relationships with the hosts

(i) vesicular-arbuscular (VA) mycorrhizas (coiled, intracellular hyphae, vesicle and arbuscules present),

(ii) ectomycprrhizas (sheath and inter-cellular hyphae present),

(iii) ectendomycorrhizas (sheath optional, inter and intra-cellular hyphae present),

(iv) arbutoidmycorrhizas (seath, inter-and coiled intracellular hyphae present),

(v) monotropoidmycorrhizas (sheath, inter- and intra- cellular hyphae and peg like haustoria present),

(vi) ericoid mycorrhizas (only coiled intracellular hyphae, long coiled hyphae present), and

(vii) orchidaceous mycorrhizas (only coiled intracellujlar hyphae present).

Type *(i)* is present in all groups of plant kingdom; Types *(ii)* and *(iii)* are found in gymnosperms and angiosperms. Types *(iv), (v)* and *(vi)* are restricted to Ericales, Monotropaceae and Ericales respectively. Types *(vii)* is restricted to Orchidaceous only. Types *(iv)* and *(v)* were previously grouped under ericoid mycorrhizaes.

Methods of Inoculum Production and Inoculation

Methods of inoculum production of VAM fungi differ; however, some of these two are briefly described here.

***(a)* Ectomycorrhizal fungi:** The basidiospores, chopped sporocarp, sclerotia, pure mycelia culture, fragmented mycorrhizal roots or soil from mycorrhizosphere region can be used as inoculum. The inoculum is mixed with nursery soils and seeds are sown.

Institute for Mycorrhizal Research and Development, U.S.A., Athens and Abbort Laboratories (U.S.A) have developed a mycelial inoculum of *Pisolithustinctorius* in a vermiculite-peat moss substrate with a trade name 'Myco-Rhiz' which is now commercially available on large quantities. In 1982, about 1.5 million pine seedlings were produced with MycoRhiz in the U.S.A. (Marx and Schenck, 1983).

***(b)* VA mycorrhizal fungi:** VA mycorrhizas can be produced on a large scale by pot culture technique. This requires the host plants, mycorrhizal fungi and natural soil. The host plants which support large scale production of inoculum are sudan grass, strawberry, sorghum, maize, onion, citrus, etc.

(i)	They increase the longevity of feeder roots, surface area of roots by forming mantle and spreading mycelia into soil and, in turn, the rate of absorption of major and minor nutrients from soil resulting in enhanced plant growth.
(ii)	They play a key role for selective absorption of immobile (P, Zn and Cu) and mobile (S, Ca, K, Fe, Mn, Cl, Br, and N) elements to plants. These are available to plants in less amount (Tinker, 1984).
(iii)	Some of the trees like pines cannot grow in new areas unless soil has mycorrhizalinocula because of limited or coarse root hairs.
(iv)	VA mycorrhizal fungi enhance water uptake in plants,
(v)	VA mycorrhizal fungi reduce plant response to soil stress such as high salt levels, toxicity associated with heavy metals, mine spoils, drought and minor element *(e.g.* Mn) imbalance.
(vi)	VA mycorrhizal fungi decrease transplant socks to seedlings. They produce organic 'glues' which bind soil particles into semistable in aggregates. Thus, they play a significant role in augmenting soil fertility and plant nutrition.
(vii)	Some of them produce metabolites which change the ability of plants to induce roots from woody plant cuttings and increase root development during vegetative propagation.
(viii)	They increase resistance in plants and with their presence reduce the effects of pathogens and pests on plant health

The starter inoculum (spores) of VA mycorrhizal fungi can be isolated from soil by wet sieving and decantation technique (Gerdeman and Nicolson, 1963). VA mycorrhizal spores are surface sterilized and brought to the pot culture. Commonly used pot substrates are sand: soil (1:1, w/w) with a little amount of moisture. An outline for inoculum production.

There are two methods of using the inoculum: *(i)* using a dried spore-root- soil to plants by placing the inoculum several centimeters below the seeds or seedlings, *(ii)* using a mixture of soil-roots, and spores in soil pellets and spores adhered to seeds with adhesives.

Commercially available pot culture of VA mycorrhizal hosts grown under aseptic conditions can provide effective inoculum. Various types of VA mycorrhiza linocula are currently produced by Native Plants, Inc (NPI), Salt Lake City.

In India, Forest Research Institute, Dehra Dun has established mycorrhizal bank in different states of the country. Inocula of these can be procured as needed and used in horticulture and forestry programmes.

Benefits from Mycorrhizas to Plants

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:

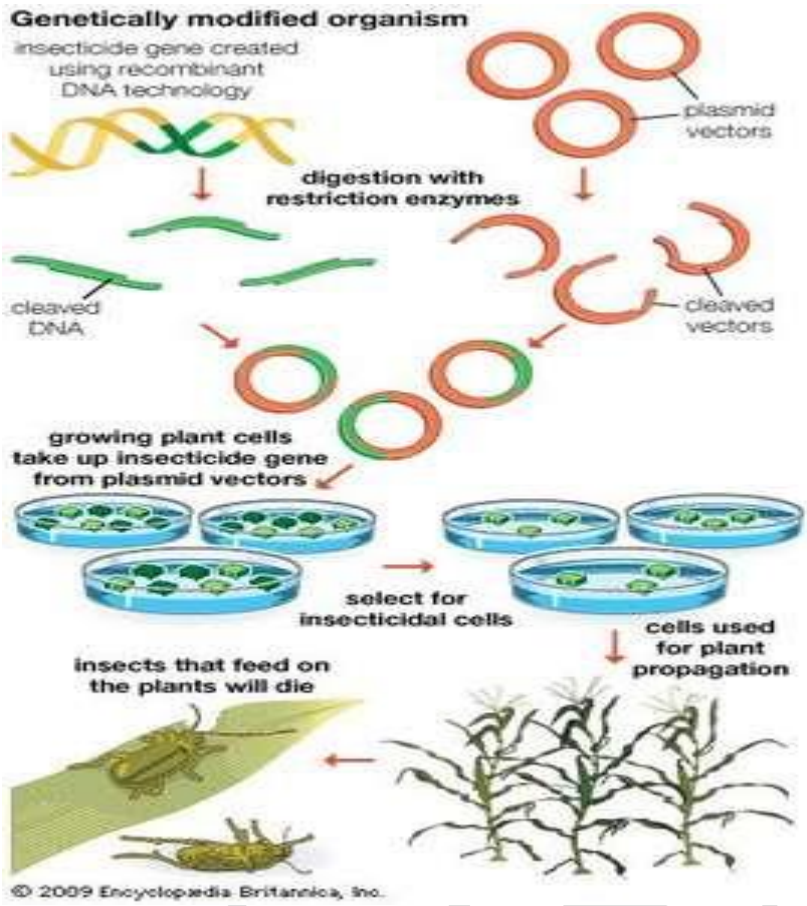
There are a number of safety issues relating to biotechnology products that differ from those raised by low molecular weight products and need to be taken into account when designing the safety evaluation programme for a biotechnology derived pharmaceutical product.

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, there are concerns about the distribution and persistence of vector sequences, the potential for expression of vector sequences in non-target cells: tissues and, in particular, the potential for inadvertent gonadal distribution and germ-line integration.

Genetically modified organism (GMO), organism whose genome has been engineered in the laboratory in order to favour the expression of desired physiological traits or the production of desired biological products. In conventional livestock production, crop farming, and even pet breeding, it has long been the practice to breed select individuals of a species in order to produce offspring that have desirable traits. In genetic modification, however, recombinant genetic technologies are employed to produce organisms whose genomes have been precisely altered at the molecular level, usually by the inclusion of genes from unrelated species of organisms that code for traits that would not be obtained easily through conventional selective breeding.

GMOs produced through genetic technologies have become a part of everyday life, entering into society through agriculture, medicine, research, and environmental management. However, while GMOs have benefited human society in many ways, some disadvantages exist; therefore, the production of GMOs remains a highly controversial topic in many parts of the world.



GMOs In Medicine And Research

GMOs have emerged as one of the mainstays of biomedical research since the 1980s. For example, GM animal models of human genetic diseases enabled researchers to test novel therapies and to explore the roles of candidate risk factors and modifiers of disease outcome. GM microbes, plants, and animals also revolutionized the production of complex pharmaceuticals by enabling the generation of safer and cheaper vaccines and therapeutics. Pharmaceutical products range from recombinant hepatitis B vaccine produced by GM baker's yeast to injectable insulin (for diabetics) produced in GM *Escherichia coli* bacteria and to factor VIII (for hemophiliacs) and tissue plasminogen activator (tPA, for heart attack or stroke patients), both of which are produced in GM mammalian cells grown in laboratory culture. Furthermore, GM plants that produce "edible vaccines" are under development. An edible vaccine is an antigenic protein that is produced in the consumable parts of a plant (e.g., fruit) and absorbed

into the bloodstream when the parts are eaten. Once absorbed into the body, the protein stimulates the immune system to produce antibodies against the pathogen from which the antigen was derived. Such vaccines could offer a safe, inexpensive, and painless way to provide vaccines, particularly in less-developed regions of the world, where the limited availability of refrigeration and sterile needles has been problematic for some traditional vaccines. Novel DNA vaccines may be useful in the struggle to prevent diseases that have proved resistant to traditional vaccination approaches, including HIV/AIDS, tuberculosis, and cancer.

Genetic modification of insects has become an important area of research, especially in the struggle to prevent parasitic diseases. For example, GM mosquitoes have been developed that express a small protein called SM1, which blocks entry of the malaria parasite, *Plasmodium*, into the mosquito's gut. This results in the disruption of the parasite's life cycle and renders the mosquito malaria-resistant. Introduction of these GM mosquitoes into the wild could help reduce transmission of the malaria parasite. In another example, male *Aedesaegypti* mosquitoes engineered with a method known as the sterile insect technique transmit a gene to their offspring that causes the offspring to die before becoming sexually mature. In field trials in a Brazil suburb, *A. aegypti* populations declined by 95 percent following the sustained release of sterile GM males.

Finally, genetic modification of humans via gene therapy is becoming a treatment option for diseases ranging from rare metabolic disorders to cancer. Coupling stem cell technology with recombinant DNA methods allows stem cells derived from a patient to be modified in the laboratory to introduce a desired gene. For example, a normal beta-globin gene may be introduced into the DNA of bone marrow-derived hematopoietic stem cells from a patient with sickle cell anemia; introduction of these GM cells into the patient could cure the disease without the need for a matched donor.

Role of GMOs in Environmental Management

Another application of GMOs is in the management of environmental issues. For example, some bacteria can produce biodegradable plastics, and the transfer of that ability to microbes that can be easily grown in the laboratory may enable the wide-scale "greening" of the plastics industry. In the early 1990s, Zeneca, a British company, developed a microbially

produced biodegradable plastic called Biopol (polyhydroxyalkanoate, or PHA). The plastic was made with the use of a GM bacterium, *Ralstoniaeutropha*, to convert glucose and a variety of organic acids into a flexible polymer. GMOs endowed with the bacterially encoded ability to metabolize oil and heavy metals may provide efficient bioremediation strategies.

Environmental Biotechnology:

Environmental biotechnology in particular is the application of processes for the protection and restoration of the quality of the environment.

Environmental biotechnology can be used to detect, prevent and remediate the emission of pollutants into the environment in a number of ways.

Solid, liquid and gaseous wastes can be modified, either by recycling to make new products, or by purifying so that the end product is less harmful to the environment. Replacing chemical materials and processes with biological technologies can reduce environmental damage.

In this way environmental biotechnology can make a significant contribution to sustainable development. Environmental Biotechnology is one of today's fastest growing and most practically useful scientific fields. Research into the genetics, biochemistry and physiology of exploitable microorganisms is rapidly being translated into commercially available technologies for reversing and preventing further deterioration of the earth's environment.

1. To adopt production processes that make optimal use of natural resources, by recycling biomass, recovering energy and minimizing waste generation.

2. To promote the use of biotechnological techniques with emphasis on bioremediation of land and water, waste treatment, soil conservation, reforestation, afforestation and land rehabilitation.

3. To apply biotechnological processes and their products to protect environmental integrity with a view to long-term ecological security.

Use of biotechnology to treat pollution problems is not a new idea. Communities have depended on complex populations of naturally occurring microbes for sewage treatment for over a century. Every living organism-animals, plants, bacteria and so forth-ingests nutrients to live and produces a waste as a by-product. Different organisms need different types of nutrients.

Certain bacteria thrive on the chemical components of waste products. Some microorganisms feed on materials toxic to others. Research related environmental biotechnology is vital in developing effective solutions for mitigating, preventing and reversing environmental damage with the help of these living forms. Growing concern about public health and the deteriorating quality of the environment has prompted the development of a range of new, rapid analytical devices for the detection of hazardous compounds in air, water and land. Recombinant DNA technology has provided the possibilities for the prevention of pollution and holds a promise for a further development of bioremediation.

Applications of Environmental Biotechnology:

Environmental protection is an integral component of sustainable development. The environment is threatened every day by the activities of man. With the continued increase in the use of chemicals, energy and non-renewable resources by an expanding global population, associated environmental problems are also increasing. Despite escalating efforts to prevent waste accumulation and to promote recycling, the amount of environmental damage caused by over-consumption, the quantities of waste generated and the degree of unsustainable land use appear likely to continue growing.

The remedy can be achieved, to some extent, by the application of environmental biotechnology techniques, which use living organisms in hazardous waste treatment and pollution control. Environmental biotechnology includes a broad range of applications such as bioremediation, prevention, detection and monitoring, genetic engineering for sustainable development and better quality of living.

Bioremediation:

Bioremediation refers to the productive use of microorganisms to remove or detoxify pollutants, usually as contaminants of soils, water or sediments that otherwise intimidate human health. Bio treatment, bio reclamation and bio restoration are the other terminologies for bioremediation. Bioremediation is not a new practice. Microorganisms have been used for many years to remove organic matter and toxic chemicals from domestic and manufacturing waste discharge. However, the focus in environmental biotechnology for fighting different pollution is on bioremediation. The vast majority of bioremediation applications use naturally occurring microorganisms to identify and filter toxic waste before it is introduced into the environment or to clean up existing pollution problems.

Some more advanced systems using genetically modified microorganisms are being tested in waste treatment and pollution control to remove difficult-to-degrade materials. Bioremediation can be performed in situ or in specialized reactors (ex situ). Bioremediation by microorganisms need appropriate environment for the cleanup of the polluted site.

Food Biotechnology

Genetically modified food is synthesized using biotechnological tools. Modern Biotechnology is also called as genetic engineering, genetic modification or transgenic technology. In this technology, Nuclear DNA is modified through insertion of gene of interest (gene encoding desired trait). This modified DNA is called as recombinant DNA. When recombinant DNA expresses, it encodes desired product. This technology, when implemented to enhance food qualities or yield is called as food technology.

Modern Biotechnology is helpful in enhancing taste, yield, shell life and nutritive values. This is also useful in food processing (fermentation and enzyme involving processes). So Biotechnology is beneficial in erasing hunger, malnutrition and diseases from developing countries and third word. Modern biotechnology products are commercially reasonable hence it can improve agriculture as well as food industry that will result in raise in income of poor farmers. Following are applications of Modern food biotechnology.

Role of Food Biotechnology in Food Processing

Breweries are synthesized through the process of fermentation. Different yeast strains are used to make breweries at commercial level. Genetic engineering has enabled us to make light wine. Yeast is genetically modified through foreign gene encoding glucoamylase. During process of fermentation yeast expresses glucoamylase that convert starch into glucose.

Yeast strains used for wine synthesis are capable of malolactic fermentation. Wine synthesis consists of two steps: 1) Primary fermentation results in conversion of glucose into alcohol using yeast. 2) Secondary fermentation uses bacteria and its product is lactic acid and this causes the rise in level of acidity. To overcome this problem different strategies are used which are costly. This problem was solved through insertion of malolactic gene *(Lactobacillus delbrueckii*) in industrial yeast strain. This gene lowers the malate conversion hence lowering acidity level of wine.

Enzymes

Enzymes are used in production and processing of food items specifically produced at industrial level. From second last decade of twentieth century, food processing companies are using enzymes that are produced through genetically modified organisms (European food information council 2015). These enzymes comprises of proteases and carbohydrases. Genes for these enzymes have been cloned so as to get higher production in less time period. These enzymes are used for making cheese, curd and flavoring food items. Major percentage of these enzymes is used in food industry as in US more than 50% of proteases and carbohydrases are used in food industry. These enzymes include rennin and α-amylase.

Following are some genetically modified enzymes used in food industry:

• Catalase used in mayonnaise production and it removes hydrogen peroxide.

• Chymosin useful in cheese production as it coagulates milk.

• Glucose oxidase is used in baking as it stabilizes the dough.

• α-amylase converts starch into maltose and used in baking for sweetness.

• Protease used for meat tenderization process, baking and dairy products

Potential Risks of GM Food

Risks to health

Some cases are studied at local level that showed some allergic reactions after usage of GM food.GM food contains foreign genes that can cause hypersensitivity and allergic reactions. One of the foreign protein is Cry9 that is encoded by gene present in soil bacteria Bacillus thuringiensis has been proved allergenic for animal feed.

Risks to environment

Another potential risk is horizontal gene transfer. Transgenic organisms when exposed to natural environment may transfer genes to other organisms resulting in spread transgene everywhere. Consequences of this spread can destroy ecosystem and other organisms. Horizontal transfer has been recorded in lab.

Use of prokaryotic and eukaryotic microorganisms in biotechnological applications

Process Biotechnology:

Bioprocess technology for the production of cell biomass and primary/secondary metabolites, such as baker's yeast, ethanol, citric acid, amino acids, exopolysacharides, antibiotics and pigments etc.; Microbial production, purification and bioprocess application(s) of industrial enzymes; Production and purification of recombinant proteins on a large scale; Chromatographic and membrane based bioseparation methods; Immobilization of enzymes and cells and their application for bioconversion processes. Aerobic and anaerobic biological processes for stabilization of solid / liquid wastes; Bioremediation.

Genetically modified microbes for industrial applications

A revolution in industrial microbiology was sparked by the discoveries of their double-stranded structure of DNA and the development of recombinant DNA technology. Traditional industrial microbiology was merged with molecular biology to yield improved recombinant processes for the industrial production of primary and secondary metabolites, protein biopharmaceuticals and industrial enzymes. Novel genetic techniques such as metabolic engineering, combinatorial biosynthesis and molecular breeding techniques and their modifications are contributing greatly to the development of improved industrial processes. In addition, functional genomics, proteomics and metabolomics are being exploited for the discovery of novel valuable small molecules for medicine as well as enzymes for catalysis. The sequencing of industrial microbal genomes is being carried out which bodes well for future process improvement and discovery of new industrial products.

Novel genetic technologies successfully applied to improvement of primary metabolite production

Genetic technologies	Metabolites
Genome-based strain reconstruction	Amino acids, vitamins, organic acids, alcohols, carotenoids.
Metabolic engineering including reverse (inverse) metabolic engineering	Amino acids, vitamins, organic acids, ethanol, 1,3-propanediol, carotenoids, 5′-inosinic acid.
Genome-wide transcript expression analysis	Riboflavin

Genetic technologies	Metabolites
Molecular breeding (whole genome shuffling)	Lactic acid, ethanol

APPLICATIONS OF BACTERIA IN INDUSTRY AND BIOTECHNOLOGY

Bacteria are used in industry in a number of ways that generally exploit their natural metabolic capabilities. They are used in manufacture of foods and production of antibiotics, probiotics, drugs, vaccines, starter cultures, insecticides, enzymes, fuels and solvents. In addition, with genetic engineering technology, bacteria can be programmed to make various substances used in food science, agriculture and medicine. The genetic systems of bacteria are the foundation of the biotechnology industry discussed below.

In the foods industry, lactic acid bacteria such as Lactobacillus, Lactococcus and Streptococcus are used in the manufacture of dairy products such as cheeses, including cottage cheese and cream cheese, cultured butter, sour cream, buttermilk, yogurt and kefir. Lactic acid bacteria and acetic acid bacteria are used in pickling processes such as olives, cucumber pickles and sauerkraut. Bacterial fermentations are used in processing of teas, coffee, cocoa, soy sauce, sausages and an amazing variety of foods in our everyday lives.

In the pharmaceutical industry, bacteria are used to produce antibiotics, vaccines, and medically-useful enzymes. Most antibiotics are made by bacteria that live in soil. Actinomycetes such as Streptomyces produce tetracyclines, erythromycin, streptomycin, rifamycin and ivermectin. Bacillus and Paenibacillus species produce bacitracin and polymyxin. Bacterial products are used in the manufacture of vaccines for immunization against infectious disease. Vaccines against diphtheria, whooping cough, tetanus, typhoid fever and cholera are made from components of the bacteria that cause the respective diseases. It is significant to note here that

the use of antibiotics against infectious disease and the widespread practice of vaccination (immunization) against infectious disease are two twentieth-century developments that have drastically increased the quality of life and the average life expectancy of individuals in developed countries.

The biotechnology industry uses bacterial cells for the production of biological substances that are useful to human existence, including fuels, foods, medicines, hormones, enzymes, proteins, and nucleic acids. The possibilities of biotechnology are endless considering the gene reservoirs and genetic capabilities within the bacteria. Pasteur said it best, "Never underestimate the power of the microbe."

Biotechnology has produced human hormones such as insulin, enzymes such as streptokinase, and human proteins such as interferon and tumor necrosis factor. These products are used for the treatment of a various medical conditions and diseases including diabetes, heart attack, tuberculosis, AIDS and SLE. Botulinum toxin and BT insecticide are bacterial products used in medicine and pest control, respectively

One biotechnological application of bacteria involves the genetic construction of super strains of organisms to perform particular metabolic tasks in the environment. For example, bacteria which have been engineered genetically to degrade petroleum products are used in cleanup of oil spills and other bioremediation efforts.

Another area of biotechnology involves improvement of the qualities of plants through genetic engineering. Genes can be introduced into plants by a bacterium *Agrobacterium tumefaciens*. Using *A. tumefaciens*, plants have been genetically engineered so that they are resistant to certain pests, herbicides, and diseases.

Finally, it should not be overlooked that industrial, pharmaceutical and food microbiology are applications of biotechnology. Archaea and bacteria are involved in production of biofuels. Bacteria are the main producers of clinically useful antibiotics; they are a source of vaccines against once dreaded diseases; they are probiotics that enhance our health; and they are primary participants in the fermentations of dairy products and many other foods.

Industrial application of yeast

Ripening yeasts, like lactic acid bacteria, are also produced in specialised fermentors under strict hygiene conditions but with different nutrient sources and growth parameters. Notably, yeast fermentation needs to be conducted under aerobic (with oxygen or air) conditions. They are concentrated and mainly available as freeze-dried powders.

The baker's yeast is commercially produced on a nutrient source which is rich in sugar (usually molasses: by product of the sugar refining). The fermentation is conducted in large tanks. Once the yeast fills the tank, it is harvested by centrifugation, giving an off-white liquid known as cream yeast. This is further processed into any of several different forms:

Compressed yeast: still widely used commercially, it is a soft beige solid block with limited storage properties.

Active dry yeast: dried yeast presented in granules or beads that needs to be rehydrated before it can be used.

Instant yeast: vacuum packed fine powder that has become popular in home bread making, as it is easy to use.

Yeasts in food production

Yeasts have two main uses in food production: baking and making alcoholic beverages. They have been used in this way since ancient times – there is evidence that ancient Egyptians used yeast in bread making, and we have been making fermented drinks like beer and wine for millennia.

Baking

Baked goods like bread rise because of the presence of yeast as a rising, or leavening, agent. The most common yeast used in bread making is *Saccharomyces cerevisiae*. It feeds on the sugars present in the bread dough, producing the gas carbon dioxide. This forms bubbles within the dough, causing it to expand. Other ingredients in the mixture have an effect on the speed of the fermentation – sugar and eggs speed it up; fats and salt slow it down.

Brewing

Several different yeasts are used in brewing beer, where they ferment the sugars present in malted barley to produce alcohol. One of the most common is *Saccharomyces cerevisiae*, the same strain used in bread making; this is used to make ale-type beers and is known as a top-fermenting yeast as it forms a foam on the top of the brew. Bottom-fermenting yeasts, such as *Saccharomyces pastorianus*, are more commonly used to make lagers. They ferment more of the sugars in the mixture than top-fermenting yeasts, giving a cleaner taste.

Winemaking

The alcohol in wine is formed by the fermentation of the sugars in grape juice, with carbon dioxide as a byproduct. Yeast is naturally present on grape skins, and this alone can be sufficient for the fermentation of sugars to alcohol to occur. A pure yeast culture, most often *Saccharomyces cerevisiae*, is usually added to ensure the fermentation is reliable. Sparkling wine is made by adding further yeast to the wine when it is bottled. The carbon dioxide formed in this second fermentation is trapped as bubbles.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB
COURSE CODE: 17MBU514A

COURSE NAME: MICROBIAL BIOTECHNOLOGY
BATCH-2017-2020

UNIT-1						
SO NO	**QUESTIONS**	**OPT 1**	**OPT 2**	**OPT 3**	**OPT 4**	**ANSWER**
1	What are the reasons for considering the yeast as a vector?	Length	Short Doubling Time (90minutes)	Can Be Grown On Complex Media	Tough Selection	Short Doubling Time (90minutes)
2	The first vinegar manufacturing industry was established in France during ----------	a) 16^{th} century	b) 14^{th} century	c) 18^{th} century	d) 7^{th} century	14^{th} century
3	In 1680 _____ scientist was discovered the microscope?	Delbruck, Heyduck and Henner berg	Watson and crick	Antony van leuwenhoek	Lederberg	Antony van leuwenhoek
4	Which are the shuttle vectors?	YE ps	PBR 322	LEu2	PUC 118	YEps
5	in 1914 to 1916 _____ discovered the large scale use of yeast food industry	Alexander Fleming	Leden berg	Delbruck Heyduck and Henner berg	Messelson and stahi	Delbruck Heyduck and Henner berg
6	In _____ year the Alexander Fleming discovered penicillin	1920	1931	1910	1922	1920
7	Which is the yeast chromosomal gene that codes for isopropyl malate dehydrozejase	2 plasmid	PBR 322	YEPs	LEU2	LEU2
8	in 1869 ____ scientist was isolate DNA from the nuclei WBC	Alexander Fleming	Louis Pasteur	Johan meischer	koch	Louis Pasteur
9	in 1910 _____ terms was coined	Thomas h.margan proved that genes carried on	molecular biotechnology	agriculture biotechnology	medical biotechnology	Thomas h.margan proved that genes carried on

		chromosome "biotechnology "				chromosome "biotechnology "
10	origin of replication in yeast having 100 bp	LEU2	ARS	2 Plasmid	ANS	ARS
11	in during period of 1912 to 1914 the three important industrial chemical _____were obtained from bacteria	acetic acid	ethanol	alcohol	acetone, butanol and glycerol	acetone, butanol and glycerol
12	in 1946 lederberg and tatum was discovered the _____	bacterial recombination	viral recombination	recombinated vaccine	recombinated plasmid	bacterial recombination
13	bacterial plasmid carrying a yeast gene	YRPs	2 plasmid	YIPs	YAC	2 plasmid
14	in _____year food and drug administration approved the first biotechnogy therapy , a human insulin drug made by genetech	1982	1980	1928	1918	1982
15	micro organism can be used to enhance to recovery of metals from low grade area and from efficient containing undesirable quantities of	low metals or other toxins	toxin	heavy metals	heavy metals and other toxins	heavy metals and other toxins
16	Phages were first discovered by	Fredrick Twort	Edward Tautum	Flein d' hericcle	Griffith	Fredrick twort
17	The field of biopharmaceutical drug developed it is the development of _____ by recombinant methods.	Therapeutic human protein	Insulin therapeutic	Gene therapies	Biopharmaceutical drug development	Therapeutic human protein

18	the main dominant forces in green biotechnology today are agro giants with a world wide area of operation such as	BASF, Bayer loop -science	Food production	Monsanto and syngenta	Both a and c	Both and c
19	The intersection of DNA fragment is accompanied with deflection of all the major part non essential region of genome the delected the region is called	Okazaki fragment	stuffer fragment	coding fragment	non coding region	stuffer fragment
20	The main emphasis in _____ is the production	Modern plant biotechnology	Medical biotechnology	Agriculture biotechnology	Green biotechnology	Modern plant biotechnology
21	Biotechnology is an amalgamation of variety of disciplines	molecular biology, bioinformation	both a and c	genetic and microbiology	gene therapies	a and c
22	The ampicillin resistant gene of Pbr 322 was derived from	pSC101	Ti plasmid	pBR 313	RSF 2124	RSF 2124
23	Gene selection through ____for biological monitoring	b and d	Mutagenesis	Bioremediation	Biosensor	b and d
24	Agriculture biotechnology is defined as that is used for	Live stock and crop improvement	Molecular breeding	Tissue culture and micro propagation	Molecular Diagnostic Tools	live stock and crop improvement
25	The size of pBR 322 IS	4.363 bp	6.600bp	10.900bp	5.300bp	4.363bp
26	Biofertilizer contain _____ of efficient strains of phosphate solubilizing , N2 fixation or cellulolytr microorganism used for the application of seeds , soli or	Live and Latent cell	Live stock	Crop improvement	Pesticides	Live and latent cell

	composting areas					
27	They promote root growth by producing _____	hormones and antibiotics	fungicides	insecticides	pesticides	hormones and antibiotics
28	In EcoRI the first two letters are known as	Genus and specific name (species)	Genus name	specific name	inventor name	Genus and specific name (species)
29	Chemical fertilizer should be applied to the soil with the gap of ____ days for better nitrogen fixation	15 to 20	10 to 15	20 to 35	5 to 10	15 to 20
30	There are various environmental factor involved such as type of soli in adequate of	Organic matter high temperature	N2 fixation	Organic matter high temperature and soil eater defict	Biosensor	Organic matter high temperature and soil eater defict
31	Retro viral infection can be applied to introduce the gene into	Fish	Mice	Plant	Bacteria	Mice
32	The most common yeast used in bread making is	*Sacchromyciscere visiae*	Yeast	Agrobacterium tumefaciens	Bacteria	*Sacchromyciscerevisiae*
33	Yeast fermentation need to be conducted under _____ condition	Aerobic	Non aerobic	Both	None of the above	Aerobic
34	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 DNA
35	Yeast of naturally present in	Apple	Orange	Grape skin	Lemon	Grape skin

36	In the pharmaceutical industry bacteria is used to produce ____	Antibodies	Antibodies, vaccine and medically - useful enzymes	Antiviral	Enzymes	antibodies, vaccine and medically-useful enzymes
37	Transfer vector is a vector	Shuttle vector	Plasmid vector	Binary vector	Co integrate vector	Shuttle vector
38	Actinomyces such as Streptomyces produce _____	Tetracycline	Bacetracin	Tetracylines,streptomycin,erythromycin, rafamycin and	Polymycin	Tetracylines, streptomycin, erythromycin rafamycin and
39	These products are used in treatment of a various medical conditions and diseases including	Diabeties,heartattack,tuberculosis, AIDS and SLE	Kidney failure	Diphtheria	Cholera	Diabeties,heartattack, tuberculosis, AIDS and SLE
40	ARS is	Autonomus replicating plasmid	Automatic replicating sequence	Automatic reproducing sequence	Autonomous reproducing sequence	Autonomous replicating plasmid
41	In industrial application of yeast having further processed into any several differential forms they are	Both b and d	Compressed yeast	Yeast in food production	Active drug and instant yeast	Both b and d
42	Type of mycorrhizae	ectomycorrhiza, ectomycorrhiza, ectendomycorrhiza	VA Mycorrhiza	ectendomycorrhiza	arbutoidmycorrhiza	Ectomycorrhiza, ectomycorrhiza, ectendomycorrhiza
43	PBR 322 is first identified and developed by	A. Chan and N. Cohen	Elinst Berlimer	T.Bolival and Rodrigues	Ishiwata	T.Bolival and Rodrigues

44	One of the foreign protein is _____that is encoded by gene present in soil bacteria *Bacillus thuringiensis* has been proved allergenic for animal feed	Cry 3Aa	Cry 9	Cry 2	Cry 1	Cry 9
45	Biotechnology is the applied science and has made advances in two major area viz _____	Molecular biology	Production of industrially important biochemical	Gene therapy	a and b	a and b
46	Genetic engineering in biotechnology stimulated hopes for therapeutic proteins drug and biological organism themselves such as	Seeds , Pesticides	Modified human cells	Engineering yeast	All the above	All the above
47	Name the animal virus used as vectors	Sv40 virus	HIV	Rabies	Polio	sv40 virus
48	In USA alone more than 225 companies has been established and successfully working like	Biogen, Cetus	Geneatech	Hybritech	All the above	All the above
49	The industrial application of molecular biotechnology is often subdivided, so that we speak of	Red , green , Gray or white biotechnology	Blue biotechnology	Orange biotechnology	Pink biotechnology	Red , green , Gray or white biotechnology
50	A lactose analogue which is involved in the screening of B galactosidase	Y -gal	X-gal	B-galactosidase	B-galactosidepermease	X-gal

51	_____percentage of companies are currently working in the field of red biotechnology	13%	22%	85%	92%	92%
52	the distinction relates to the use of the technology in	medical field ,Agriculture , the environment and industry	white biotechnology	biopharmaceutical	molecular biotechnology	medical field ,Agriculture , the environment and industry
53	baculo virus is a	Parasite	Obligate parasite	Saprophytes	Pathogen	Obligate parasite
54	in _____ the first genetically engineered food product , the flavrSavr tomato, gained FDA approval	1994	1992	1982	1996	1994
55	Biotechnology-derived pharmaceutical may be derived from a variety of expression system such as	E.coli	Yeast , mammalian	Insect, plant cells and transgenic animals or other organism	All the above	All the above
56	The type of biotechnology that deals with applications in human and animal medicine is called as	A) Green	B)Red	C)Blue	D)White	B)Red
57	Which of the following is an insertion vector	EHBL 4	Charon 16A	GEM12	Charon 4a	Charon 16A
58	some technical advantages such as an optimized ____	Pharmocokinetie	Pharmacodynamics profile	A and b	Pharmaceutical	a and b
59	It is important for the toxicologist to be aware of the nature of the product to be tested in terms of	Primary	Secondary	Tertiary	All the above	All the above

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB COURSE NAME: MICROBIAL BIOTECHNOLOGY
COURSE CODE: 17MBU514A BATCH-2017-2020

60	The _____form in this second fermentation is trapped as bubbles	Nitrogen	Carbon dioxide	Oxygen	None of the above	Carbon dioxide
61	Endomycorrhizas are found in	Gymnosperms	Angiosperms	All plants	Gymnosperms and Angiosperms	All plants
62	α-amylase converts starch into …… used in baking for sweetness?	Maltose	Fructose	Glucose	Sucrose	Maltose
	Which foreign protein has proved to be allergen for animal feed	Cry4	Cry3	Cry9	Cry2	Cry9
63	Who said these words "Never underestimate the power of the microbe.	Leewn hook	Pasteur	Joseph Lister	Edward Jenner	Pasteur
64	Lagers are commonly made using	*Saccharomyces cerevisiae*	*Saccharomyces pastorianus*	*Saccharomyces boulardii*	*Saccharomyces carlsbergensis*	*Saccharomyces pastorianus*
65	Which species produces bacitracin and polymyxin	*Paenibacillus*	*E.coli*	*Bacillus cereus*	*Bacillus coagulens*	*Paenibacillus*
66	Enzyme used for meat tenderization process	Protease	α-amylase	Catalase	Maltose	Protease
67	Catalase is used in the production of?	Cheese	Butter	mayonnaise	Beer	mayonnaise
68	**Zeneca**, a British company, developed a microbially produced	Biopol	Biopla	Plabio	Plasbio	Biopol

	biodegradable plastic called as					
69	Forest Research Institute, Dehra Dun has established …….bank in different states of the country?	Mycorrhizae	Gene	Yeast	Bacterial	Mycorrhizae
70	By 2050, food supply has to be increased by ……per cent to fulfill the demand of overgrowing population	60	70	80	90	70
71	Alexander Fleming discovered penicillin in the year?	1920	1919	1921	1922	1920
	In 1914 to 1916, Delbruck, Heyduck and Hennerberg discovered the large-scale use of ……. in food industry?	*Bacillus*	*Yeast*	Enzymes	Proteins	Yeast
72	Restriction enzymes mostly preferred for genetic engineering are of type	Blue colour	Green colour	Colorless	greenish yellow	Colorless

Recombinant DNA Vaccine

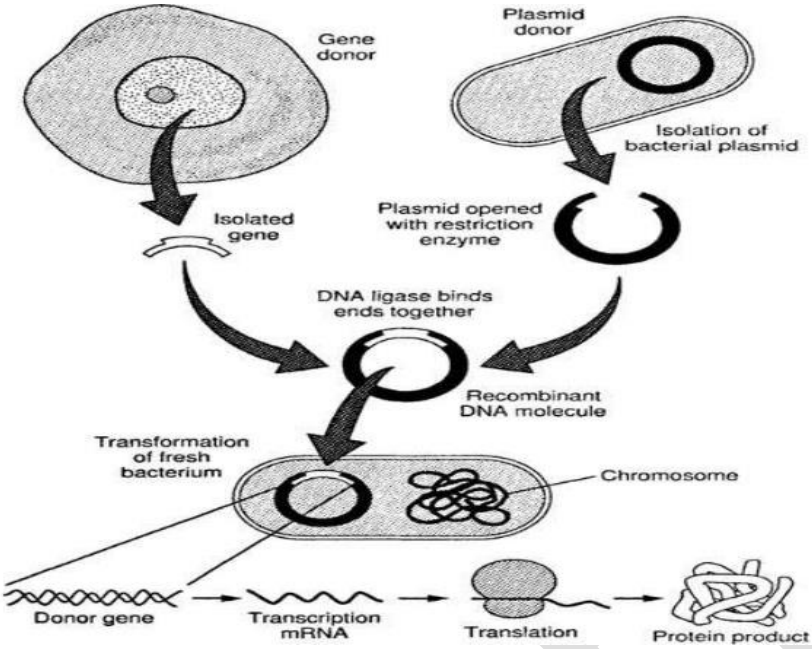
Recombinant DNA (**rDNA**) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

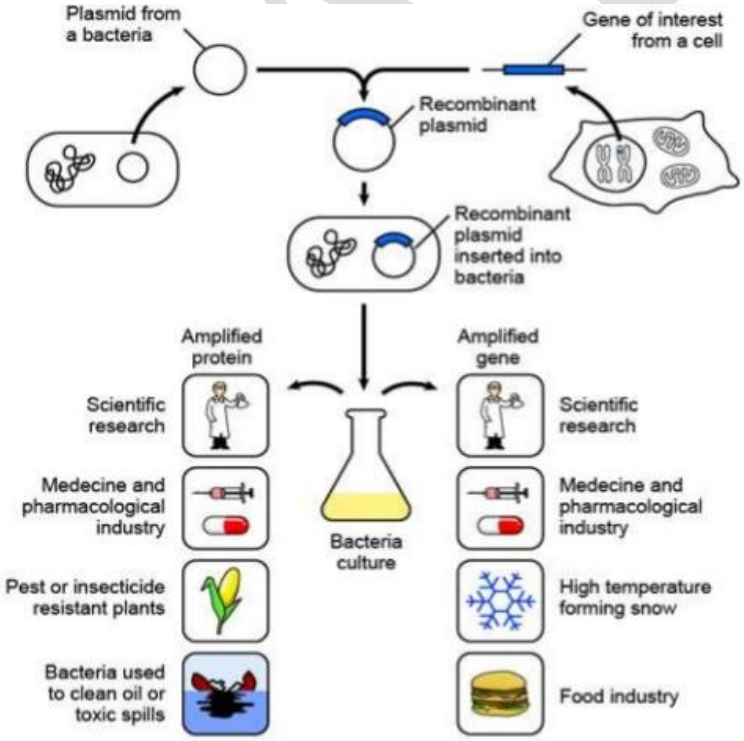
Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes called **chimeric DNA**, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

Proteins that can result from the expression of recombinant DNA within living cells are termed *recombinant proteins*. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced.[1]Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences.

Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms

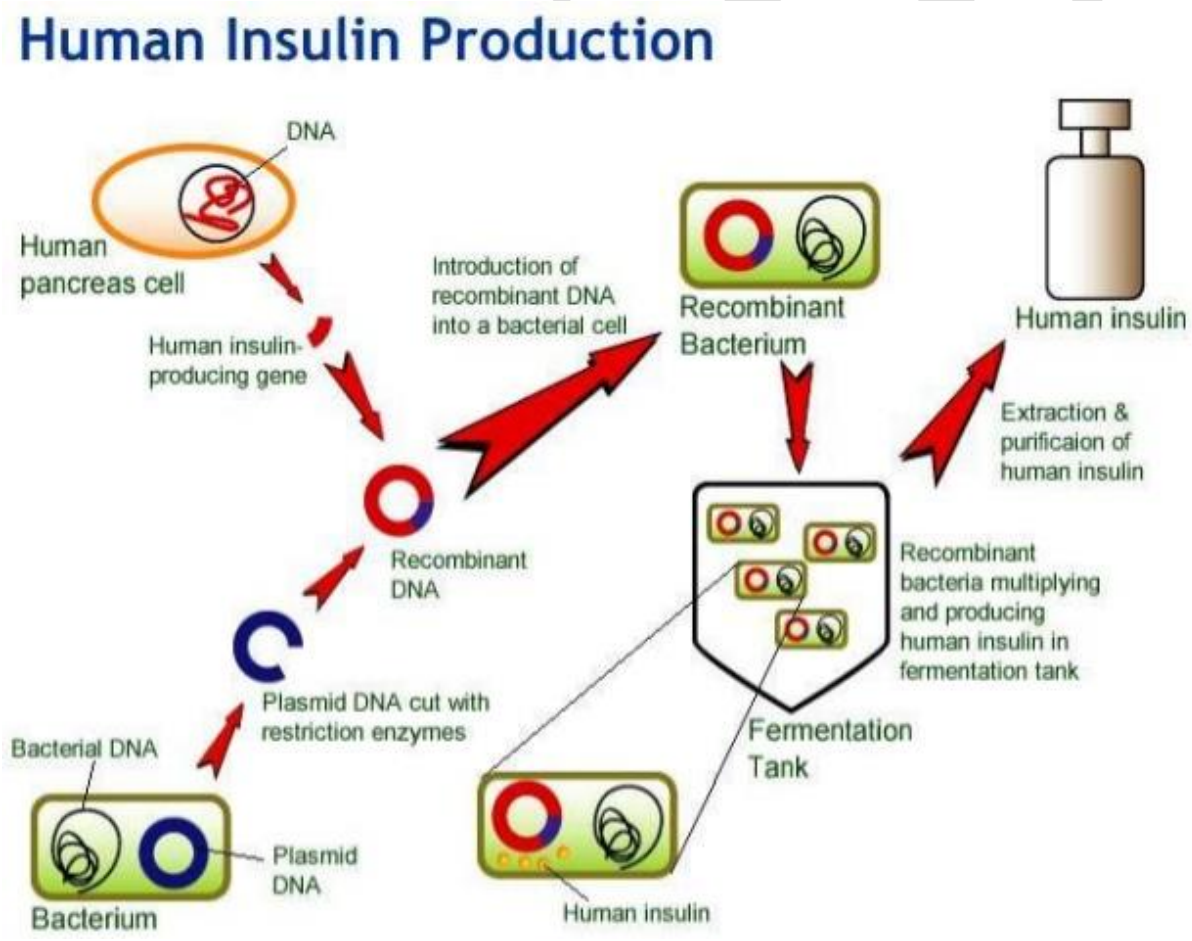

Gene donor
Plasmid donor
Isolation of bacterial plasmid
Isolated gene
Plasmid opened with restriction enzyme
DNA ligase binds ends together
Recombinant DNA molecule
Transformation of fresh bacterium
Chromosome
Donor gene
Transcription mRNA
Translation
Protein product


Plasmid from a bacteria
Gene of interest from a cell
Recombinant plasmid
Recombinant plasmid inserted into bacteria
Amplified protein
Amplified gene
Scientific research
Medecine and pharmacological industry
Pest or insecticide resistant plants
Bacteria used to clean oil or toxic spills
Bacteria culture
Scientific research
Medecine and pharmacological industry
High temperature forming snow
Food industry

Producing Recombinant Insulin

• First, scientists synthesized genes for the two insulin A & B chains. They were then inserted into plasmids along with a strong lac Zpromoter. The genes were inserted in such a way that the insulin & B-galactosidase residues would be separated by a methionine residue. This is so that the insulin A & B chains can be separated easily by adding cyanogen bromide.

The vector was then transformed into E.coli cells. Once inside the bacteria, the genes were "switched-on" by the bacteria to translate the code into either the "A" chain or the "B" chain proteins found in insulin. The purified insulin A and B chains were then attached to each other by disulphide bond formation under laboratory conditions



Recombinant microbial production of Streptokinase

Introduction:

•A blood clot (thrombus) developed in the circulatory system can cause vascular blockage leading to serious consequences including death.

• A healthy hemostatic system suppresses the development of blood clots in normal circulation, but reacts extensively in the event of vascular injury to prevent blood loss.

Outcomes of a failed homeostasis include stroke, pulmonary embolism, deep vain thrombosis and acute myocardial infarction.

Streptococcal fibrinolysis These enzyme activates a fibrinolytic enzyme in human serum, which splits fibrin into smaller fragments, thus it causes rapid dissolution of blood clots and fibrinous exudates. Therefore, streptokinase acts indirectly upon a substrate of fibrin or fibrinogen.

Source this enzyme is produced by certain bacteria. The most frequently employed for manufacture are: haemolytic streptococci, particularly those of the Lancefield group A, human C and G. Steptodornase is a related enzyme, which act directly upon a substrate of deoxy ribo nucleoproteins (DNA).

These are main constituents of nuclei. They also present upto 30-70% in purulent exudates. Steptodornase splits them into free purine bases and pyrimidine nucleosides thereby decrese the viscosity of purulent exudates.

Manufacturing process It is produced by fermentation process 'which involves the following steps:-

1. Preparation of medium
2. Fermentation
3. Purification of the product.

PREPARATION OF MEDIUM

Ingredients: Casein digest solution: It is prepared by dissolving casein in water in specified proportion. It is heated to 100 C and maintained the same temperature, till the solution is clear. The resultant solution is rapidly cooled to 15 C and filtered through a coarse filter paper. Toluene

in small quantity is added for the purpose of preservation. It is stored for four days at 20 C and filtered to remove insoluble material.

Dextrose (a carbohydrate source) 3. Amino acids a) Cysteine in 10%HCl b) Glycine c) Tryptophan. 4. Vitamins a)Thiamine hydrocloride b)Riboflavin c)Nicotinic acid d)Pyridoxine e) Calcium pentothenate a)

Trace elements – MgSO4, CuSO4, MnSO4, FeSO4, Potassium bicarbonate, Potassium dihydrogen phosphate, Uracil, Adenyl sulphate, Thioglycolic acid

FERMENTATION Sterilized medium is inoculated with seed inoculation of bacterium; S. haemolyticus having a bacterial count of 20 billions/ml. fermentation is carried out in a tank for 14hrs at 37C. During this period no pH adjustment, aeration or modification are made.

Later, dextrose 50% is added and pH is adjusted to 6 at 15 min. interval with 5N sodium hydroxide. After each adjustment of pH, 50% dextrose is added. Fermentation is continued till bacterial count ceases to increase (about 3 hrs).At this stage fermentation medium contains appx. 1000 units/ml

PURIFICATION Crude streptokinase is first dialysed against phosphate buffer then it is applied on modified cellulosic columns and eluted with phosphate buffer with increasing pH and molarity (increasing pH 5.8 to 8.5 and molarity 0.005to 0.1 M).

At 0.1 M: streptokinase is eluted ′ pH and molarities are the important factors in purification. ′ > ′0.1 M: improper adsorption and separation pH > ′8.5: adsorption capacity of cellulose decreases < At pH 8 and molarity 0.75, impurities are eluted.′5.8:streptokinase is precipitated

PURIFICATION BY DEAE CELLULOSE: Crude streptokinase, phosphate buffer 0.2 M and DEAE cellulose in the proportion of 3:2:1 are stirred for 1 hour and filtered. Cake is washed with phosphate buffer 0.025 m by stirring for 30 mins. It is again filtered and cake is suspended in 0.1 m phosphate buffer by stirring for 1 hour. Filterate is collected which contains pure streptokinase.

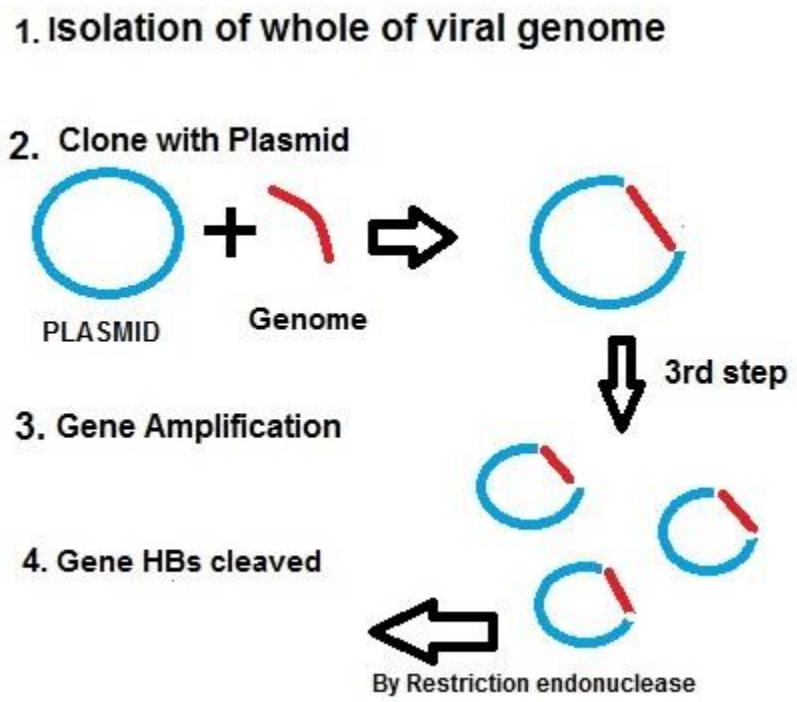
USES: Treatment of thromboembolic disorders for the lysis of pulmonary emboli, arterial thrombus, deep vein thrombus and acute coronary artery thrombosis.

Recombinant vaccine

A recombinant vaccine is a vaccine produced through recombinant DNA technology. This involves inserting the DNA encoding an antigen (such as a bacterial surface protein) that stimulates an immune response into bacterial or mammalian cells, expressing the antigen in these cells and then purifying it from them.

Hepatitis B Vaccine

Hepatitis is an infectious viral disease which damages the liver. Hepatitis arises from the words "Hepato+ liver and its inflammation."It is one of the deadly infections of virus which affects almost 7 million people a year it can cause acute and also chronic liver infection and damage. Since the liver is a seat of all the body reactions and metabolism, its damage leads to death. It is a severe disorder and very contagious. But it can be prevented by use of hepatitis-B vaccination. The production of hepatitis B vaccine was done by use of hepatitis virus previously. The vaccine was a live vaccine or an attenuated vaccine one



The live vaccine is one where the live hepatitis virus is used. But due to attenuation, its virulence is removed.

So they no longer can be pathogenic when the weak virus is introduced into the body. But these vaccines are unsafe and non-reliable due to problems of virulence in immune-compromised patients. Also, there is a trend of decline in immunity of humans.

So once administered the vaccines can turn virulent and cause infection.

So to avoid this, and provide effective prevention, hepatitis-B subunit vaccines are used.

These vaccines are manufactured by using genetic engineering technology.

The subunits vaccines are the antigens of the virus and can evoke an immune reaction.

Further, these subunits are not the virus as a whole so no chances of virulence.

But the subunits are hard to collect and even multiply as they are not organisms. Further their size is also very small.

So the steps in the production of hepatitis B vaccine is quite lengthier than routine steps of five steps of genetic engineering.

Production of Hepatitis B Vaccine

1. Isolation of the Whole genome of the hepatitis-B virus:

2. Cloning of the genome with plasmid and its multiplication

3. The release of the sequence coding for HBs antigen.

4. Ligate with yeast expression vector

5. Transform in Saccharomyces and allow for vaccine formation.

Isolation of genome from the virus is the first step. An entire genome of 2kb is isolated which codes for HB ag on the virus body. But the quantity can be less and virus is also not readily multiplied in labs. So the gene is multiplied by the use of plasmid. The plasmid has the inherent tendency to multiply. These plasmids with genome are introduced into bacteria for replication and multiplication. Many plasmids with the gene are produced.
From these sufficient genomes, the required gene coding for HBs is cleaved by use of restriction endonucleases.
These are the enzymes which cut DNA molecules at precise points.

Once the required gene is formed, it is again taken and cloned with another vector namely yeast expression vector.

Here the yeast expression vector has alcohol dehydrogenase-1 (a strong promoter gene) by the side. Also, there is leucine 2 as a marker.

The enzyme DNA ligase is used for ligation (cloning) of the gene with yeast vector.

Then this newly formed vector is transformed into Saccharomyces cerevisiae bacteria.
The transformed cells are allowed to grow in culture media and promote the gene expression. The vaccine is formed and is present in the body of the bacterial cell.
The bacterial cells are lysed, and the solution is centrifuged.
By this, the HBs gene is obtained in the supernatant. This vaccine is isolated and allowed to form aggregates with silver.
Unlike routine steps of rDNA technology, here we use two vectors. One for the multiplication of viral genes. The other is to transfer the HBs gene into bacteria from where it is expressed.
The hepatitis B vaccine is given as an intramuscular injection. It is administered as three doses and the second and third doses are given after one month and six months respectively.

Polysaccharides:

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

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

Applications of Microbial Polysaccharides:

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

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

Production of Microbial Polysaccharides:

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

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

Biosynthesis of polysaccharides:

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

Recovery of polysaccharides:

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

Microbial polysaccharides versus plant polysaccharides:

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

General Features of Microbial Polysaccharides:

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

TABLE 30.1 Commercially important microbial polysaccharides and their applications

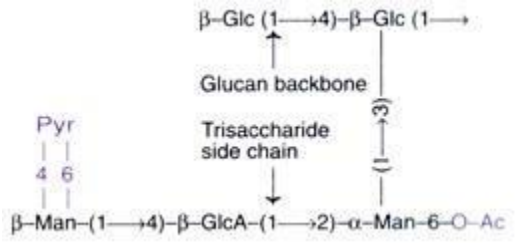
Polysaccharide	*Producing organism(s)*	*Application(s)*
Xanthan	*Xanthomonas campestris*	As a food additive for stabilization, gelling and viscosity control, i.e. for the preparation of soft foods e.g. ice cream, cheese. In oil industry for enhanced oil recovery. In the preparation of toothpastes, and water based paints.
Dextran	*Leuconostoc mesenteroides,* *Acetobacter* sp, *Streptococcus mutans*	Blood plasma expander Used in the 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:

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) as depicted below.



Basically, xanthan is a branched polymer with β (1 → 4) linked glucan (glucose polymer) backbone bound to a trisaccharide (Man, GIcA, Man) side chain on alternate glucose residues. The mannose has either acetate or pyruvate groups.

The number of acetate or pyruvate molecules in xanthan is variable and is dependent on the bacterial strain used. The culture conditions and the recovery processes also influence the quantities of pyruvate and acetate residues. It is believed that the viscosity of xanthan gum is influenced**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.

by the contents of pyruvate and acetate.

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. uridinediphosphate 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, Xanthomonascampestris. 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 Xanthomonascampestris 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. Leuconostocmesenteroides 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:

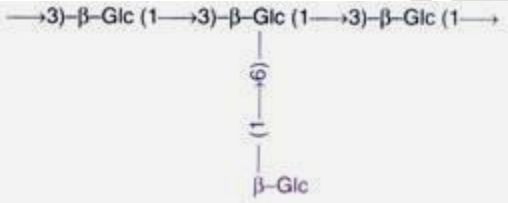
Alginate is a linear polymer composed of mannuronic acid and glucuronic acid (both of them being uronic acids) in a proportion ranging from 4: 1 to 20: 1. Some of the mannuronic acid residues are acetylated. Alginate is commercially produced by Gram-negative bacteria, Pseudomonas aeruginosa and Azobactervinelandii.

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 Sclerotiumglucanicum, 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 deacetylatedgellan which forms firm and brittle gels under the trade name Celrite has been developed by a reputed company in USA (KalcoInc). 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, Aureobasidiumpollulans. 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:

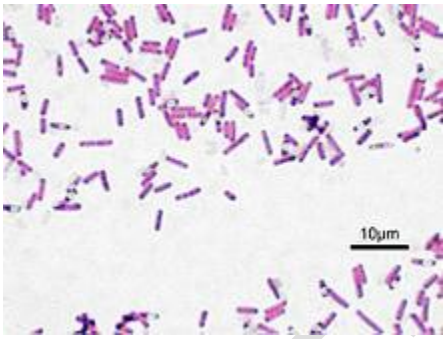
Curdlan is a β-glucose polymer (β-glucan). The glucose residues are held together by β 1 —>3 glycosidic bonds. The exopolysaccharidecurdlan is commercially produced by employing Alcaligenesfaecalis. Curdlan-like polysaccharides are also produced by other microorganisms such as Agrobacterium rhizogenes 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.

Polyhydroxyalkanoates or **PHAs** are polyesters produced in nature by numerous microorganisms, including through bacterial fermentation of sugar or lipids.[1] When produced by bacteria they serve as both a source of energy and as a carbon store. More than 150 different monomers can be combined within this family to give materials with extremely different properties.[2] These plastics are biodegradable and are used in the production of bioplastics.

They can be either thermoplastic or elastomeric materials, with melting points ranging from 40 to 180 °C.

The mechanical properties and biocompatibility of PHA can also be changed by blending, modifying the surface or combining PHA with other polymers, enzymes and inorganic materials, making it possible for a wider range of applications

Biosynthesis[edit]



Certain strains of *Bacillus subtilis*bacteria can be used to produce polyhydroxyalkanoates

To produce PHA, a culture of a micro-organism such as *Cupriavidusnecator* is placed in a suitable medium and fed appropriate nutrients so that it multiplies rapidly. Once the population has reached a substantial level, the nutrient composition is changed to force the micro-organism to synthesize PHA. The yield of PHA obtained from the intracellular granule inclusions can be as high as 80% of the organism's dry weight.

The biosynthesis of PHA is usually caused by certain deficiency conditions (e.g. lack of macro elements such as phosphorus, nitrogen, trace elements, or lack of oxygen) and the excess supply of carbon sources.[4]

Polyesters are deposited in the form of highly refractive granules in the cells. Depending upon the microorganism and the cultivation conditions, homo- or copolyesters with different hydroxyalkanic acids are generated. PHA granules are then recovered by disrupting the cells.[5] Recombinant *Bacillus subtilis* str. pBE2C1 and *Bacillus subtilis* str. pBE2C1AB were used in production of polyhydroxyalkanoates (PHA) and it was shown that they could use malt waste as carbon source for lower cost of PHA production.

PHA synthases are the key enzymes of PHA biosynthesis. They use the coenzyme A - thioester of (r)-hydroxy fatty acids as substrates. The two classes of PHA synthases differ in the specific use of hydroxy fatty acids of short or medium chain length.

The resulting PHA is of the two types:

- Poly (HA SCL) from hydroxy fatty acids with short chain lengths including three to five carbon atoms are synthesized by numerous bacteria, including *Cupriavidusnecator* and *Alcaligeneslatus* (PHB).
- Poly (HA MCL) from hydroxy fatty acids with medium chain lengths including six to 14 carbon atoms, can be made for example, by *Pseudomonas putida*.

A few bacteria, including *Aeromonashydrophila* and *Thiococcuspfennigii*, synthesize copolyester from the above two types of hydroxy fatty acids, or at least possess enzymes that are capable of part of this synthesis.

Another even larger scale synthesis can be done with the help of soil organisms. For lack of nitrogen and phosphorus they produce a kilogram of PHA per three kilograms of sugar.

The simplest and most commonly occurring form of PHA is the fermentative production of poly-beta-hydroxybutyrate (poly-3-hydroxybutyrate, P3HB), which consists of 1000 to 30000 hydroxy fatty acid monomers.

Industrial production

In the industrial production of PHA, the polyester is extracted and purified from the bacteria by optimizing the conditions of microbial fermentation of sugar or glucose.

In the 1980s, Imperial Chemical Industries developed poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) obtained via fermentation that was named "Biopol". It was sold under the name "Biopol" and distributed in the U.S. by Monsanto and later Metabolix.[6]

As raw material for the fermentation, carbohydrates such as glucose and sucrose can be used, but also vegetable oil or glycerine from biodiesel production. Researchers in industry are working on methods with which transgenic crops will be developed that express PHA synthesis routes from bacteria and so produce PHA as energy storage in their tissues. Several companies are working to develop methods of producing PHA from waste water, including start-up Micromidas and Veolia subsidiary Anoxkaldnes.

PHAs are processed mainly via injection molding, extrusion and extrusion bubbles into films and hollow bodies.

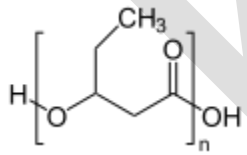
Material properties

PHA polymers are thermoplastic, can be processed on conventional processing equipment, and are, depending on their composition, ductile and more or less elastic. They differ in their properties according to their chemical composition (homo-or copolyester, contained hydroxy fatty acids).

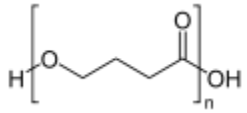
They are UV stable, in contrast to other bioplastics from polymers such as polylactic acid, partial ca. temperatures up to 180 °C, and show a low permeation of water. The crystallinitycan lie in the range of a few to 70%. Processability, impact strength and flexibility improves with a higher percentage of valerate in the material. PHAs are soluble in halogenated solvents such chloroform, dichloromethane or dichloroethane.[9]

PHB is similar in its material properties to polypropylene (PP), has a good resistance to moisture and aroma barrier properties. Polyhydroxybutyric acid synthesized from pure PHB is relatively brittle and stiff. PHB copolymers, which may include other fatty acids such as beta-hydroxyvaleric acid, may be elastic.

Applications

- 

Structure of poly-3-hydroxyvalerate (PHV)

- 

Structure of poly-4-hydroxybutyrate (P4HB)

Due to its biodegradability and potential to create bioplastics with novel properties, much interest exists to develop the use of PHA-based materials. PHA fits into the green economyas a means to create plastics from non-fossil fuel sources. Furthermore, active research is being carried out for the biotransformation "upcycling" of plastic waste (e.g., polyethylene terephthalate and polyurethane) into PHA using *Pseudomonas putida* bacteria.

A PHA copolymer called PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) is less stiff and tougher, and it may be used as packaging material.

In June 2005, a US company (Metabolix, Inc.) received the US Presidential Green Chemistry Challenge Award (small business category) for their development and commercialisation of a cost-effective method for manufacturing PHAs.

There are potential applications for PHA produced by micro-organisms within the medical and pharmaceutical industries, primarily due to their biodegradability.

Fixation and orthopaedic applications have included sutures, suture fasteners, meniscus repair devices, rivets, tacks, staples, screws (including interference screws), bone plates and bone plating systems, surgical mesh, repair patches, slings, cardiovascular patches, orthopedic pins (including bone.lling augmentation material), adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, atrial septal defect repair devices, pericardial patches, bulking and filling agents, vein valves, bone marrow scaffolds, meniscus regeneration devices, ligament and tendon grafts, ocular cell implants, spinal fusion cages, skin substitutes, dural substitutes, bone graft substitutes, bone dowels, wound dressings, and hemostats.

Biopesticides

Biopesticides, a contraction of 'biological pesticides', include several types of pest management intervention: through predatory, parasitic, or chemical relationships. The term has been associated historically with [biological control] – and by implication – the manipulation of living organisms. Regulatory positions can be influenced by public perceptions, thus:

in the EU, biopesticides have been defined as "a form of pesticide based on micro-organisms or natural products".

the US EPA states that they "include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs".

They are obtained from organisms including plants, bacteria and other microbes, fungi, nematodes, etc. They are often important components of integrated pest management (IPM) programmes, and have received much practical attention as substitutes to synthetic chemical plant protection products (PPPs).

Types

Biopesticides can be classified into these classes-

- Microbial pesticides which consist of bacteria, entomopathogenic fungi or viruses (and sometimes includes the metabolites that bacteria or fungi produce). Entomopathogenic nematodes are also often classed as microbial pesticides, even though they are multi-cellular.
- Bio-derived chemicals. Four groups are in commercial use: pyrethrum, rotenone, neem oil, and various essential oils are naturally occurring substances that control (or monitor in the case of pheromones) pests and microbial diseases.
- Plant-incorporated protectants (PIPs) have genetic material from other species incorporated into their genetic material (*i.e.* GM crops). Their use is controversial, especially in many European countries.RNAi pesticides, some of which are topical and some of which are absorbed by the crop.

Biopesticides have usually no known function in photosynthesis, growth or other basic aspects of plant physiology. Instead, they are active against biological pests. Many chemical compounds

have been identified that are produced by plants to protect them from pests so they are called antifeedants. These materials are biodegradable and renewable alternatives, which can be economical for practical use. Organic farming systems embraces this approach to pest control

RNA

RNA interference is under study for possible use as a spray-on insecticide by multiple companies, including Monsanto, Syngenta, and Bayer. Such sprays do not modify the genome of the target plant. The RNA could be modified to maintain its effectiveness as target species evolve tolerance to the original. RNA is a relatively fragile molecule that generally degrades within days or weeks of application. Monsanto estimated costs to be on the order of $5/acre.

RNAi has been used to target weeds that tolerate Monsanto's Roundup herbicide. RNAi mixed with a silicone surfactant that let the RNA molecules enter air-exchange holes in the plant's surface that disrupted the gene for tolerance, affecting it long enough to let the herbicide work. This strategy would allow the continued use of glyphosate-based herbicides, but would not per se assist a herbicide rotation strategy that relied on alternating Roundup with others.

They can be made with enough precision to kill some insect species, while not harming others. Monsanto is also developing an RNA spray to kill potato beetles One challenge is to make it linger on the plant for a week, even if it's raining. The Potato beetle has become resistant to more than 60 conventional insecticides.

Monsanto lobbied the U.S. EPA to exempt RNAi pesticide products from any specific regulations (beyond those that apply to all pesticides) and be exempted from rodent toxicity, allergenicity and residual environmental testing. In 2014 an EPA advisory group found little evidence of a risk to people from eating RNA.

However, in 2012, the Australian Safe Food Foundation alleged that the RNA trigger designed to change wheat's starch content might interfere with the gene for a human liver enzyme. Supporters countered that RNA does not appear to make it past human saliva or stomach acids. The US National Honey Bee Advisory Board told EPA that using RNAi would put natural systems at "the epitome of risk". The beekeepers cautioned that pollinators could be

hurt by unintended effects and that the genomes of many insects are still unknown. Other unassessed risks include ecological (given the need for sustained presence for herbicide and other applications) and the possible for RNA drift across species boundaries.

Monsanto has invested in multiple companies for their RNA expertise, including Beeologics (for RNA that kills a parasitic mite that infests hives and for manufacturing technology) and Preceres (nanoparticle lipidoid coatings) and licensed technology from Alnylam and Tekmira. In 2012 Syngenta acquired Devgen, a European RNA partner. Startup Forrest Innovations is investigating RNAi as a solution to citrus greening disease that in 2014 caused 22 percent of oranges in Florida to fall off the trees.

Examples

Bacillus thuringiensis, a bacterial disease of Lepidoptera, Coleoptera and Diptera, is a well-known insecticide example. The toxin from *B. thuringiensis* (Bt toxin) has been incorporated directly into plants through the use of genetic engineering. The use of Bt Toxin is particularly controversial. Its manufacturers claim it has little effect on other organisms, and is more environmentally friendly than synthetic pesticides.

Other microbial control agents include products based on:

- entomopathogenic fungi (*e.g. Beauveriabassiana, Isariafumosorosea, Lecanicillium* and *Metarhizium* spp.),
- plant disease control agents: include *Trichoderma* spp. and *Ampelomycesquisqualis* (a hyper-parasite of grape powdery mildew); *Bacillus subtilis* is also used to control plant pathogens.[4]
- beneficial nematodes attacking insect (*e.g. Steinernemafeltiae*) or slug (*e.g. Phasmarhabditishermaphrodita*) pests
- entomopathogenic viruses (*e.g.. Cydiapomonella* granulovirus).
- weeds and rodents have also been controlled with microbial agents.

Various naturally occurring materials, including fungal and plant extracts, have been described as biopesticides. Products in this category include:

- Insect pheromones and other semiochemicals
- Fermentation products such as Spinosad (a macro-cyclic lactone)
- Chitosan: a plant in the presence of this product will naturally induce systemic resistance (ISR) to allow the plant to defend itself against disease, pathogens and pests.
- Biopesticides may include natural plant-derived products, which include alkaloids, terpenoids, phenolics and other secondary chemicals. Certain vegetable oils such as canola oilare known to have pesticidalproperties. Products based on extracts of plants such as garlic have now been registered in the EU and elsewhere

Applications

Biopesticides are biological or biologically-derived agents, that are usually applied in a manner similar to chemical pesticides, but achieve pest management in an environmentally friendly way. With all pest management products, but especially microbial agents, effective control requires appropriate formulationand application.

Biopesticides for use against crop diseases have already established themselves on a variety of crops. For example, biopesticides already play an important role in controlling downy mildew diseases. Their benefits include: a 0-Day Pre-Harvest Interval (see: maximum residue limit), the ability to use under moderate to severe disease pressure, and the ability to use as a tank mix or in a rotational program with other registered fungicides. Because some market studies estimate that as much as 20% of global fungicide sales are directed at downy mildew diseases, the integration of biofungicides into grape production has substantial benefits in terms of extending the useful life of other fungicides, especially those in the reduced-risk category.

A major growth area for biopesticides is in the area of seed treatments and soil amendments. Fungicidal and biofungicidal seed treatments are used to control soil borne fungal pathogens that cause seed rots, damping-off, root rot and seedling blights. They can also be used to control internal seed–borne fungal pathogens as well as fungal pathogens that are on the

surface of the seed. Many biofungicidal products also show capacities to stimulate plant host defence and other physiological processes that can make treated crops more resistant to a variety of biotic and abiotic stresses.

Disadvantages

- High specificity: which may require an exact identification of the pest/pathogen and the use of multiple products to be used; although this can also be an advantage in that the biopesticide is less likely to harm species other than the target
- Often slow speed of action (thus making them unsuitable if a pest outbreak is an immediate threat to a crop)
- Often variable efficacy due to the influences of various biotic and abiotic factors (since some biopesticides are living organisms, which bring about pest/pathogen control by multiplying within or nearby the target pest/pathogen)
- Living organisms evolve and increase their resistance to biological, chemical, physical or any other form of control. If the target population is not exterminated or rendered incapable of reproduction, the surviving population can acquire a tolerance of whatever pressures are brought to bear, resulting in an evolutionary arms race.
- Unintended consequences: Studies have found broad spectrum biopesticides have lethal and nonlethal risks for non-target native pollinators such as *Meliponaquadrifasciata* in Brazil.

Bioplastics

Bioplastics are plastics derived from renewable biomass sources, such as vegetable fats and oils, corn starch, straw, woodchips, food waste, etc. Bioplastic can be made from agricultural by-products and also from used plastic bottles and other containers using microorganisms. Common plastics, such as fossil-fuel plastics (also called petrobased polymers) are derived from petroleum or natural gas. Not all bioplastics are biodegradable nor biodegrade more readily than commodity fossil-fuel derived plastics. Bioplastics are usually derived from sugar derivatives, including starch, cellulose, and lactic acid. As of 2014, bioplastics represented approximately 0.2% of the global polymer market (300 million tons)

Applications

Flower wrapping made of PLA-blend bio-flex

Bioplastics are used for disposable items, such as packaging, crockery, cutlery, pots, bowls, and straws. Few commercial applications exist for bioplastics. In principle they could replace many applications for petroleum-derived plastics, however cost and performance remain problematic. As a matter of fact, their usage is favourable only if supported by specific regulations limiting the usage of conventional plastics Typical is the example of Italy, where biodegradable plastic bags and shoppers are compulsory since 2011 with the introduction of a specific law.[8] Beyond structural materials, electro active bioplastics are being developed that promise to be used to carry electric current.

Biopolymers are available as coatings for paper rather than the more common petrochemical coatings

Types

Starch-based plastics

Thermoplastic starch currently represents the most widely used bioplastic, constituting about 50 percent of the bioplastics market Simple starch bioplastic can be made at home. Pure starch is able to absorb humidity, and is thus a suitable material for the production of drug capsules by the pharmaceutical sector. Flexibiliser and plasticiser such as sorbitol and glycerine can also be added so the starch can also be processed thermo-plastically. The characteristics of the resulting bioplastic (also called "thermo- plastical starch") can be tailored to specific needs by adjusting the amounts of these additives.

Starch-based bioplastics are often blended with biodegradable polyesters to produce starch/polylactic acid, starch / polycaprolactone or starch/Ecoflex (polybutyleneadipate-co-terephthalate produced by BASF). blends. These blends are used for industrial applications and are also compostable. Other producers, such as Roquette, have developed other starch/polyolefin blends. These blends are not biodegradable, but have a lower carbon footprint than petroleum-based plastics used for the same applications.

Due to the origin of its raw material, starch is cheap, abundant, and renewable.

Starch based plastics are complex blends of starch with compostable plastics such as Polylactic acid, PolybutyleneAdipate Terephthalate, Polybutylene Succinate, Polycaprolactone, and Polyhydroxyalkanoates. These complex blends improve water resistance as well as processing and mechanical properties.

Starch-based films (mostly used for packaging purposes) are made mainly from starch blended with thermoplastic polyesters to form biodegradable and compostable products. These films are seen specifically in consumer goods packaging of magazine wrappings and bubble films. In food packaging, these films are seen as bakery or fruit and vegetable bags. Composting bags with this films are used in selective collecting of organic waste.

Further, a new starch-based film was developed by Agricultural Research Service scientists can even be used as a paper.

Cellulose-based plastics

A packaging blister made from cellulose acetate, a bioplastic

Cellulose bioplastics are mainly the cellulose esters, (including cellulose acetate and nitrocellulose) and their derivatives, including celluloid.

Cellulose can become thermoplastic when extensively modified. An example of this is cellulose acetate, which is expensive and therefore rarely used for packaging. However, cellulosic fibers added to starches can improve mechanical properties, permeability to gas, and water resistance due to being less hydrophilic than starch.

A group at Shanghai University was able to construct a novel green plastic based on cellulose through a method called hot pressing.

Protein-based plastics

Bioplastics can be made from proteins from different sources. For example, wheat gluten and casein show promising properties as a raw material for different biodegradable polymers.

Additionally, soy protein is being considered as another source of bioplastic. Soy proteins have been used in plastic production for over one hundred years. For example, body panels of an original Ford automobile were made of soy-based plastic.

There are difficulties with using soy protein-based plastics due to their water sensitivity and relatively high cost. Therefore, producing blends of soy protein with some already-available biodegradable polyesters improves the water sensitivity and cost.[24]

Some aliphatic polyesters

The aliphatic biopolyesters are mainly polyhydroxyalkanoates (PHAs) like the poly-3-hydroxybutyrate (PHB), polyhydroxyvalerate (PHV) and polyhydroxyhexanoate (PHH).

Polylactic acid (PLA)

Mulch film made of polylacticacid(PLA)-blend bio-flex

Polylactic acid (PLA) is a transparent plastic produced from corn[25] or dextrose. Superficially, it is similar to conventional petrochemical-based mass plastics like PS. It has the distinct advantage of degrading to nontoxic products. Unfortunately it exhibits inferior impact strength, thermal robustness, and barrier properties (blocking air transport across the membrane).[6] PLA and PLA blends generally come in the form of granulates with various properties, and are used in the plastic processing industry for the production of films, fibers, plastic containers, cups and bottles. PLA is also the most common type of plastic filament used for home fused deposition modeling.

Poly-3-hydroxybutyrate

The biopolymer poly-3-hydroxybutyrate (PHB) is a polyester produced by certain bacteria processing glucose, corn starchor wastewater. Its characteristics are similar to those of the petroplastic polypropylene. PHB production is increasing. The South Americansugar industry, for example, has decided to expand PHB production to an industrial scale. PHB is distinguished primarily by its physical characteristics. It can be processed into a transparent film with a melting point higher than 130 degrees Celsius, and is biodegradable without residue.

Polyhydroxyalkanoates

Polyhydroxyalkanoates are linear polyesters produced in nature
by bacterial fermentation of sugar or lipids. They are produced by the bacteria to store carbon and energy. In industrial production, the polyester is extracted and purified from the bacteria by

optimizing the conditions for the fermentation of sugar. More than 150 different monomers can be combined within this family to give materials with extremely different properties. PHA is more ductile and less elastic than other plastics, and it is also biodegradable. These plastics are being widely used in the medical industry.

Polyamide 11

PA 11 is a biopolymer derived from natural oil. It is also known under the tradenameRilsan B, commercialized by Arkema. PA 11 belongs to the technical polymers family and is not biodegradable. Its properties are similar to those of PA 12, although emissions of greenhouse gases and consumption of nonrenewable resources are reduced during its production. Its thermal resistance is also superior to that of PA 12. It is used in high-performance applications like automotive fuel lines, pneumatic airbrake tubing, electrical cable antitermite sheathing, flexible oil and gas pipes, control fluid umbilicals, sports shoes, electronic device components, and catheters.

A similar plastic is Polyamide 410 (PA 410), derived 70% from castor oil, under the trade name EcoPaXX, commercialized by DSM. PA 410 is a high-performance polyamide that combines the benefits of a high melting point (approx. 250 °C), low moisture absorption and excellent resistance to various chemical substances.

Bio-derived polyethylene

Main article: Renewable Polyethylene

The basic building block (monomer) of polyethylene is ethylene. Ethylene is chemically similar to, and can be derived from ethanol, which can be produced by fermentation of agricultural feedstocks such as sugar cane or corn. Bio-derived polyethylene is chemically and physically identical to traditional polyethylene – it does not biodegrade but can be recycled. The Brazilian chemicals group Braskem claims that using its method of producing polyethylene from sugar cane ethanol captures (removes from the environment) 2.15 tonnes of CO_2 per tonne of Green Polyethylene produced.

Genetically modified feedstocks

With GM corn being a common feedstock, it is unsurprising that some bioplastics are made from this.

Under the bioplastics manufacturing technologies there is the "plant factory" model, which uses genetically modified crops or genetically modified bacteria to optimise efficiency.

Polyhydroxyurethanes

Recently, there have been a large emphasis on producing biobased and isocyanate-free polyurethanes. One such example utilizes a spontaneous reaction between polyamines and cyclic carbonates to produce polyhydroxurethanes Unlike traditional cross-linked polyurethanes, cross-linked polyhydroxyurethanes have been shown to be capable of recycling and reprocessing through dynamic transcarbamoylation reactions.

Lipid derived polymers

A number bioplastic classes have been synthesized from plant and animal derived fats and oils. Polyurethanes, polyesters, epoxy resins and a number of other types of polymers have been developed with comparable properties to crude oil based materials. The recent development of olefin metathesis has opened a wide variety of feedstocks to economical conversion into biomonomers and polymers. With the growing production of traditional vegetable oils as well as low cost microalgae derived oils, there is huge potential for growth in this area.

Biodegradation of Bioplastics

Packaging air pillow made of PLA-blend bio-flex

Biodegradation of any plastic is a process that happens at solid/liquid interface whereby the enzymes in the liquid phase depolymerize the solid phase Bothbioplastics and conventional plastics containing additives are able to biodegrade. Bioplastics are able to biodegrade in different environments hence they are more acceptable than conventional plastics. Biodegradability of bioplastics occurs under various environmental conditions including soil, aquatic environments and compost. Both the structure and composition of biopolymer or bio-composite have an effect on the biodegradation process, hence changing the composition and structure might increase biodegradability. Soil and compost as environment conditions are more efficient in biodegradation due to their high microbial diversity. Composting not only biodegrades bioplastics efficiently but it also significantly reduces the emission of greenhouse gases. Biodegradability of bioplastics in compost environments can be upgraded by adding more soluble sugar and increasing temperature. Soil environments on the other hand have high diversity of microorganisms making it easier for biodegradation of bioplastics to occur. However, bioplastics in soil environments need higher temperatures and a longer time to biodegrade. Some bioplastics biodegrade more efficiently in water bodies and marine systems; however, this causes danger to marine ecosystems and freshwater. Hence it is accurate to conclude that biodegradation of bioplastics in water bodies which leads to the death of aquatic organisms and unhealthy water can be noted as one of the negative environmental impacts of bioplastics

Year	Bioplastic Discovery or Development
1862	Parkesine - Alexander Parkes
1868	Celluloid - John Wesley Hyatt

1897	Galalith - German chemists
1907	Bakelite - Leo Baekeland
1912	Cellophane - Jacques E. Brandenberger
1920s	PolylacticACid (PLA) - Wallace Carothers
1926	Polyhydroxybutyrate (PHB) - Maurice Lemoigne
1930s	Soy bean-based bioplastic car - Henry Ford
1983	Biopal - Marlborough Biopolymers
1989	PLA from corn - Dr. Patrick R. Gruber; Matter-bi - Novamount
1992	PHB can be produced by Arabidopsis thaliana (a small flowering plant)
1998	Bioflex film (blown, flat, injection molding) leads to many different applications of bioplastic
2001	PHB can be produced by elephant grass

2007	Mirel (100% biodegradable plastic) by Metabolic inc. is market tested
2012	Bioplastic is developed from seaweed
2013	Bioplastic made from blood and a cross-linking agent which is used in medical procedures
2014	Bioplastic made from vegetable waste
2016	Car bumper made from banana peel bioplastic
2017	Bioplastics made from lignocellulosic resources (dry plant matter)
2018	Bioplastic furniture, bio-nylon, packaging from frui

Biosensor

A biosensor is an analytical device, used for the detection of a chemical substance that combines a biological component with a physicochemical detector. The sensitive biological element, e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc., is a biologically derived material or biomimetic component that interacts, binds, or recognizes with the analyte under study. The biologically sensitive elements can also be created by biological engineering. The transducer or the detector element, which transforms one signal into another one, works in a physicochemical way: optical, piezoelectric, electrochemical, electrochemiluminescence etc., resulting from the interaction of the analyte with the biological

element, to easily measure and quantify. The biosensor reader device with the associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way. This sometimes accounts for the most expensive part of the sensor device, however it is possible to generate a user friendly display that includes transducer and sensitive element (holographic sensor). The readers are usually custom-designed and manufactured to suit the different working principles of biosensors.

Type of Microbial Biosensors (Transducers)

Optical Biosensor

An optical biosensor is a device that makes use of an optical transducer to produce changes in diverse optical properties such as adsorption, fluorescence, luminescence, or refractive index, which are proportional to the concentration of the analytes. Fluorescence, bioluminescence, and colorimeter based biosensors are widely investigated due to their properties of compactness, selectivity, sensitivity, flexibility, resistance to electrical nose and small probe size .

Fluorescent Microbial Biosensor

Fluorescent microbial biosensors are widely used in analysis processes, which can emit fluorescent light that is directly proportional to the analytes concentration at a low level . The basis of the fluorescent microbial biosensor is to fuse an inducible promoter to a reporter gene to encode a fluorescent protein which can emit detectable fluorescence in a genetically engineered microorganism . Due to the advantages of stability and sensitivity, green fluorescent protein is most commonly used in fabrication of fluorescent microbial biosensors. Recombinant Escherichia coli cells which are transformed with plasmids, harboring three tandem copies of the promoter/operator-the gene for gfp, were developed for the detection of arsenic. Compared to cells that used plasmids harboring only one copy, the recombinant Escherichia coli cells doubled the signal-to-noise ratio and decreased the detection limit form 20 to 7.5 μg/L. The recombinant yeast, Green Screen, has the ability to emit fluorescence by expressing green fluorescent proteins

when it is exposed to genotoxins. Based on this mechanism, a microfluidic chip which retained yeast within the chip was developed for the detection of toxic compounds.

Bioluminescent Microbial Biosensor

Bioluminescence based microbial biosensors have been extensively used in environmental monitoring for detection of toxicity due to its ability to closely reflect to toxicity. As a proportional response to the concentration of the analytes, the changes in the density of the bioluminescence emitted by the living cells can be measured by the bioluminescent microbial biosensor. According to the mechanism of production of bioluminescence, the method to control the expression of the lux gene can be divided into two manners: the constitutive manner and the inducible manner. In the constitutive manner, the bioluminescence caused by lux gene-coded luciferase exists constitutively as long as the organism is active. As the density of the bioluminescence can be affected by the additional compounds such as the toxicity, it can be used as a parameter to determine the additional compounds. In the inducible manner, the lux gene is fused with a promoter regulated by the concentration of the analytes. Based on this mechanism, the bioluminescence cannot be detected until the concentration of the analytes approaches a critical value. Several bioluminescent microbial biosensors have been developed in recent years. A whole-cell bioluminescent biosensor, based on genetically engineered Escherichia coli bacteria, carrying a promoter-reporter fusion, was developed for the detection of water toxicity. The constructed a biosensor for the detection of water pollutions, based on Pseudomonas putida TVAS, harboring chromosomal tod-lux CDABE fusion. By immobilizing bioluminescent bacteria, TV1061 strain, in wells of a microtiter plate, fabricated a microbial biosensor for air toxicity monitoring and achieved a good response to a low concentration of chloroform (6.65 ppb).

Colorimetric Microbial Biosensor

Colorimetric microbial biosensors make use of the changes in the color of the special compound to determine the concentration of the target analytes. Methyl parathion can be hydrolyzed by bacterium into chromophoric product, p-nitrophenol (PNP), which can be

measured by a colorimetric method. Based on this mechanism, colorimetric transducers have been widely used in developing microbial biosensors for the detection of methyl parathion. A colorimetric microbial biosensor based on the immobilization of Flavobacterium˚ sp. in glass fiber filter was constructed for the detection of methyl parathion with a detection limit of 0.3 μM and a linear range from 4 - 80 μM. The immobilized *Sphingomonas* bacteria onto the surface of the wells of polystyrene microplates (96 wells) to construct a colorimetric microbial biosensor, which had the same linear range to methyl parathion but achieved an advantage of multiple detections. By immobilizing the Sphingomonas bacteria on inner epidermis of onion bulb scale, a colorimetric microbial biosensor for detection of methyl parathion was developed and achieved a stable characteristic.

MFC Biosensor

The ability to convert organic substrates into electricity through microbial catabolism makes it possible for microbial fuel cells (MFCs) to work as a transducer in microbial biosensors. A typical two-chamber MFC consists of an anodic and a cathodic chamber which are separated by a proton exchange membrane. In the anodic chamber, fuel is oxidized by microbes, generating electrons and protons which are transferred to the cathodic chamber through the external electric circuit and the membrane separately. They combine with oxygen to form water in the cathodic chamber. MFCs have been widely used as biosensors, especially for measuring biochemical oxygen demand and water toxicity, because of its portability, long-term stability and fast response. However, MFC biosensors suffer from low sensitivity because the power generated from MFCs is very low. We have improved an array of microliter-sized MFCs, generating 100 μW and 1.8 V output voltage, which contributes to achieve a higher sensitivity. There are several new MFC biosensors.

The linear relationship between the current density generated by the MFCs and the BOD concentration makes MFCs work as BOD biosensors. Compared to the conventional methods for BOD analysis which take 5 or 7 days, the microbial BOD biosensors have fast response.

The novel MFC BOD sensor system operated by integrating an anaerobic bioreactor for continuous supply of stable anaerobic consortium, which had a

Analyte	Microorganism	Linearity range	Current density	Reference
Dissolved oxygen (DO)	SBMFC	Up to 8.8 ± 0.3 mg/L	5.6 ± 0.5 - 462.2 ± 0.5 mA/m²	[49]
BOD	Single-chamber MFC	Up to 350 mg/cm³		[50]
BOD	MFC	17 ± 0.5 mg/L to 78 ± 7.6 mg/L	282 ± 23 mA/m²	[47]
BOD	MFC	32 to 1280 mg/L		[52]
VFA	wall-jet MFC			[51]
Glucose	Single-chamber MFC	Up to 25 g/L		[48]

response time around 3 - 5 min without the need to wait for the metabolic recovery of anaerobic

MFC biosensors.

Consortium in the anodic compartment. Further, a simple method for monitoring the dissolved oxygen based on a submersible microbial fuel cell (SBMFC) assured the maximum response time of less than 4 minutes. A MFC-type of BOD biosensor is advantageous over other types of BOD biosensors because they have a high reproducibility, long-term stability and wide linear range. Lorenzo used a single-chamber MFC with an air cathode to fabricate a BOD sensor which had a linear range up to 350 ppm and was still stable after 7 months with a total variation of only 15 %.

The toxicity in the water caused by pesticides and the waste water from the industry has been a big risk to human health. Conventional methods for detection of toxic compounds have a lot of limitations such as time consumption and high cost. A silicon-based MFC utilized as a toxicity biosensor was validated to minimize the time and the cost.

VFA, particularly acetate and propionate, as the important inter mediator of anaerobic digestion (AD), can be used as a process indicator. Liu et al. developed a wall-jet MFC biosensor to reflect the real time microbial activity by the detection of acetate

Applications Environmental Monitoring

Pollutants in the environment are great risks for the health of human beings. Several microbial biosensors for detection of organic and inorganic toxicities are shown in. Being extensively used in industry, heavy metal becomes a main toxicant in waste water. The non-biodegradability of metal ions results in its accumulation in living organisms and causes various diseases. A low cost, specific, simple and quick tool is needed for monitoring heavy metals. The microbial biosensor provides an opportunity to solve this problem. The constitutive manner

(light-off) and the inducible manner (light-on) are two general strategies for developing a microbial biosensor for monitoring heavy metal toxicity. In the constitutive manner, the lux gene exists constitutively. The presence of the toxic heavy metal affects the expression of the lux gene and reduces the light density. As it can respond to any substance that is toxic to the microbe, this microbial biosensor is nonspecific. Specific biosensors, which are based on inducible promoters fused to reporter genes, are more sophisticated and sensitive. Only the specific biosensor can be used for in situ measurement of contaminants. Heavy metal ions can act as an acute enzyme inhibitor and then cause some changes that can be used as the signal for detecting heavy metal ions. As mercury can inhibit the activity of alkaline phosphate enzymes present in the cell wall of Chlorella sp., Singh et al. developed a biosensor for determination of mercury by immobilizing Chlorella sp. on a glassy carbon surface. The use of genetically engineered bacteria, which can produce measurable signals when contacted with bio-components, is the best approach for detecting heavy metal [57].

Food and Fermentation

As the quality of the products is required by both the customers and the government, rapid and affordable methods to assure the quality of products and process controls are needed. The recent developed microbial biosensors used in food and fermentation. Fermentation is widely used for the production of foodstuffs and drinks, which requires a carefully performed fermentation system operation . Microbial biosensors are used to monitor the materials in order to control the fermentation process. Because ethanol is very important and necessary in different fermentation process, microbial biosensors have been used for sensitive determination of ethanol in order to monitor the fermentation process. An amperometric biosensor based on Candida tropicalis cells immobilized in gelatin by using glutaraldehyde was developed for the determination of ethanol in the range from 0.5 mM to 7.5 mM. The constructed a new microbial amperometric biosensor for the measurement of ethanol in flow injection analysis, which achieved a linear response to ethanol in the range from 10 μM to 1.5 mM in 3 minutes. Furthermore, based on the immobilization of Methylo bacterium organophilium on eggshell

membrane and an oxygen electrode, an ethanol biosensor got a linear range of 0.050 - 7.5 mmol/L and a detection limit of 0.035 mmol/L to ethanol.

The control of food quality and freshness is of growing interest for both the consumer and the food industry. The demand for quick and specific analytical tools is needed for monitoring nutritional parameters and food contaminants. Microbial biosensors work as a rapid and affordable method to assure the quality of products. As an index in the determination of the quality of coffee, caffeine needs to be detected sensitively and rapidly

Clinical Diagnostics

Conventional techniques for the diagnosis of various diseases suffer from slow response time, time consumption, and complicated process, which make critical care during emergencies difficult. Compared to enzyme based biosensors, microbial biosensors require no purification which is time consuming and expensive. Microbial biosensors provides a rapid, accurate and inexpensive way for diagnosis of hormones, pathogens and DNA, which are important parameters of a living individual. The fabricated a novel microbial biosensor for the determination of epinephrine by immobilizing white rot fungi (Phanerochaetechrysosporium ME446) in gelatin using glutaraldehyde cross linking agent on a Ptelectrode, which achieved a linear range of 5 - 100 μM and a detection limit of 1.04 μM. In this biosensor, epinephrine was turned into epinephrine quinone through a redox activity catalyzed by lactase in the fungal cells, causing an increase in the current. As a cause of virus diseases, the detection of pathogens plays an important role in clinical diagnostics. Rat basophilic leukemia (RBL) mast cells which could produce a dramatic exocytotic response within minutes of antigen addition were used to fabricate a microbial biosensor for the detection of pathogens. DNA damage which can affect DNA replication, repair and gene expression can lead to many diseases including cancer. An E. coli SOS-EGFP based on SOS response was constructed for detection of DNA damage. The SOS response could be triggered by harmful chemicals for DNA to produce fluorescent protein controlled by recAgene promoter.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB
COURSE CODE: 17MBU514A

COURSE NAME: MICROBIAL BIOTECHNOLOGY
BATCH-2017-2020

UNIT-2						
S.NO	**QUESTION**	**A**	**B**	**C**	**D**	**ANSWER**
1	_____are the enzymes are used to cut the target DNA fragment	Ligase	Restriction endonicleases	Methylases	Exonucleases	Restriction endonicleases
2	_____ are also known as DNA ligases	Klenow fragment	Molecular sutures	Molecular scissors	Holo enzymes	Molecular sutures
3	_____ enzymes mediates nick translation	DNA polymerase 1	DNA polymerase 2	DNA polymerase 3	RNA polymerase	DNA polymerase 1
4	Enzymes used to remove unanneated regions of RNA from DNA: RNA hybrids	exonuclease 3	endonuclease 2	endonuclease	ribonuclease T1	ribonuclease T1
5	RNA is tightly associated with	lipids	amino acid	protein	carbohydrate	protein
6	Precipitation of RNA can be taken place by	lipids	ethanol	alcohol	methane	ethanol
7	Commonly used reagent in RNA	EDTA	NAOH	SDS	Guana diniumthiocyanate	Guana diniumthiocyanate
8	Celluloid was discovered by	Heory ford	Leo	John Wesleyhyatt	Alexander	John Wesleyhyatt
9	Bakelite was introduced in year	1907	1928	1917	1937	1907
10	Bioplastic made from _____ waste was founded in 2014	vegetable	meat	fruits	flowers	vegetable
11	Expansion of PLA	Polylactic acid	Polyacetic acid	Polyactic acid	Polyanacline acid	Polyactic acid
12	Soya bean- based bioplastic car was introduced by	R.gruber	Henry ford	Patrick	Wallace	Henry ford
13	Galalith is a type of_____	Biopolymer	Bioplastic	Biosensor	All the above	Bioplastic

Prepared by R.Dineshkumar, Assistant Professor, Dept of Microbiology, KAHE.

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14	Bioplastic is developed from scawed in	2010	2018	2014	2012	2012
15	Enzymes activates a fibrinolysis enzymes in human serum	*Staphylococcal fibrinolysis*	*Streptococcal fibrinolysis*	*Fibrinolysis*	*All the above*	*Streptococcal fibrinolysis*
16	Most frequently employed for manufacture of streptokinase is	*Haemolytic streptococci*	*G. steptodornase*	*Streptococcus pyogrnes*	All the above	*Haemolytic streptococci*
17	Vaccines are manufactured by using _____ technology	molecular biology	genetic engineering	r DNA Technology	none of the above	genetic engineering
18	The enzymes DNA ligase is used is for_____	Restriction	Ligation	Both	None	Ligation
19	The neutral microbial polymerase	Dextron	Xanthan	Gellan	Polyelectrolytes	Dextron
20	The acidic microbial polysaccharides	gellan and Xanthan	Dextron	Gellan alone	Scleroglucan	Gellan and Xanthan
21	Expansion of MESR	Microbial endooraganic recovery	Microbial ethnic organism recovery	Microbial exooraganic recovery	Microbial Enhanced oil recovery	Microbial enhanced oil recovery
22	First polysaccharides that commercially available	Alginate	Gellan	Xanthan	Polluan	Xanthan
23	Xanthan is commercially produced by	Gram positive only	Gram negative only	Both	None	Gram negative only
24	_____ is used in oil industry for enhanced recovery	Gellan and xanthan	Curdlan	Emulsan	Dextran	Emulsan
25	Which one is blood plasma expander	Dextron	Emulsan	Alginate	Xanthan	Dextron
26	The molecular weight of dextron range form	50,000-5,00,000	15,000-50,000	50,000-55,000	15,000-5,00,000	15,000-5,00,000
27	Dexton can be produced by a wide range of	Gram negative	Gram positive	None	Both	both

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28	_____ is a linear polymer composed of mannural acid &glucuronic acid	Dextron	Aliginate	Gellan	Emulsan	Aliginate
29	Scleroglucan is used in all expect_____	Paint	Oil	Printing	Drilling muds	Oil
30	Gellan is produced by _____	*Pseudomonas aeruginosa*	*Pseudomonas clodea*	*Bacillus cereus*	*Bacillus anthrax*	*Pseudomonas clodea*
31	Pollu is produced by using _____	Fungi	Bacteria	Algae	Parasite	Fungi
32	Curdian is a _____ polymer	Alpha-glucose	Beta-glucose	Gamma-glucose	Sigma-glucose	Beta-glucose
33	_____ was distributed in the U.Sbymonsanic& later metabolix	Partcesine	Galalith	Biopol	Bioplastic	Biopol
34	Expansion of PIP	plant incorporation protein	plant incorporation protectants	plant incorporation produces	plant incorporation protection	plant incorporation protectants
35	Expansion of ISR	None of the above	Induce synthetic response	Induce systemic resistance	Induce systemic response	Induce systemic resistance
36	Bioplastic are plastics derived from all expection	Negative	Food waste	Straw	Plastics	Plastics
37	Types of biosensor	3 types	4 types	2 types	5 types	5 types
38	Microbial biosensor also called as	Trans detector	Transducers	Both	None	Transducers
39	The type 1 restriction enzymes need	Methyl gas	Sulfur gas	Fe+	S	Methyl gas
40	The cutting of DNA takes place with the enzymes sites	Identification site	Cleavage	Restriction site	Clear site	Restriction site

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41	Site specific clearage is carried out by	Type3	Type 1	Type2	Type 4	Type 2
42	_____ are the enzymes are used to cut the target DNA fragment	Ligases	Restriction endonucleases	Exo nucleases	methylases	Restriction endonucleases
43	The presence of restriction enzymes postulated by	Werner	Watson	Smith	Nathan	Nathan
44	Two restriction enzymes which have the same recognition sequence but leave at different sites	Epimers	isomers	Neoschizomers	Isoschizomers	Neoschizomers
45	The recognition / clearage sites of types2 enzymes have	Adjacent sizes	Same site	Modification site	Different sites	Same site
46	pH of stacking gel is	6.8	4.8	7.8	5.8	6.8
47	Agarose is made up of	Lactose	Glucose	Agarobiose	Polyacrylamide	Agarobiose
48	First, scientists synthesized genes for the two insulin	B and C chains	A and B chains	A and C chains	None	A and B chains
49	Manufacturing process of streptokinase	Both	Fermentation	Pausteurization	None	Fermentation
50	Hepatitis is an _____ infection	Bacteria	Viral	Fungal	None	Viral
51	Treatment of hepatitis B viruses	Antibacterial	Hepatitis B vaccination	Antiviral	Antifungal	Hepatitis B vaccination
52	Administration of doses	5 Doses	2 Doses	3 Doses	7 Doses	3 Doses
53	Microbial production of polysaccharides is carried out mostly by	All the above	Stock culture	Mother culture	Batch culture	Batch culture

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54	Commonly used reagent in RNA	EDTA	NAOH	SDS	Guanadiniumthiocyanate	Guanadiniumthiocyanate
55	Streptokinase is a kind of	Protein	Carbohydrate	Lipid	Enzymes	Enzymes
56	Streptokinase is used in the treatment of	Myocardial disorder	liver disorder	bone disorder	kidney disorder	Myocardial disorder
57	Which bacteria produces streptokinase	*Vibrio*	*Streptococci*	*Staphylococci*	*E.coli*	*Streptococci*
58	How much L of human serum sample was required to produce single drop of Hepatitis B vaccine	30	40	50	60	40
59	The hepatitis B virus genome consists of?	largely double-stranded DNA	)largely single-stranded DNA	smally double-stranded DNA	Smally single-stranded DNA	largely double-stranded DNA

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). 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 bioconversions of steroids and sterols have been reported ever since, focusing mainly on steroid hydroxylations, 1-dehydrogenation and sterol side-chain cleavage. These biotransformation, 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 Solanumturvum, 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 dehydro epiandro sterone sulfate (DHEAS), progesterone, pregnenolone and their sulfate derivatives.

Steroid transformations by microbial systems A great deal of work has been carfied QUI on 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 anti-inflammatory, 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-hydroxylation 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 stigma sterol (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. It is so designed that microbial transformation is conducted 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 anti-inflammatory commercial product. Industrial applications The 11c-. 11 b- and 16a-hydroxylalions. 6'-tlehydrogenation and side chain 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 Cunninghamellablakesleena and Curvularialunata 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-ketoSteroids 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 pregame side chain has replaced partially the use of dysgenic and solasodine as base materials. A partial side-chain degradation of sterols to 2Ocarboxylic 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 commercially as 16-dehydropregnenolone

BIOCATALYSIS PROCESS TECHNOLOGY

A key technology for the next generation of processes will be biocatalysts (the use of one or more enzymes or cells containing one or more enzymes for the production of chemicals, pharmaceuticals and fuels). Biocataltyic processes offer the advantage of un-paralleled selectivity under mild reaction conditions. Nevertheless when using biocatalysts on non-natural reactants rates are often low and conditions frequently sub-optimal. This has led to the development of improved enzymes via recombinant DNA technology.

At PROCESS we work with both academic and industrial partners who supply recombinant enzymes and cells for us to use and test in entirely new processes. In order to complement this we focus on the novel process concepts which are also required for the final effective implementation of such processes (especially where the reaction thermodynamics is unfavorable). The work is mostly experimental, based both in the laboratory (in miniaturized and scaled-down equipment) as well as the pilot plant.

In addition some research is focused on methodology to implement and develop biocatalytic processes in the most effective way including economic and environmental evaluations, which are essential to ensure the sustainability of such new processes. The sub-group currently works on several classes enzymes such as transaminases (for the synthesis of (chiral) amines), lipases (for the synthesis of biodiesel), oxidases and oxygenases (for the selective introduction of oxygen into molecules). Process concepts include multi-enzymatic processes, substrate feeding, *in-situ* product removal and multi-phase processes. The work is funded by several EC projects, including BIOINTENSE which we launched in 2012.

An excellent example of the type of research carried out in the biocatalysts sub-group is that of the work done with transaminases, where enormous progress has been made in the last year. Transaminases are one of the most effective ways of making optically pure chiral amines. In particular because the conditions used mean that protection and subsequent de-protection of functional groups is not required. Optically pure chiral amines are of great importance in the pharmaceutical industry. Indeed interest is such that in 2013 we will have helped organize the

first international conference on the application of transaminases together with KTH (Stockholm, Sweden), with attendance of over 100 from Europe, USA and Japan.

Particularly interesting in 2012 has been the completion of the first stage of work examining the thermodynamics of such reactions, which are frequently unfavorable in the synthetic direction (creating the chiral center). Shifting the equilibrium is a basic requirement for implementation and we have now learnt that a combination of approaches is required, such as use of an excess amine donor alongside selective and effective *in-situ* co-product removal (IScPR). In this case effective co-product removal means stripping of a volatile by-product (such as acetone) or selective enzymatic removal of a by-product (such as pyruvate). The enzymatic method in combination with an excess donor holds great promise. Alongside this much progress has been made on a methodology to help select the donor molecule, reactor and control technology for substrate and (co)product feeding and removal, respectively, based on modeling and fundamental data. The results have been published in a series of articles and at several international conferences in 2012 and more exciting results in this area can be expected within 2013.

High fructose corn 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 Aspergillus, 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. 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. 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, better solubility 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. 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. Analysis of food consumption patterns using USDA (2008) food consumption tables for the US from 1967 to 2000 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.

Cocoa Butter

Cocoa butter (cacao fat) naturally occurs in the cocoa bean at about 50% of the cocoa nib (EU). Cocoa butter is resistant to oxidation due to high levels of natural tocopherols and fatty acid composition.

Typical Fatty acid composition (%)

Stearic	(C18:0)	34.5%
Oleic	(C18:1)	34.5%
Palmitic	(C16:0)	26.0%
Linoleic	(C18:2)	3.2%
Arachidic	(C20:0)	1.0%
Palmitoleic	(C16:1)	0.3%

Other Fatty Acids 0.5%

When tempered properly to achieve the correct crystal formulation, cocoa butter creates a finished chocolate with a high gloss and texture snap. Additionally, cocoa butter contracts when solidified, which enables efficient molding production.

Cocoa Butter Alternatives:

Often the ingredients used for cocoa butter replacement undergo additional processing steps which raise the price. But with demand for cocoa butter on the rise and difficulty achieving high crop yields, the price of these alternatives will be more in line with productivity.

Cocoa Butter Substitute (EU) (CBS)

- Palm oil or coconut oil based and normally contains lauric fatty acids.
- Does not require tempering.
- Lauric fat in the presence of enzymes like lipase (found in cocoa beans), under the right conditions (moisture, temperature), can react and produce a soapy off-note.
- Not compatible with cocoa butter, although can be mixed in at a low percentage.

Cocoa butter Replacer (EU) (CBR)

- Non-lauric containing fats like palm oil, soybean oil, rapeseed oil and cottonseed oil.
- Does not require tempering.
- Partially compatible with cocoa butter.

Cocoa Butter Equivalent (EU) (CBE).

- Can contain shea, illipe, and sal nut oils as well as palm, mango kernel fat and palm oil.
- Have physical properties and a fatty acid profile similar to cocoa butter.
- Requires tempering.
- Compatible with cocoa butter.

Beyond availability and potential cost reduction, cocoa butter alternatives have some advantages when used in manufacturing.

First, cocoa butter alternatives improve the finished product's fat composition profile and fat stability. This enables consistent products, reduces fat migration in multi-layer products, and counters softness resulting from nut or milk ingredients.

Second, because many cocoa butter alternatives do not require tempering, it's much easier to achieve essential texture characteristics like gloss and snap. The chances of fat bloom over shelf life are also reduced.

Other Alternatives

Coconut Oil

Like cocoa butter, coconut oil is solid at room temperature. It's also widely available and relatively inexpensive -- and, also like cocoa butter, has a pleasing scent. Coconut oil has been used for skin and hair care for centuries and has excellent emollient properties. It's also packed with a hefty dose of vitamin E, an antioxidant that helps with skin repair.

Shea Butter

Shea butter is derived from the kernel of the karite tree, which is native to Africa. Solid at room temperature, it is renowned as an excellent skin moisturizer and is rich in protective antioxidants as well, including vitamins A and E. Shea butter has a higher melting point than coconut oil or cocoa butter and tends to be a little stiffer; it has a pleasant nutty scent. Look for unrefined shea butter, which is less processed than other forms

Consider Naming Requirements & Melt Profile

- In the US, the standard of identity (21 CFR 163) requires exclusive use of cocoa butter as the fat source for a product to be called chocolate. Any addition of a cocoa butter alternative will require you to change the name of your product from chocolate. However, some products have transitioned into these alternate naming requirements by using the history of an established brand name.
- The European Union has a little more flexibility outlined in the European Chocolate Directive 2000/36/EC, allowing up to 5% replacement of cocoa butter, while still maintaining chocolate as the product name.
- Cocoa butter is unique in that it quickly melts at 34° C (93° F), which is just under body temperature. This melting point gives a smooth, creamy texture in the mouth and increases flavor release. When using alternatives for cocoa butter, the fats that melt closer to body temperature will give a similar experience. Those with a higher melting point

will give the chocolate waxy mouths feel. With an alternative melt profile, flavor release will be perceived differently.

Phage

A **bacteriophage**, also known informally as a ***phage*** , is a virus that infects and replicates within bacteria and archaea. The term was derived from "bacteria" and the Greek (*phagein*), "to devour". Bacteriophages are composed of proteins that encapsulate a DNA or RNA genome, and may have structures that are either simple or elaborate. Their genomes may encode as few as four genes and as many as hundreds of genes. Phages replicate within the bacterium following the injection of their genome into its cytoplasm.

Bacteriophages are among the most common and diverse entities in the biosphere. Bacteriophages are ubiquitous viruses, found wherever bacteria exist. It is estimated there are more than 10. bacteriophages on the planet, more than every other organism on Earth, including bacteria, combined. One of the densest natural sources for phages and other viruses is seawater, where up to $9x10^8$ virions per millilitre have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages.

Phages have been used for more than 90 years as an alternative to antibiotics in the former Soviet Union and Central Europe, as well as in France. They are seen as a possible therapy against multi-drug-resistant strains of many bacteria (see phage therapy). Phages of *Inoviridae* have been shown to complicate biofilms involved in pneumonia and cystic fibrosis and to shelter the bacteria from drugs meant to eradicate disease, thus promoting persistent infection

Phage typing

Phage typing is a method used for detecting single strains of bacteria. It is used to trace the source of outbreaks of infections. Phages are viruses that attack bacteria. When this happens the phage (bacterial virus) will copy itself within the bacteria and either kill the bacteria or stay in the bacteria in a dormant form. When this happens the phage will also be copied when the

bacteria divides. Bacteria may thus have several phages sitting in them. Such phages will affect the ability of other phages to infect the bacteria. In other words, the ability to be infected by different phages varies between different strains of bacteria, even if they are fairly closely related. This fact forms the basis of phage typing. The bacterial strains are grown and then subjected to attack by a series of different known phages. Some phages will kill the bacteria (this is clearly visible and therefore measurable) and others won't be able to kill a given bacteria. Depending on which groups of phages can kill or not kill a bacterial strain (the reaction pattern) the bacteria is given a number, the phage type. Phage typing is most often used for the two common salmonella types, *S. Enteritidis* and *S. Typhimurium*. But phage typing systems also exist for some other salmonella serotypes and a few other bacteria.

There are several such phage typing systems around. Probably the most widely used systems are those originally developed in England, by which *S. Enteritidis* isolates are called pt and then a number (e.g. *S. Enteritidis* pt4) and *S. Typhimurium* isolates often DT (Definitive Type) or U followed by a number (e.g. *S. Typhimurium* DT104). Phage typing is not a DNA method (and is therefore sometimes said to be phenotypic rather than genotypic) and has been around for several decades. The phage type designations are therefore often well known and much used in the microbiological community. The method itself requires that the different phages are available and it's therefore a method that can generally only be performed at reference laboratories. Also it requires substantial technical expertise to perform.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB
COURSE CODE: 17MBU514A

COURSE NAME: MICROBIAL BIOTECHNOLOGY
BATCH-2017-2020

UNIT-3						
S. No	**QUESTION**	**A**	**B**	**C**	**D**	**ANSWER**
1	Steroids are structurally derived from_____	Cyclopentanoperhydrophenantrene	Dehydroepiandrosterone	Cunning haemellablankeslеena	Hydrocortisona	Cyclopentanoperhydrophenantrene
2	The steps taken to kill insects in plant root is - _____	To inject BT toxin into roots	To introduction BT gene into cells of root	Spray the BT toxin	Introduce BT toxin gene into bacterial species of rhizosphere	Introduce BT toxin gene into bacterial species of rhizosphere
3	Methods for biological protection of plants	Transgenic plant	Chemical insecticides	trimming of plants	avoid plant damages	transgenic plant
4	The transgenic plants transformed with highly modified synthesticprotoxin gene had _____ level of expression than wild type	10 fold	100 fold	1000 fold	10000 fold	100 fold
5	The different biological manipulation that would cause a crop plant to be herbicide resistant are _____	Over production of herbicide sensitive target protein	Improve the ability of herbicides	Resistant protein to bind to herbicide	Metabolic activation of herbicide	over production of herbicide sensitive target protein
6	The EPSPS plays important role in the synthesis of _____- amino acids in both bacteria& plant	Aromatic	Aliphatic	Both a &b	None	Aromatic

7	Delay of fruit ripening can be alone by_____	Antisense RNA technology	Biofarming	Refrigeration	Incubation	Antisense RNA technology
8	The plant growth promoter induces the experience of a number of genes involved in fruit riponing&senescence	Auxin	Cytokinin	Gibberellins	Ethylene	Ethylene
9	EPSPS	5-Enolpypyruvylshikimate-3-phosphate synthase	3-enolpyruvylshikimate-5-phosphate synthase	5-endophosphate-2-shyruvin phoshato synthase	none of the above	5-Enolpypyruvylshikimate-3-phosphate synthase
10	Expansion of DHEA	Dyclopentanoperhydrophenantrene	Dytrocortisone	dehydroepiandrosterone	Dehydroendoandrosterone	Dehydroepiandrosterone
11	The important of microbial transformations was realized for the first time in _____ patented the process of I/U-hydrexylation of progesterone by a rhizopus	1968, murray&peterson ,upjohn company	Both a and c	1952, murray&peterson upjohn company	None	1952, murray&peterson upjohn company
12	Removal of the side chain of cholesterol by a chemical method was reported in _____	1967	1974	1988	1975	1975
13	The work is funded by se4veraln EC projects including _____ which we launched in 2012	Biointerface	Biointense	Biointegrat	None	BOIINTENSE

14	EXP ansion of HFCS	High fuel control system	High fructose corn solution	High fructose corn syrup	High functions syrup	High fructose corn syrup
15	The term was derived from _____ and in greek to devour	bacteria	mold	fungi	yeast	bacteria
16	Phage typing is a method used for detection _____ strains of bacteria	Single strain	Three strains	Double strain	Four strain	Single strain
17	Phage typing is most often used for the two common salmonella types	*S.aureus and s. typhimurium*	*Salmonella sps*	*S. enteritidis*	*S. enteritidis& S.typhimurium*	*S. enteritidis & S.typhimurium*
18	Recently a steriodal glycoside, torvoside 2,isolated from the _____ of solanumturvum	Vegetables	Fruits	Microorganism	All the above	Fruits
19	Biotechnology application dealing with steroids may be classified broadly into_____ categories	4	5	3	2	2
20	Fatty acid composition of palmitic in cocoa butter	34.50%	26.00%	36.00%	26.50%	26.00%
21	_____ of palmitolic is present in cocoa butter	0.60%	2.00%	0.30%	1.80%	0.30%
22	_____ of 3.2% present in cocoa butter	Palmitic	Linoleleic	Palmitoleic	Stearic	Linoleleic
23	Cocoa butter consist of _____	Palmitic	Linoleleic	Palmitoleic	All the above	All the above

24	Expansion of CBS	Cocoa butter solvent	Cocoa butter solution	Cocoa butter substitute	None of the above	Cocoa butter substitute
25	In cocoa butter arachidic composed of _____	0.10%	2.00%	1.00%	0.20%	1.00%
26	Cocoa butter naturally occurs in the cocoa bean at about _____%	50%	10%	55%	60%	50%
27	Ratio of stearic acid in cocoa butter	(C18:0)	(C16:0)	(C18:1)	None	(c18:0)
28	Oleic acid composed of _____% in cocoa butter	30.6	34.6	34.5	30.5	34.5
29	Palm oil or coconut oil based &normally contains_____	Lauric fatty acid	Oleic fatty acid	Palmitoleic fatty acid	All the above	Lauric fatty acid
30	Coconut oil is	Solid	Semisolid	Liquid	None	Solid
31	Cocoa butter is unique in that it quickly melts at _____ degree	33degree	32 degree	35 degree	34 degree	34 degree
32	Expanison of CCD	Colony collapse disorder	Colony counter disorder	Colony count display	None of the above	Colony collapse disorder
33	Bacteriophage composed of_____	Amino acids	Proteins	Lipids	All the above	Proteins
34	HFCS has increased sinse its introduction as a_____	Flavour	Spickle	Sweetener	None	Sweetener
35	Coconut oil is packed with a hefty dose of _____	Vitamin A	Vitamin B	Vitamin C	Vitamin E	Vitamin E
36	Coconut oil been used for_____ and_____	Skin & hair care	Hair care	Skin	None	Skin &hair care

37	Cocoa butter coconut oil is solid at _____temperature	50-60	15-20	25-30	95	25-30
38	The third & relatively expensive enzymes used in the process is_____	Fructose isomerase	Glucose isomerase	A &b	None	Glucose isomerase
39	_____ may also prove to be useful commercial sources for sterols	Yeast	Hyphae	Mold	Yeast & Mold	Yeast & Mold
40	The production of _____&_____is one of the best example of the successful application of microbial technology in large scale industrial processes	Steroids drugs& hormones	Drugs& hormones	Cortisol& progesterone	All the above	Steroids drugs& hormones
41	The production of steroid drugs &hormones to the values of _____ million involves biotechnological production	$400 million	$ 100 million	$300 million	$500 million	$ 400 million
42	Lauric fat in the prances of enzymes like_____	Nuclease	Protease	Lipase	None	Lipase
43	In first , cocoa butter alternatives improve the	Gloss &snap	Fat composition profiles& fat	Fatty acid profile& fat	None	Fat composition profiles& fat stability

	finished products _____&_____ _____		stability	properties		
44	The european union has a little more flexibility outlined in the euyropeancccholate directive_____	2000/36/EC	2007/24/EC	2000/35/EC	2000/34/EC	2000/36/EC
45	An industrial produced from _____	*Bacillus* species	*Pseudomonas* species	*Strephylococcus* species	*Streptococcus* species	*Bacillus* species
46	A partial side-chain degradation of sterds to _____ structures also has been developed and is in commercial use	20 Carboxylic acid	60 Carboxylic acid	10 Carboxylic acid	25 Carboxylic acid	20 carboxylic acid
47	Parasporal crystals sensitive to _____-	Sunlight	Chemicals	Acid pH	Lipase	Sunlight
48	Parasporal crystals are lived in the environment	Long	Short	Moderately	Limited	Short
49	In second, because many cocoa butter alternatives do not require tempering it's easier to achieve essential texture characteristics like	Gloss& snap	Physical properties & fatty acid	Nut or milk	None	gloss& snap
50	Proteases for the commercial conversation of sterols to 17 kerosterotds by mutants of	*Streptococcus* & *Staphylococcus*	*Mycobacterium* and *Corynbacteria*	*Rhizopus* & *Aspergillus*	None	*Mycobacterium* and *Corynbacteria*

	_____&_____ _____ have been developed & are in practical use					
51	Expand ISCPR	In-situ co-produce removal	Insect co-produce removal	In -shifting co-product remain	All the above	In-situ co-produce removal
52	The manufacture steroid compound have a coids range of therapeutic purpose, namely as_____	Anti inflammatory	Contraceptive	Immunosuppressive	All the above	All the above
53	Exhibited antiviral activity on _____ types	Herpes simplex virus	HIV	AIDS	Anti fungal agent	Herpes simplex virus
54	A variety of steroids are widely used as_____	Inflammatory diuretic	Aninflammatory, diuretic	Aninflammatory	None	aninflammatory, diuretic
55	as such the _____ microbial process immediately was considered for industrial exploitation	Multi-step high-yielding	Double-step short-yielding	Single-step high-yielding	None	Single-step high-yielding
56	The synthetic protocol of a_____	Drugs	Steroid drugs/ hormones	Hormones	None	Steroid drugs/ hormones
57	Selective cleavage of the acetyl side chain of pregnanes by microorganisms to the _____ steroids	C-19	C-10	C-15	C-16	C-19
58	The chemical methods for preparation of progesterone	Diosgenin &stigmasterol	Mexicanyam &soyabeans	A &b	none	A &b

	from widely available plant steroids example					
59	Prednisolonea highly selective _____ commercial product	Aninflammatory	Inflammatory	Anti-inflammatory	Anti-aninflammatory	Anti-inflammatory
60	_____ of 3.2% present in cocoa butter	Palmitic	Linoleleic	Palmitolelic	Stearic	Linoleleic

MICROBIAL PRODUCT PURIFICATION

Downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste

The five stages of downstream processing are:

(1) Solid-Liquid Separation

(2) Release of Intracellular Products

(3) Concentration

(4) Purification by Chromatography and

(5) Formulation.

the major steps in downstream processing

This article throws light upon the five stages in downstream processing. The five stages are:

(1) Solid-Liquid Separation

(2) Release of Intracellular Products

(3) Concentration

(4) Purification by Chromatography and

(5) Formulation**.**

AN OUTLINE OF THE MAJOR STEPS IN DOWNSTREAM PROCESSING IS GIVEN.

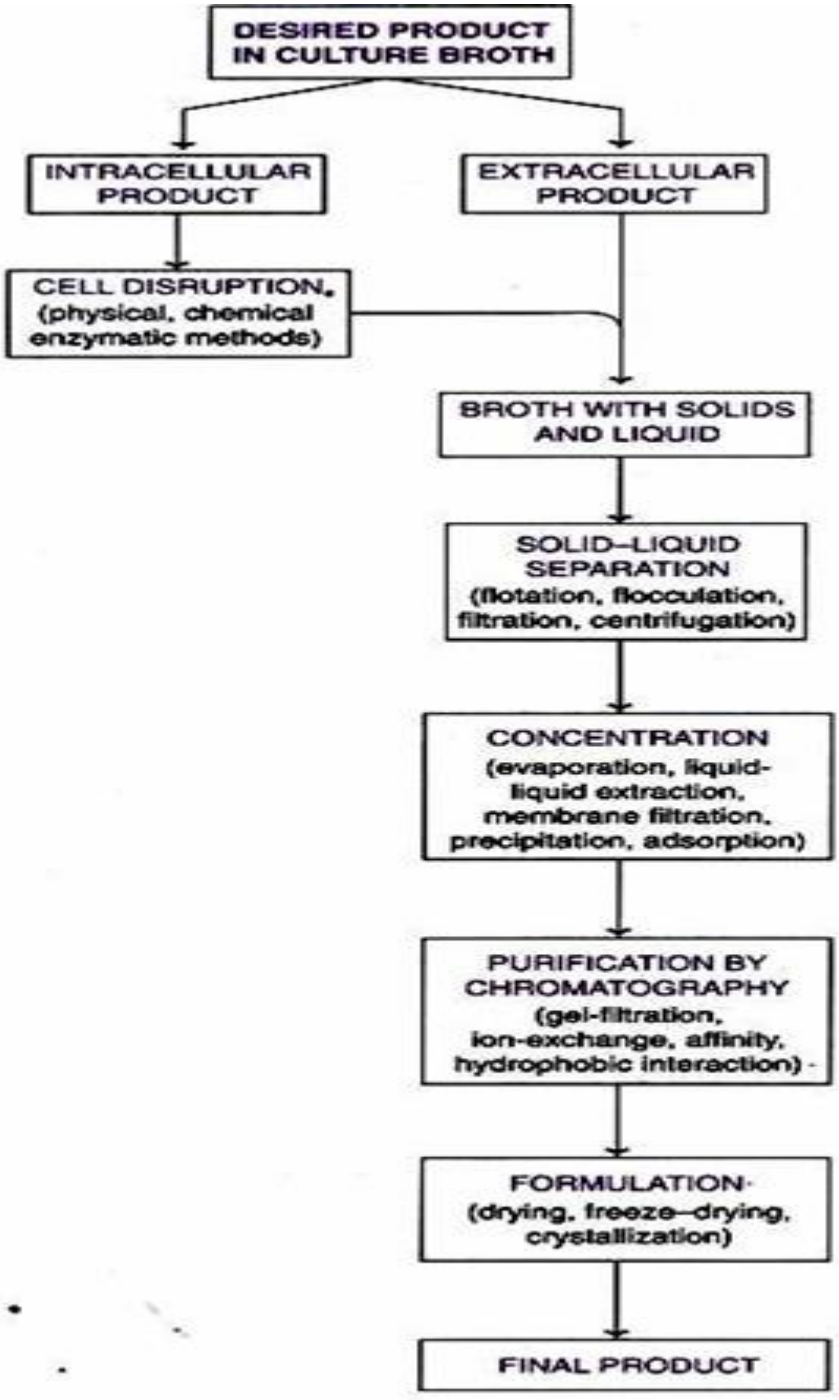

Fig. 20.1 : A summary of the major steps in downstream processing.

Stage # 1. Solid-Liquid Separation:

The first step in product recovery is the separation of whole cells (cell biomass) and other insoluble ingredients from the culture broth (Note: If the desired product is an intracellular metabolite, it must be released from the cells before subjecting to solid-liquid separation). Some authors use the term harvesting of microbial cells for the separation of cells from the culture medium. Several methods are in use for solid-liquid separation. These include flotation, flocculation, filtration and centrifugation.

Flotation:

When a gas is introduced into the liquid broth, it forms bubbles. The cells and other solid particles get adsorbed on gas bubbles. These bubbles rise to the foam layer which can be collected and removed. The presence of certain substances, referred to as collector substances, facilitates stable foam formation e.g., long chain fatty acids, amines.

Flocculation:

In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

Filtration:

Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors— the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters such as depth filters, absolute filters, rotary drum vacuum filters and membrane filters are in use.

Depth Filters:

They are composed of a filamentous matrix such as glass wool, asbestos or filter paper. The particles are trapped within the matrix and the fluid passes out. Filamentous fungi can be removed by using depth filters.

Absolute Filters:

These filters are with specific pore sizes that are smaller than the particles to be removed. Bacteria from culture medium can be removed by absolute filters.

Rotary Drum Vacuum Filters:

These filters are frequently used for separation of broth containing 10-40% solids (by volume) and particles in the size of 0.5-10µm. Rotary drum vacuum filters have been successfully used for filtration of yeast cells and filamentous fungi. The equipment is simple with low power consumption and is easy to operate. The filtration unit consists of a rotating drum partially immersed in a tank of broth. As the drum rotates, it picks up the biomass which gets deposited as a cake on the drum surface. This filter cake can be easily removed.

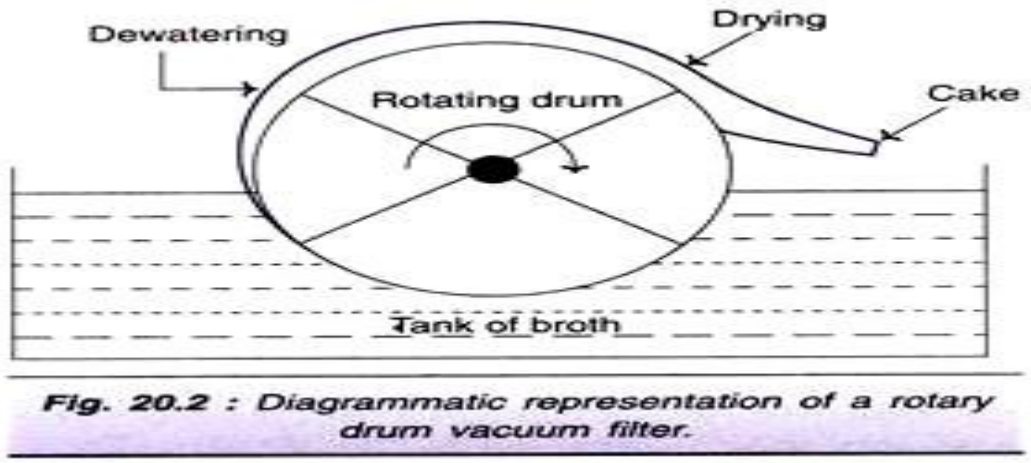

Fig. 20.2 : Diagrammatic representation of a rotary drum vacuum filter.

Membrane Filters:

In this type of filtration, membranes with specific pore sizes can be used. However, clogging of filters is a major limitation. There are two types of membrane filtrations—static filtration and cross-flow filtration (Fig. 20.3). In cross-flow filtration, the culture broth is pumped in a crosswise fashion across the membrane. This reduces the clogging process and hence better than the static filtration.

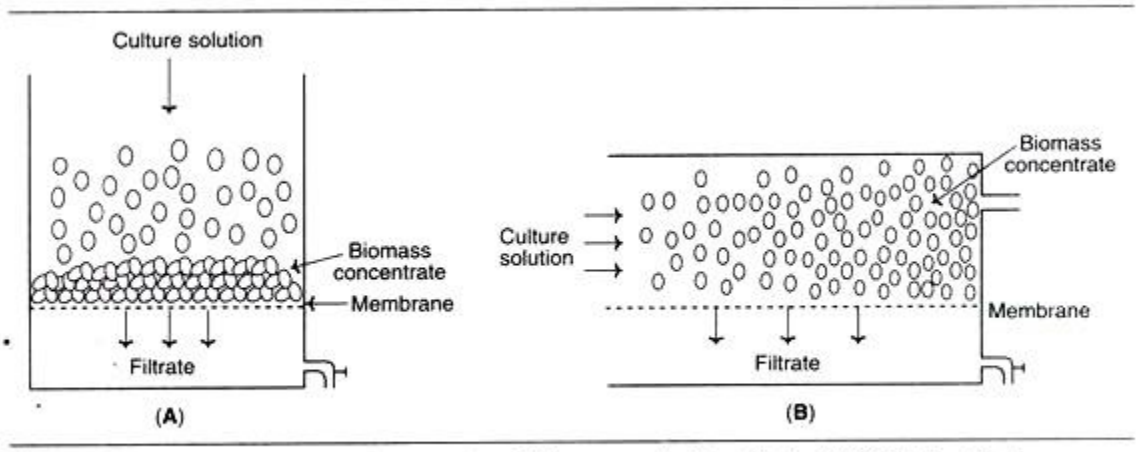

Fig. 20.3 : Filter systems for separation of biomass and culture filtrate (A) Static–flow filtration (B) Cross–flow filtration.

Types of filtration processes:

There are 3 major types of filtrations based on the particle sizes and other characters (Table 20.1). These are microfiltration, ultra filtration and reverse osmosis.

TABLE 20.1 Major types of filtration processes with characteristic features

Type	Sizes of particles separated	Compound or particle separated
1. Microfiltration	0.1–10 μm	Cells or cell fractions, viruses.
2. Ultrafiltration	0.001–0.1 μm	Compounds with molecular weights greater than 1000 (e.g. enzymes).
3. Reverse osmosis (hyperfiltration)	0.0001–0.001 μm	Compounds with molecular weights less than 1000 (e.g. lactose).

Centrifugation:

The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. Thus, centrifugation is mostly used for separating solid particles from liquid phase (fluid/particle separation). Unlike the centrifugation that is conveniently carried out in the laboratory scale, there are certain limitations for large scale industrial centrifugation.

However, in recent years, continuous flow industrial centrifuges have been developed. There is a continuous feeding of the slurry and collection of clarified fluid, while the solids deposited can be removed intermittently. The different types of centrifuges are depicted in Fig. 20.4, and briefly described hereunder.

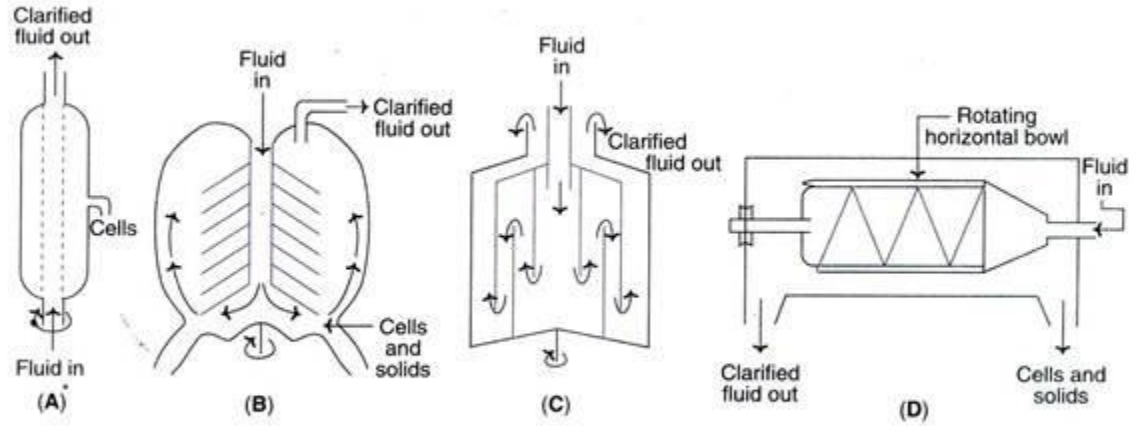

Fig. 20.4 : Centrifuges commonly used in downstream processing (A) Tubular bowl centrifuge (B) Disc centrifuge (C) Multichamber centrifuge (D) Scroll centrifuge (decanter).

Tubular bowl centrifuge:

This is a simple and a small centrifuge, commonly used in pilot plants. Tubular bowl centrifuge can be operated at a high centrifugal speed, and can be run in both batch or continuous mode. The solids are removed manually.

It consists of several discs that separate the bowl into settling zones. The feed/slurry is fed through a central tube. The clarified fluid moves upwards while the solids settle at the lower surface.

This is basically a modification of tubular bowl type of centrifuge. It consists of several chambers connected in such a way that the feed flows in a zigzag fashion. There is a variation in the centrifugal force in different chambers. The force is much higher in the periphery chambers, as a result smallest particles settle down in the outermost chamber.

It is composed of a rotating horizontal bowl tapered at one end. The decanter is generally used to concentrate fluids with high solid concentration (biomass content 5-80%). The solids are deposited on the wall of the bowl which can be scrapped and removed from the narrow end.

Stage # 2. Release of Intracellular Products:

As already stated, there are several biotechnological products (vitamins, enzymes) which are located within the cells. Such compounds have to be first released (maximally and in an active form) for their further processing and final isolation. The microorganisms or other cells can be disintegrated or disrupted by physical, chemical or enzymatic methods. The outline of different techniques used for breakage of cells is given in Fig. 20.5.

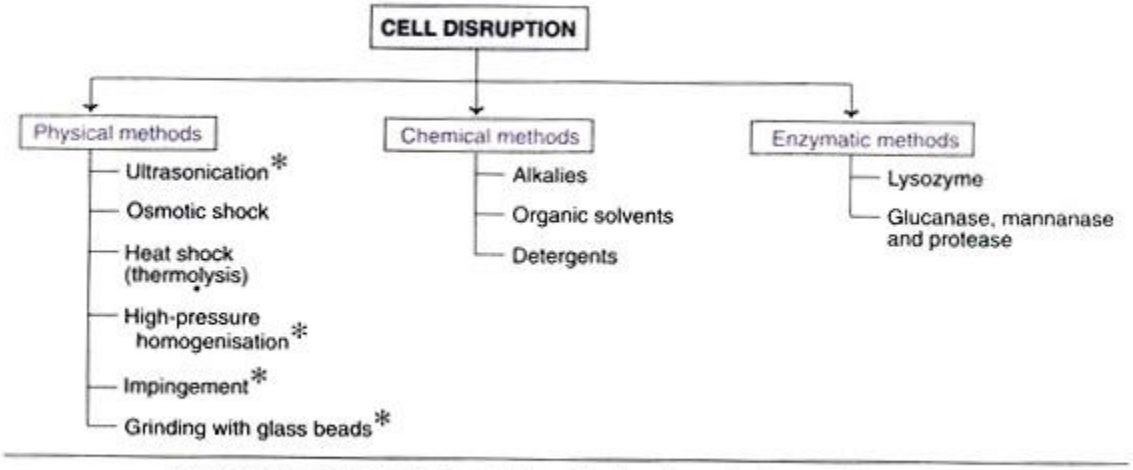

Fig. 20.5 : Major methods for cell disruption to release the intracellular products (indicate mechanical methods while all the remaining are non-mechanical).*

The selection of a particular method depends on the nature of the cells, since there is a wide variation in the property of cell disruption or breakage. For instance, Gram-negative bacteria and filamentous fungi can be more easily broken compared to Gram-positive bacteria arid yeasts.

Cell Disruption:

Physical methods of cell disruption:

The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.

Ultra sonication:

Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

Osmotic shock:

This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis):

Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

High pressure homogenization:

This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.

Impingement:

In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

Grinding with glass beads:

The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells.

A diagrammatic representation of a cell disrupter employing glass beeds is shown in Fig. 20.6. It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

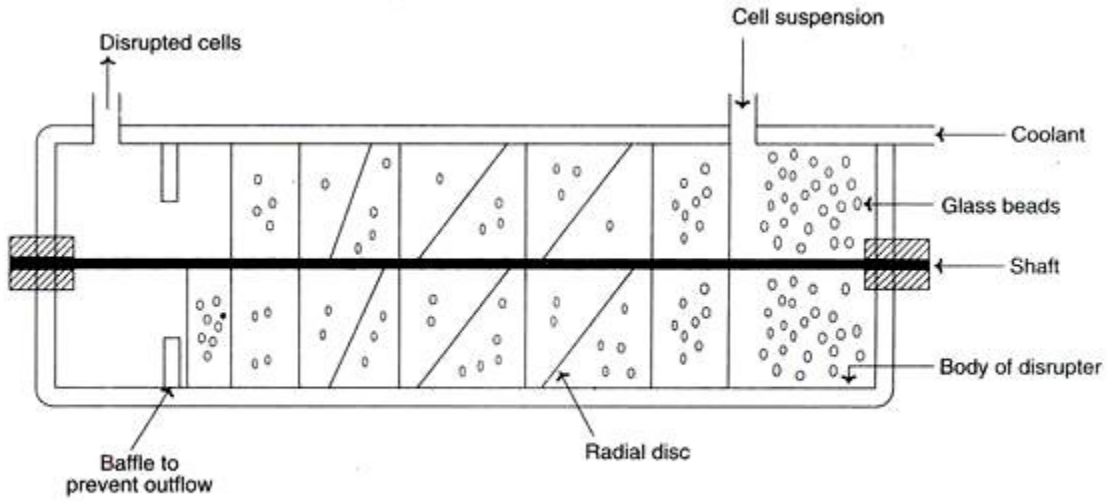

Fig. 20.6 : Diagrammatic representation of a cell disrupter.

Mechanical and non-mechanical methods:

Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods (described below) are non- mechanical in nature.

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Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

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Organic solvents:

Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that

toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

Detergents:

Detergents that are ionic in nature, cationic-cetyltrimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt precipitation. This limitation can be overcome by using ultra filtration or ion-exchange chromatography for purification.

Enzymatic methods of cell disruption:

Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram- positive bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme.

For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

Combination of methods:

In order to increase the efficiency of cell disintegration in a cost-effective manner, a combination of physical, chemical and enzymatic methods is employed.

Stage # 3. Concentration:

The filtrate that is free from suspended particles (cells, cell debris etc.) usually contains 80-98% of water. The desired product is a very minor constituent. The water has to be removed to achieve the product concentration. The commonly used techniques for concentrating biological products are evaporation, liquid-liquid extraction, membrane filtration, precipitation and adsorption. The actual procedure adopted depends on the nature of the desired product (quality and quantity to be retained as far as possible) and the cost factor.

Evaporation:

Water in the broth filtrate can be removed by a simple evaporation process. The evaporators, in general, have a heating device for supply of steam, and unit for the separation of concentrated product and vapour, a condenser for condensing vapour, accessories and control equipment. The capacity of the equipment is variable that may range from small laboratory scale to industrial scale. Some of the important types of evaporators in common use are briefly described.

Plate evaporators:

The liquid to be concentrated flows over plates. As the steam is supplied, the liquid gets concentrated and becomes viscous.

Falling film evaporators:

In this case, the liquid flows down long tubes which gets distributed as a thin film over the heating surface. Falling film evaporators are suitable for removing water from viscous products of fermentation.

Forced film evaporators:

The liquid films are mechanically driven and these devices are suitable for producing dry product concentrates.

Centrifugal forced film evaporators:

These equipment evaporate the liquid very quickly (in seconds), hence suitable for concentrating even heat-labile substances. In these evaporators, a centrifugal force is used to pass on the liquid over heated plates or conical surfaces for instantaneous evaporation.

Liquid-Liquid Extraction:

The concentration of biological products can be achieved by transferring the desired product (solute) from one liquid phase to another liquid phase, a phenomenon referred to as liquid-liquid extraction. Besides concentration, this technique is also useful for partial purification of a product.

The efficiency of extraction is dependent on the partition coefficient i.e. the relative distribution of a substance between the two liquid phases. The process of liquid-liquid extraction may be broadly categorized as extraction of low molecular weight products and extraction of high molecular weight products.

Extraction of low molecular weight products:

By using organic solvents, the lipophilic compounds can be conveniently extracted. However, it is quite difficult to extract hydrophilic compounds. Extraction of lipophilic products can be done by the following techniques.

Physical extraction:

The compound gets itself distributed between two liquid phases based on the physical properties. This technique is used for extraction of non-ionising compounds.

Dissociation extraction:

This technique is suitable for the extraction of ionisable compounds. Certain antibiotics can be extracted by this procedure.

Reactive extraction:

In this case, the desired product is made to react with a carrier molecule (e.g., phosphorus compound, aliphatic amine) and extracted into organic solvent. Reactive extraction procedure is quite useful for the extraction of certain compounds that are highly soluble in water (aqueous phase) e.g., organic acids.

Supercritical fluid (SCF) extraction:

This technique differs from the above procedures, since the materials used for extraction are supercritical fluids (SCFs). SCFs are intermediates between gases and liquids and exist as fluids above their critical temperature and pressure. Supercritical CO_2, with a low critical temperature and pressure is commonly used in the extraction. Supercritical fluid extraction is rather expensive, hence not widely used (SCF has been used for the extraction of caffeine from coffee beans, and pigments and flavor ingredients from biological materials).

Extraction of high molecular weight compounds:

Proteins are the most predominant high molecular weight products produced in fermentation industries. Organic solvents cannot be used for protein extraction, as they lose their biological activities. They are extracted by using an aqueous two-phase systems or reverse micelles formation.

Aqueous two-phase systems (ATPS):

They can be prepared by mixing a polymer (e.g., polyethylene glycol) and a salt solution (ammonium sulfate) or two different polymers. Water is the main component in ATPS, but the two phases are not miscible. Cells and other solids remain in one phase while the proteins are transferred to other phase. The distribution of the desired product is based on its surface and ionic character and the nature of phases. The separation takes much longer time by ATPS.

Reverse miceller systems:

Reverse micelles are stable aggregates of surfactant molecules and water in organic solvents. The proteins can be extracted from the aqueous medium by forming reverse micelles. In fact, the enzymes can be extracted by this procedure without loss of biological activity.

Membrane Filtration:

Membrane filtration has become a common separation technique in industrial biotechnology. It can be conveniently used for the separation of bimolecular and particles, and for the concentration of fluids. The membrane filtration technique basically involves the use of a semi permeable membrane that selectively retains the particles/molecules that are bigger than the pore size while the smaller molecules pass through the membrane pores.

Membranes used in filtration are made up of polymeric materials such as polyethersulfone and polyvinyl di-fluoride. It is rather difficult to sterilize membrane filters. In recent years, micro-filters and ultrafiIters composed of ceramics and steel are available. Cleaning and sterilization of such filters are easy. The other types of membrane filtration techniques are described briefly.

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They are micro- or macro porous membranes with ion exchange groups and/or affinity ligands. Membrane adsorbers can bind to proteins and retain them. Such proteins can be eluted by employing solutions in chromatography.

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This is a technique in which volatile products can be separated by a process of permeation through a membrane coupled with evaporation. Pervaporation is quite useful for the extraction, recovery and concentration of volatile products. However, this procedure has a limitation since it cannot be used for large scale separation of volatile products due to cost factor.

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Precipitation:

Precipitation is the most commonly used technique in industry for the concentration of macromolecules such as proteins and polysaccharides. Further, precipitation technique can also be employed for the removal of certain unwanted byproducts e.g. nucleic acids, pigments.

Neutral salts, organic solvents, high molecular weight polymers (ionic or non-ionic), besides alteration in temperature and pH are used in precipitation. In addition to these non-specific protein precipitation reactions (i.e. the nature of the protein is unimportant), there are some protein specific precipitations e.g., affinity precipitation, ligand precipitation.

Neutral salts:

The most commonly used salt is ammonium sulfate, since it is highly soluble, nontoxic to proteins and low-priced. Ammonium sulfate increases hydrophobic interactions between protein molecules that result in their precipitation. The precipitation of proteins is dependent on several factors such as protein concentration, pH and temperature.

Organic solvents:

Ethanol, acetone and propanol are the commonly used organic solvents for protein precipitation. They reduce the dielectric constant of the medium and enhance electrostatic interaction between protein molecules that lead to precipitation. Since proteins are denatured by organic solvents, the precipitation process has to be carried out below 0°C.

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And for concentration of low molecular weight compounds (vitamins, antibiotics, peptides) polystyrene, methacrylate and acrylate based matrices are used. The process of adsorption can be carried out by making a bed of adsorbent column and passing the culture broth through it. The desired product, held by the adsorbent, can be eluted.

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The biological products of fermentation (proteins, pharmaceuticals, diagnostic compounds and research materials) are very effectively purified by chromatography. It is basically an analytical technique dealing with the separation of closely related compounds from a mixture. Chromatography usually consists of a stationary phase and mobile phase.

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TABLE 20.2 Chromatographic techniques along with the principles for separation of proteins

Chromatography	*Principle*
Gel-filtration (size exclusion)	Size and shape
Ion-exchange	Net charge
Chromatofocussing	Net charge
Affinity	Biological affinity and molecular recognition
Hydrophobic interaction	Polarity (hydrophobicity of molecules)
Immobilized metal-ion affinity	Metal ion binding

Gel-filtration chromatography:

This is also referred to as size-exclusion chromatography. In this technique, the separation of molecules is based on the size, shape and molecular weight. The sponge-like gel beads with pores serve as molecular sieves for separation of smaller and bigger molecules. A solution mixture containing molecules of different sizes (e.g. different proteins) is applied to the column and eluted.

The smaller molecules enter the gel beads through their pores and get trapped. On the other hand, the larger molecules cannot pass through the pores and therefore come out first with the mobile liquid (Fig. 20.7). At the industrial scale, gel-filtration is particularly useful to remove salts and low molecular weight compounds from high molecular weight products.

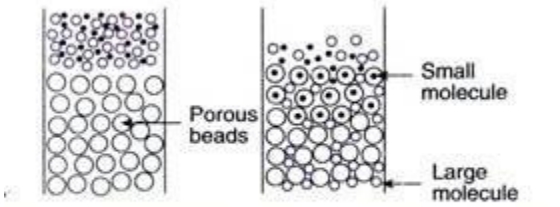

Fig. 20.7 : The principle of gel-filtration chromatography.

Ion-exchange chromatography:

It involves the separation of molecules based on their surface charges. Ion-exchangers are of two types (cation- exchangers which have negatively charged groups like carboxymethyl and sulfonate, and anion- exchangers with positively charged groups like diethylaminoethyl (DEAE). The most commonly used cation-exchangers are Dowex HCR and Amberlite IR, the anion-exchangers are Dowex SAR and Amberlite IRA.

In ion-exchange chromatography, the pH of the medium is very crucial, since the net charge varies with pH. In other words, the pH determines the effective charge on both the target molecule and the ion-exchanger. The ionic bound molecules can be eluted from the matrix by changing the pH of the eluant or by increasing the concentration of salt solution. Ion-exchange chromatography is useful for the purification of antibiotics, besides the purification of proteins.

Affinity chromatography:

This is an elegant method for the purification of proteins from a complex mixture. Affinity chromatography is based on an interaction of a protein with an immobilized ligand. The ligand can be a specific antibody, substrate, substrate analogue or an inhibitor. The immobilized ligand on a solid matrix can be effectively used to fish out complementary structures.

In Table 20.3, some examples of ligands used for the purification of proteins are given. The protein bound to the ligand can be eluted by reducing their interaction. This can be achieved by changing the pH of the buffer, altering the ionic strength or by using another free ligand molecule. The fresh ligand used has to be removed in the subsequent steps.

TABLE 20.3 Some examples of ligands used for separation of proteins by affinity chromatography

Ligand	*Type of protein*
Antibody	Antigen
Cofactor	Enzyme
Receptor	Hormone
Hapten	Antibody
Inhibitor	Enzyme
Lectins	Glycoproteins
Heparin	Coagulation factors
Metal ions	Metal ion binding proteins

Hydrophobic interaction chromatography (HIC):

This is based on the principle of weak hydrophobic interactions between the hydrophobic ligands (alkyl, aryl side chains on matrix) and hydrophobic amino acids of proteins. The differences in the composition of hydrophobic amino acids in proteins can be used for their separation. The elution of proteins can be done by lowering the salt concentration, decreasing the polarity of the medium or reducing the temperature.

Stage # 5. Formulation:

Formulation broadly refers to the maintenance of activity and stability of a biotechnological product during storage and distribution. The formulation of low molecular weight products (solvents, organic acids) can be achieved by concentrating them with removal of most of the water. For certain small molecules, (antibiotics, citric acid), formulation can be done by crystallization by adding salts.

Proteins are highly susceptible for loss of biological activity; hence their formulation requires special care. Certain stabilizing additives are added to prolong the shelf life of protein. The stabilizers of protein formulation include sugars (sucrose, lactose), salts (sodium chloride, ammonium sulfate), polymers (polyethylene glycol) and polyhydric alcohols (glycerol). Proteins may be formulated in the form of solutions, suspensions or dry powders.

Drying:

Drying is an essential component of product formulation. It basically involves the transfer of heat to a wet product for removal of moisture. Most of the biological products of fermentation are sensitive to heat, and therefore require gentle drying methods. Based on the method of heat transfer, drying devices may be categorized as contact, convection, radiation dryers. These three types of dryers are commercially available.

Spray drying:

Spray drying is used for drying large volumes of liquids. In spray drying, small droplets of liquid containing the product are passed through a nozzle directing it over a stream of hot gas. The water evaporates and the solid particles are left behind.

Freeze-drying:

Freeze-drying or lyophilization is the most preferred method for drying and formulation of a wide-range of products—pharmaceuticals, foodstuffs, diagnostics, bacteria, viruses. This is mainly because freeze-drying usually does not cause loss of biological activity of the desired product.

Lyophilization is based on the principle of sublimation of a liquid from a frozen state. In the actual technique, the liquid containing the product is frozen and then dried in a freeze-dryer under vacuum. The vacuum can now be released and the product containing vials can be sealed e.g., penicillin can be freeze dried directly in ampules.

Integration of Different Processes:

It is ideal to integrate the fermentation and downstream processing to finally get the desired product. However, this has not been practicable for various reasons. Integration of certain stages in downstream processing for purification of product has met with some success. For instance, protein concentration by extraction into two phase systems combined with clarification and purification can be done together.

Methods of Immobilization of Cells and Enzymes and their Applications

Introduction

What do you mean by "Immobilization"?

Word "immobilize" means to make anything unable to move at its own. We can entrap or fix Something on certain supportive material for their immobilization. However, during this process it is very important to maintain their physiological conditions.

In 1950, for the first time intentionally enzyme was immobilized on solid material. Immobilization of Enzyme defined, as „the imprisonment of an enzyme in a phase that allows interaction with substrate effectors or inhibitor molecules but it is separate from them".

What should be immobilized - cell or enzyme?

The selection of immobilization of cell or enzyme depends on so many criteria like number of step in the process requirement of coenzyme importance of contaminating reactions, cost, stability and catalytic specificity.

When can we use enzyme for immobilization?

When, the reaction is a single step

When, co-enzyme is not involved

When, there is use of single enzyme

What are the benefits of enzyme immobilization?

It is Cost effective

It requires smaller reactor

It needs shorter process time

Benefits of Cell Immobilization

Do not require coenzyme

Enzyme remains stable in the cell

For reactions, those are more complex, immobilized cells are used, rather than immobilized enzyme.

NMEICT-MHRD (Govt. of India) Project on - Creation of e-Contents on Fermentation Technology

Project control No: RE-02091011297, Christ College, Rajkot, Gujarat, India

Methods of Immobilization of enzymes or cells

Immobilization achieved by any of the following methods

1. Adsorption
2. Physical entrapment
3. Covalent cross-linking
4. Micro-encapsulation

Adsorption

In this method, enzymes adsorbed onto supporting matrix. A range of specific or non-specific bond force may use like electrostatic force, hydrophobic interactions, or affinity bonding to specific ligand.

Advantages of Adsorption

It is Easy

It is Simple

It gives High yield

Limitations of Adsorption

Enzyme will leach with change in pH, or ionic strength

Substrate with same charge as polymer may not gain access to enzyme except at high Concentration, which in turn causes loss of enzyme

Physical Entrapment

Enzyme entrapped in Polymer matrix. The biocatalyst dissolved in a solution of the polymer‟s precursors, and polymerization initiated. Two types of polymers are used Polyacrylamide type gel and naturally occurring type gel materials such as, cellulose triacetate, agar, gelatin, carrageenan and alginate.

Advantages of Entrapment

It is Simple

It provides variability of pore size for the immobilization of cells or enzyme

Mild conditions are used in the preparation of immobilized cells or enzymes

Limitations of Entrapment

Viable cells bursts from gel material

NMEICT-MHRD (Govt. of India) Project on - Creation of e-Contents on Fermentation Technology

Project control No: RE-02091011297, Christ College, Rajkot, Gujarat, India

Free radicals formed during polymerization□ are toxic

Covalent cross-linking

Enzyme or cell bounded covalently with matrix. Enzyme can bind directly with reactive group of polymer or enzyme and polymer bridged by the use of bi-functional reagent. The principle groups on the enzyme through which it coupled are hydroxyl and amino group and to a lesser extent sulfhydryl groups.

Many commercially available activated polymers are hydrogels such as, celluloses or, polyacrylamide, onto which some reactive group like, diazo, carbodiimide or azide attached.

Bi-functional group used are of two types

1. Gluteraldehyde
2. Cyanuric chloride or

Metal like Titanium Four

Gluteraldehyde is simple, and it bound to polymer and enzyme.

Cyanuric chloride is multifunctional group, which react with cellulose and hydroxy substituted compound or enzyme.

Titanium four is multivalent group, which react with glass and used for the immobilization of cells or enzyme.

Advantages of covalent cross-linking

This technique is Easy

It gives High yield

There is no Leaching of Enzyme

Limitations of covalent cross-linking

Enzymes frequently inactivated

Toxic reagents are used

Preparations are complex

Micro-Encapsulation

Enzymes enclosed in a semipermeable membrane capsule.

Materials used for the preparation

of capsule are nylon or biodegradable polylactate or liposomes.

Advantages of Micro-encapsulation

NMEICT-MHRD (Govt. of India) Project on - Creation of e-Contents on Fermentation Technology

Project control No: RE-02091011297, Christ College, Rajkot, Gujarat, India

Surface area to volume ratio is high

Replacement of enzyme is easy

Highly viscosity substrate may be use

Limitations of Micro-encapsulation

Membrane bound enzyme can denature

This technique used for low molecular weight compound

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

Immobilisation of whole Cells:

Immobilisation of whole cells has been defined as the physical confinement or localisation of intact cells to a certain defined region of space with preservation of some, or most, catalytic activity.The increased stability under extreme conditions of pH and temperature, as well

as the re-use and applicability in continuous processing systems that enclose immobilised cells instead of soluble enzymes make the cells a preferred, versatile tool in both food industry and medicine.

There are several different approaches to the classification of immobilised biocatalysts, but the most frequently employed classification is based upon the method of immobilisation selected for a specific application.

The selection of immobilisation method depends, therefore, upon the application, the nature of the microorganism being immobilised, as well as the resources available. Table 1 shows several, possible immobilisation methods that available for whole microbial cells. Most immobilisation methods can be applied either to whole cells or to enzymes.

Some of the advantages of whole-cell immobilisation in comparison with enzyme immobilisation are: the higher stability and enzyme activity, multivariate enzyme applications, and the lower cost.

On the other hand, disadvantages of using whole cell immobilisation in comparison with enzyme immobilisation are linked to the increased diffusional barriers caused by the much larger sizes of cells in comparison with enzymes.

Table 1. Immobilisation Methods for Whole Microbial Cells.

Binding	Weak bonds	Flocculation
		Adsorption
		Ionic
	Strong bonds	Covalent
		Cross-linking
Physical retention	Entrapment	Thermal Gelation
		Ionotropic Gelation
		Polymerization
	Membrane Retention	Dialysis Culture
		Ultrafilters

Adsorption is the least expensive and mildest immobilisation method. It uses weak interaction forces such as hydrogen bonds, hydrophobic interactions and Van der Waal forces to immobilise cells or enzymes.

However, the sensitivity of this interaction to pH makes the leakage of cells immobilised by this technique quite common. Important applications of this technique are related to the production of fructose and vinegar, and also waste water treatments.

Ionic binding uses the properties of negatively charged microbial cells to interact with positively charged ion exchangers. The results obtained with this technique are also sensitive to extreme pH values, and the binding strength is greater in comparison with adsorption. However, the mild conditions employed by this technique make it suitable for use for immobilisation of both enzymes and whole cells.Covalent binding and cross- linking offer better strength than the previous techniques; however, there is an encountered toxicity in the reagents that are used to produce immobilisation. Entrapment techniques are, however, the most commonly encountered in the industry and they are based on the formation of thermally reversible gels, ionotropic gels and polymerisation.

Application of Immobilisation of Cells:

Entrapment of cells in a gel-like matrix by ionotropic gelation using alginates and carrageenans is certainly the most useful method for industrial purposes. The properties of the gel-like matrix allow the cell to remain viable and with its catalytic ability for a long period of time.For example, an increment in the yeast concentration obtained through immobilisation techniques has helped the brewing industry to reduce fermentation process times and the size of their storage facilities. Unfortunately, because of the high concentration of diacetyl, and the low concentration of higher alcohols and esters, the flavour of the fast fermented beer has been compromised.

Even the amino acid profile has been altered. The main factor causing this uncommon imbalance is the insufficient mass transfer in the older designs of fermentation reactors; thus, the use of new reactor designs, and combining technologies could improve the quality of the products obtained through such fermentation reactions. Nutraceuticals are defined as food components that have health benefits beyond traditional nutritional value.

Novel biotechnology tools like immobilisation were also applied for the isolation and incorporation of such food components in ordinary foods. The synthesis of nutraceuticals was reported to be successful by employing immobilised lipases, such as those from Candida antartica and Lactobacillus ruteri. The introduction of conjugated linoleic acid (CLA) in dairy foods has been made possible through the immobilisation of lipases.

There is a quite extensive list of immobilisation technique applications in medicine. A very important group of such applications is concerned with the regulation of equilibrium between coagulation and dissolution of coagulated blood (fibrinolysis) through the use of immobilised enzymes.

The high probability of death caused by thrombosis (involving the formation of clots in the blood vessels), has committed physicians to the use of fibrinolytic therapy for the treatment of occlusions in those parts of the body where a surgical intervention would be too risky.

Amongst the most important enzymes that have been immobilised for use in such therapy are Plasmin and Heparin. The use of biotechnology as well as microscopic techniques has helped refine and greatly improve such therapeutical means.

Current regulations for the disposal of toxic chemicals in the environment as well as the detoxification of water used in any agricultural and industrial process brings the need for novel biotechnology tools to be developed in order to solve such problems in a cost-efficient manner.

Enzymes have been isolated from genetically manipulated microorganism strains with the purpose of accelerating the rate of degradation of organic and some inorganic compounds in wastewater as well as in soils.

Mechanisms of RNA interference

The discovery of dsRNA-induced gene silencing in *C elegans* allowed genetic screens to be performed that led to the identification of genes required for RNAi in the nematode. Comparison of these genes to those from other species involved in silencing phenomena known as posttranscriptional gene silencing, cosuppression, quelling, and RNAi revealed that all of these events follow a similar core pathway. Nevertheless, there are features of the pathway that show species-specific differences as well as differences depending on the source of the dsRNA trigger.

dsRNA molecules, whether introduced experimentally or present as naturally occurring viral byproducts, endogenous miRNAs, or aberrant transgene transcripts, are recognized and cleaved into 21-23 nucleotide siRNAs by the RNase III-like enzyme termed Dicer. Dicer homologues can be found in *S pombe* (but not *S cerevisiae*), *C elegans*, *Drosophila*, plants, and mammals. Different species contain different numbers of Dicer homologues and/or associated proteins containing dsRNA binding domains that function to recognize dsRNAs from different sources. For example, in *Drosophila*, Dicer-1 processes miRNA precursors and Dicer-2 processes long dsRNAs. In *Arabidopsis thaliana* there are 4 Dicer homologues that function together with associated proteins to cleave dsRNAs of different types. To date, only one Dicer gene has been identified in mammals, and interacting proteins regulating Dicer function remain to be identified. dsRNA cleavage by Dicer generates siRNAs that contain a 2-nucleotide 3′ overhang and a 5′-phosphorylated terminus, both of which are required for activity. Processing by *Drosophila* Dicer is adenosine triphosphate (ATP) dependent and requires a functional RNA helicase domain. In contrast, it appears that human Dicer may not require ATP. Processing of miRNAs requires a preliminary processing step in which the long primary transcript is processed by another RNaseIII-like endonuclease, Drosha, within the nucleus. The miRNA precursor is then exported to the cytoplasm where it is processed further by Dicer.

The small dsRNA products of Dicer cleavage are then incorporated into multi-subunit effector complexes. Depending on the species and the source of the dsRNA, different effector complexes are formed with different end results on the target RNA. rasiRNAs are integrated into the RNA-induced transcriptional silencing (RITS) complex and guide chromatin modification. miRNA-containing complexes are usually referred to as miRNPs and direct target RNA degradation (in plants) or translational repression (in animals). Synthetic siRNAs or those derived from naturally occurring long dsRNAs are incorporated into the RNA-induced silencing complex (RISC) and guide its cleavage of target RNAs. RISC has helicase, exonuclease, endonuclease, and homology-searching domains. The initial RISC remains inactive until it is transformed into an active form by the unwinding of the siRNA duplex, through RISC-mediated helicase activity, and loss of the sense strand of the dsRNA molecule. This solves the problem of how a stable dsRNA is converted to a form that is capable of using base pairing to search among

cellular RNAs for homologous regions. Based on studies aimed at determining the functional characteristics of siRNAs, Zamore and colleagues determined that the antisense strand is mainly responsible for target recognition and silencing activity. The active siRNA/RISC complex targets the mRNA of homologous sequence for degradation and the mRNA is reliably cleaved at regions homologous to the siRNA. This degradation is mediated by the endonuclease activity of active RISC.

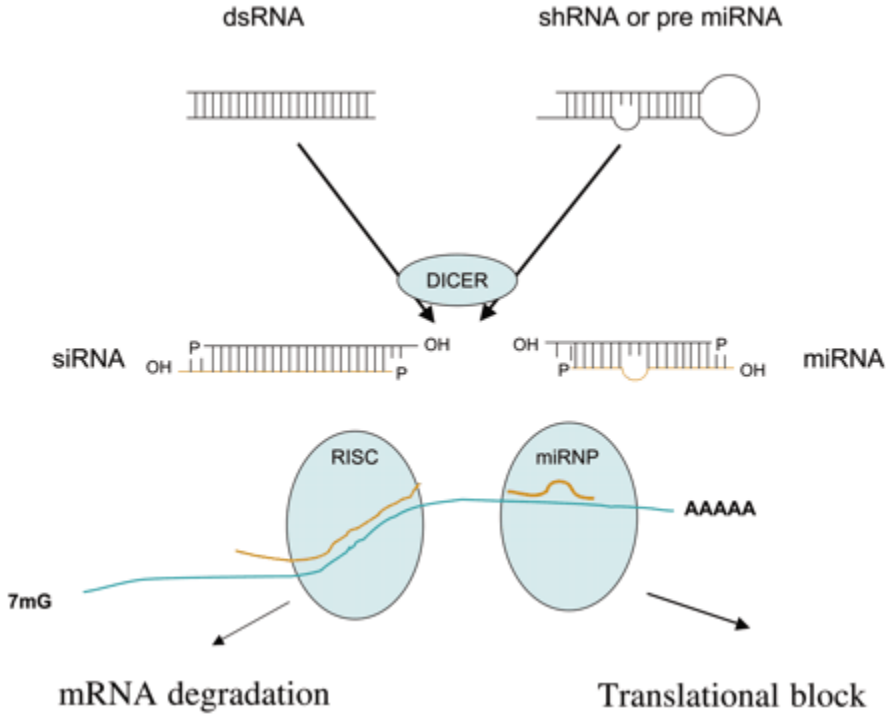

Figure 1.RNA silencing pathways. Long dsRNA and shRNA or miRNA precursors are processed to siRNA or miRNA by the double-stranded RNA binding and RNase III–like enzyme Dicer into 21 to 23 nucleotide dsRNA intermediates. These are subsequently unwound and assembled into the RNA-induced silencing complex (RISC; siRNAs), which directs RNA cleavage, or into anmiRNA-effector complex termed miRNP, which directs translational repression (mRNA target identified by the 5′cap, 7 mG, and poly A tail).

RNA interference in mammalian cells

In order to study gene function in any experimental system, it is useful to eliminate the expression of specific genes and note the resulting effects. In mammalian systems, this has been achieved through the development of knock-out models in mice. While effective, this method

has its disadvantages. First, it eliminates gene expression throughout the entire organism, and cannot be used for studying developmental or cell type–specific effects unless tissue-specific knock-outs are employed. Second, generating knock-out models is both labor and time intensive. In theory, these problems could be resolved by adapting RNAi techniques to silence gene expression in vivo in mammalian systems. It was initially thought that this would not be possible since introduction of dsRNA molecules into normal, mature mammalian cells activates an innate antiviral immune response that results in a general inhibition of protein translation and proinflammatory gene expression (Figure 2).21 However, in 2001, Elbashir and colleagues22 and Caplen and colleagues23 were able to demonstrate RNAi in mammalian systems by the intracellular expression of artificially synthesized mimics of Dicer products, 21-23 base pair siRNA duplexes, which are delivered into cultured cells by transfection. This technique has also been applied to adult mouse models.24 By eliminating long dsRNAs from the process, it was hoped to prevent activation of the innate immune response controlled by the interferon system and its regulator, the dsRNA-activated protein kinase PKR.

Stable RNA interference

Introduction of 21 bpsiRNAs has allowed for the successful application of RNAi technology to mammalian systems. However, assays using this method are transient in nature and the suppressed phenotype can be lost within several doubling times, most likely due to the dilution of the siRNA. While this approach is reliable for short-term studies of gene expression, it cannot replace knock-out mouse models or allow for precise loss-of-function genetic screens. This is not a concern in organisms such as C elegans or N crassa because they direct ongoing synthesis of siRNAs through the action of an RNA-dependent RNA polymerase. In these systems, the suppressed phenotype is not only maintained, but is also passed on to future generations,25 although the effect gradually diminishes. An additional disadvantage of transient siRNA studies in mammalian systems is that synthesis of the required siRNAs is costly, limiting the benefits from this technique. To address these issues, a system for the stable expression of siRNAs has been developed. Taking clues from the structure of endogenous miRNAs, mammalian expression vectors were designed to direct the intracellular synthesis of siRNAs.26,27 In most cases, the target-specific insert is made up of a 19-nucleotide sequence

complementary to the target, followed by a short spacer and the reverse complement of the same target sequence. Once transcribed, a 19 bp stem-loop structure, termed short-hairpin RNA (shRNA), is processed by Dicer into ansiRNA that can direct the down-regulation of target gene expression via the elements of RNAi machinery (Figure 1). Polymerase III promoters, such as T7 or U6, were initially used in these constructs, as they produce siRNAs that mimic the requirements for an efficient siRNA. These requirements include, but are not limited to, the absence of a poly A tail and a termination signal that yields a transcript with a 3′ overhang. Polymerase II–driven shRNA expression vectors have also been developed, which will allow for the regulated expression of siRNAs.

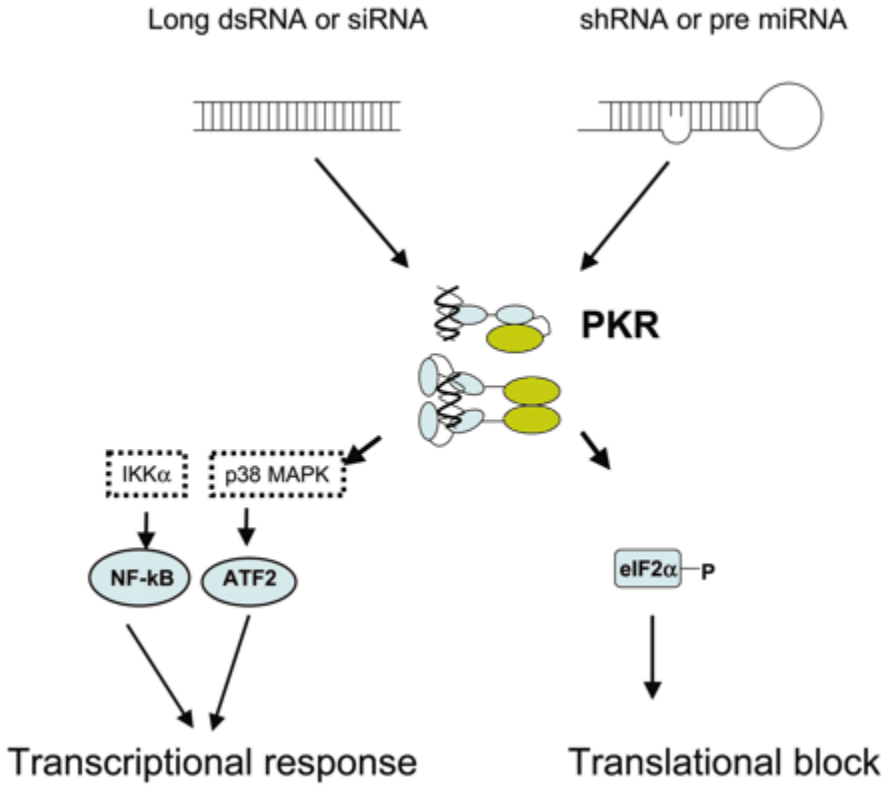

Figure 2.PKR activation by dsRNA. Long dsRNA, shRNA, or pre-miRNA can activate protein kinase R (PKR) via binding to the dsRNA binding motifs, causing a conformational change, dimerization, and autophosphorylation. Activated PKR can inhibit protein synthesis by phosphorylation of translational initiation factor eIF2α. PKR can also function as a signal-transducing kinase interacting with IκB kinase (IKK) and p38 mitogen-activated protein kinase

(p38) regulating gene transcription by activation of different transcription factors including NFκB and ATF2

Research applications of RNAi

For many years, homology-dependent, RNA-mediated gene silencing techniques have been used as a basic research tool, introducing RNA into cells to interfere with the function of an endogenous homologous gene. These studies assumed that the observed effects resulted from simple antisense mechanisms that depend on Watson-Crick base pairing between the introduced RNA and the endogenous messenger RNA transcripts.32 However, it is now apparent that the presence of dsRNA accounted for many of the observed robust silencing effects.

In 1998, Fire and colleagues1 determined that dsRNA was a more efficient inhibitor of sequence-specific suppression of gene expression in C elegans, as opposed to previously employed antisense methods. RNAi can be induced in the nematode worm by direct injection of the dsRNA, by feeding the worm bacteria that have been generated to express siRNAs, or simply by soaking the worm in siRNA-containing media. From that point forward, the emphasis has been not only on understanding how this phenomenon occurs, but also how it can be harnessed as a research tool.33 Through these investigations, RNAi has been shown to greatly facilitate both “reverse genetic” experiments (identifying the function of a known gene) and “forward genetic” experiments (identifying the gene responsible for a given phenotype). From an application point of view, RNAi may also be useful as a therapy for diseases arising from aberrant gene expression.

Typical reverse genetic experiments involve designing siRNAs (chemically or enzymatic ally synthesized) or shRNA-expressing constructs targeting a gene of interest. Following transient transfection of siRNAs or selection of shRNA-expressing stable transfect ants, the phenotype of the cells is assessed using appropriate functional assays. As discussed below, it is critical in this type of experiment to use adequate controls to ensure that the observed phenotype is due solely to targeting of the gene of interest. Both transient and long-term silencing of the expression of a given gene have also been used in vivo studies, primarily in mice (reviewed in Paroo and Corey34).

Analyses testing gene function through RNAi have been performed in a large number of experimental systems, including hematologic disorders such as acute myeloid leukemia and chronic myelogenous leukemia. Normally, the acute myeloid leukemia (AML) gene product is part of a transcription factor complex whose activity is required for normal hematopoiesis. Chromosomal translocations in hematopoietic malignancies where the AML1 gene is the most frequent target are common in human leukemias.35 Specifically, the translocation t(8;21) resulting in the AML1/MTG8 (myeloid translocation gene) translocation product accounts for 10% to 15% of all de novo cases of myeloid leukemia, although the exact role of the chimeric protein in the development of leukemia was not completely understood.36 To address this RNAi, technology has been used to specifically silence the expression of the AML1/MTG8 translocation product. Electroporation with AML1/ MTG8-specific siRNAs successfully suppressed the expression of the fusion proteins, without interrupting the expression of wild-type AML1 mRNA in 2 t(8;21)–positive cell lines. In this type of functional analysis, Heidenreich and colleagues36 were able to determine the role for the AML1/MTG8 fusion protein in preventing differentiation of the leukemia cells. Suppression of chimeric protein expression in t(8;21)–positive cell lines increased their susceptibility to growth factors that lead to their ultimate differentiation, determined both by changes in cell shape, the display of surface marker proteins, and the up-regulation of the CAAT/enhancer binding protein (C/EBP) marker gene for differentiation. Mismatched siRNAs were used as the controls in these experiments to conclude that these observations were the direct result of siRNA silencing activity, and not the result of nonspecific, off-target effects, as discussed below, under “Nonspecific and off-target effects of RNA interference.”

Clearly, RNAi techniques will also be helpful in investigating related chromosomal abnormalities associated with myeloid malignancies, such as the t(16;21) translocation reported by Heidenreich and colleagues in a recent issue of Blood.36 Chronic myelogenous leukemia (CML) and variants of acute lymphoblastic leukemia (ALL) that arise from a t(9;22) chromosomal translocation resulting in a constitutively active Bcr-Abl tyrosine kinase are also targets for siRNA approaches (Table 1). Scherr and colleagues38 determined that siRNAs recognizing the breakpoint of the fusion protein are effective at specifically silencing its

suppression in both established cell lines and primary CML cells, without interfering with the expression of wild type c-abl or c-bcr.

Therapeutic applications

RNAi likely developed as an endogenous host defense mechanism directed against viral infections. Evidence for this has been seen in both plants41,42 and animals, such as C elegans43 and Drosophila.44 Once RNAi techniques had been successfully developed to suppress endogenous gene expression in mammalian systems, the next obvious question was whether these strategies could be used for therapeutic purposes. Early results from cell culture and animal models suggest this application has the potential to revolutionize the treatment of such diseases as viral infection and cancer, by expressing siRNAs that specifically target components of the virus or the disease for silencing.

Promising studies have already been performed against multiple viruses, including human immunodeficiency virus, influenza virus, and human papiloma virus, preventing the establishment of productive infection in susceptible cells. In addition to pathogenic viruses, RNAi technology has also been used to target specific cancer genes in melanoma,45 pancreatic adenocarcinoma,46 and leukemia, as discussed here. These studies further illustrate the potential for an RNAi-based gene therapeutic approach.

The RNAi-mediated functional analysis of the AML1/MTG8 fusion protein that was performed by Heidenreich and colleagues36 succeeded not only in advancing our understanding of the basic biology of myeloid leukemia, but also in opening the door for the potential use of RNAi technology in treating this disease. In the case of Bcr-Abl–positive leukemias, the tyrosine kinase inhibitor imatinibmesylate is currently the primary therapeutic agent for this disease. However, suboptimal therapeutic response or resistance is common, preventing disease control. The success reported by Scherr and colleagues38 in suppressing the expression of the Bcr-Abl fusion protein through siRNA expression has provided a potential alternative avenue for treating this disease. In a recent report, Wohlbold and colleagues47 were able to silence the expression of the Bcr-Abl fusion protein in leukemia cell lines, resulting in an increased sensitivity to imatinib and γ radiation. In a similar study, Wilda and colleagues48 reported an increase in apoptosis in

CML cells transfected with ansiRNA targeting Bcr-Abl. Clearly there are opportunities to develop RNAi therapeutic approaches to hematologic malignancies.

Host pathogen interaction by siRNAs

Interferons (IFNs) are cytokines that function as the first line of defense against viral infection in mammals.58 They can be induced in response to molecular patterns specific to pathogens, including bacteria, viruses, and fungi, and are secreted to provide paracrine protection against virus infection and to mediate adaptive immune responses. Activation of this innate immune response is efficiently triggered by dsRNA, a pathogen-associated molecular pattern (PAMP) commonly formed during the replication cycle of most viruses. dsRNA is responsible for the initiation of 3 known types of signaling events involved in the antiviral response. Initially, dsRNA binds to constitutively expressed dsRNA recognition proteins. These proteins act either to directly mediate antiviral events or to initiate signaling cascades that result in the up-regulation of IFNs and other antiviral proteins (Figure 3). dsRNA can also directly activate the transcription of specific genes through alternative, IFN-independent pathways (Figure 3).

IFNs induce the transcriptional activation of interferon-stimulated genes (ISGs), whose protein products confer cellular antiviral or antiproliferative activity. Many protein products of these antiviral genes are constitutively expressed in uninfected cells. These proteins, such as PKR and 2′-5′A synthetase, are not only up-regulated through IFN-mediated transcriptional activation, but are also present at basal levels to directly respond to the initial infection. Following activation by dsRNA, PKR dimerizes, autophosphorylates, and subsequently phosphorylates its substrates, the best characterized of which is the alpha subunit of eukaryotic initiation factor 2 (eIF2α). Phosphorylation of eIF2α by PKR renders the initiation factor unavailable for further rounds of translation. As a result, PKR inhibits both viral replication59 and cellular protein synthesis.60 PKR also acts as a signal transducer in pathways leading to the activation of NFκB and ATF2.61

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COURSE CODE: 17MBU514A **BATCH-2017-2020**

UNIT-4						
S.NO	**QUESTIONS**	**OPT 1**	**OPT 2**	**OPT 3**	**OPT 4**	**ANSWER**
1	_____has been used for filtration of yeast cells and filamentous fungi	Absolute filters	Rotary drum vacuum filters	Membrane filters	Micro filters	Rotary drum vacuum filters
2	The patentable product involving R-DNA technology	Vaccine	Transposons	Base pairs	Genes	Vaccine
3	Plant patents has been granted by	England patent office	American patent office	Japan patent	European patent	European patent
4	Who got the patent for Pseudomonas	Dr. Chakrabarty	Edward Jenner	Louis Pastuer	Robert Koch	Dr. Chakrabarty
5	Pct is	Patent control term	Patent cooperation treaty	Public cooperation team	Private cooperation team	Patent cooperation team
6	Rotary drum vacuum filters are frequently used for separation of broth contain in _____% solids	40-50%	10-40%	40-10%	10-45%	10-40%
7	Indian copy right Act was published in	1956	1997	1957	1965	1957
8	What is the mode of revolution of patent	State government	Union government	UN	Central government	Central government

Prepared by R.Dineshkumar, Assistant Professor, Dept of Microbiology, KAHE.

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9	The first step in product recovery is the _____ of whole cells and other insoluble ingredients from the culture broth	Filtration	Centrifugation	Separation	Flotation	Separation
10	The word patent derived from _____	Patent	Latin word patere	Pantor	Patentor	Latin word patere
11	The colonial theory was proposed by	J. Cbose	Haeckel	John	Anand Chakraboty	Haeckel
12	Rotary drum vacuum filters are filter the particles in the size of _____mu	0.5-1.0mu	0.5-2mu	0.5-10mu	0.5-100mu	0.5-10mu
13	In which year copyright was brought enforced	1999	1993	1994	2000	1999
14	In which year Treade mark bill was passed in Inidia	1958	1978	1948	2002	1958
15	_____technique involves forcing of cell suspension at high pressure through a very narrow oritice	High pressure homogenizaion	Osmotic shock	Ultra sonication	Heat shock	High pressure homogenization
16	Pseudomonas was patented by ____	Dubey	Anand Chakraboty	Khorana	T.J.C.Bose	Anand Chakraboty
17	EMR stands for _____	Exclusive market rights	Extend market rights		Exclusive market rate	Exclusive market rights

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18	_____are stable aggregate of surfactant molecules and water in organic solvents.	Reverse Micelles	Micellus	Aquous two phase cells	Aqueous micells	Reverse Micells
19	A patent should contain_____	Name of the inventor	Description of patent	Name of the patent	All the above	All the above
20	Development of crop varities are protected through_____	Plant breeder's rights	Plant biotech regulation	Plant biotech rights	Prevent breeders rights	Plant breeder's rights
21	The duration of Indian patent is _____	7 years	6 years	5 years	3 years	5 years
22	The commonly used techniques for concentration the biological products is	Evaporation	Liquid -liquid extraction	Membrane filtration	All the above	Central government
23	Scientific theories, methamatical methods and therapeutic treatments are	Easily patentable	Non patentable	Uncopyrightable	Long time procesing	Non patentable
24	IPR clasified in to	2 catagories	5 catagories	3 catagories	7 catagories	2 catagories
25	How many types of patents available?	2	3	6	8	3
26	The principle of gel-filtration chromotography is based on the_____	Shape	Size	Size and shape	Net charge	Size and shape
27	How many years USPTO can issue a patent	3 years	2 years	1 years	7 years	3 years
28	In which year copyright was amended	1993	1994	2000	1997	1994

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29	Gel-filtration chromatography also called as _____	Size-shape exclusion chromatography	Ion exchange chromatography	Size exclusion chromatography	Chromatofocusing chromatography	Size exclusion chromatography
30	Which symbol was used distinguish one trade to other?	Copy right	Patent	Tread mark	Tread secrete	Tread mark
31	In which year the colonial theory proposed	1894	1874	1876	1884	1874
32	Ligands are used for the separation of protein in _____chromatography	Ion-exchange	Affinity	Gel - filtration	None of the above	Affinity
33	_____is patentable process involving are - DNA technology	Modifying genomic sequences	Enzyme sequences	Plant genes	Gene sequence	Modifying genomic sequences
34	Lectin used for the separation of _____	Enzymes	Glycoproteins	hormones	antigen	Glycoproteins
35	Affinity chromatography is based on a _____	Interaction of protein with an immobilized ligand	Interaction of protein with antigen	Interaction of protein with metal ions	Interaction of protein antibody	Interaction of protein with an immobilized ligands
36	A elegant method for the purification of protein from the complex mixture?	Ion-exchange chromatography	Affinity chromatography	Gel - filtration chromatography	None of the above	Affinity chromatography
37	In ion exchange chromatography DEAE stand for ____	Diethylaminoethyl	Double ethylaminoethyl	Diethylamioacid ethyl	All the above	Diethylamionoethyl
38	Which are patentable	Invention	Treatments	Scientific theories	All the above	Invention

39	Formulation broadly refers to the maintains of activity and stability of biotechnological product during ___ and _____	Storage and distribution	Fermentation and storage	Storage and packing	All the above	Storage and distribution
40	_____is a essential component of product formulation	Separation	Preservation	Drying	Fermentation	Drying
41	Before the release of genetically engineered microbial pesticides should be approved by _____	EPA	EPD	GATT	EDA	EPA
42	_____method is used for drying large volumes of liquids	Spray drying	Freeze drying	Drying	All the above	Spray drying
43	In _____for the first time intentionally enzyme was immobilized on solid material	1950	1940	1970	2000	1950
44	Most immobilization methods can be applied either to whole cells or to _____	Proteins	Enzymes	Antigen	Antibodies	Enzymes
45	Immobilized enzyme used in _____	Food industry	Biosensor	Industries	All the above	All the above
46	Introduction of _____has allowed for the successful application of RNA	21bpsi RNA	24bpsiRNA	24pbsiRNA	All the above	24pbsiRNA

	technology mammalian system					
47	Grant specification and climbs are the parts of _____	Copy right	IPR	Patent	IRR	Patent
48	_____stages in downstream process	5	4	3	1	5
49	Downstream processing refers to the recovery and purification of _____products	Biosynthetic product	Pharmaceutical product	Animal and plant tissue	All the above	All the above
50	The Indian patent act include _____but not _____	Product and invention	Process patents and product patent	FDA but not IPR	None of the above	Process patent and product patent
51	In which year industrial design bill was passed	1940	1999	2010	1980	1999
52	In micro filtration _____size of particles seperated	1-10mu	0.1-10mu	2.0-3.0mu	1.0-1.5mu	0.1-10mu
53	Types of cell disstruption	Physical method	Chemical method	Enzymatic methods	All the above	All the above
54	When the pasis convention for pretection of industrial property signed	1884	1883	1878	1748	1883
55	Duration of patent	20 years	40 years	all the above	200 years	20 years
56	Example of non ionic	Trion x-100	Trion x-50	Tion x-60	Trion x-70	Trion x-100

	detergents ___					
57	Techniques will be helpful in investigating related chromosomal abnormalities associated with myeloid malignancies	RNAi	SiRNA	DNA	mRNA	RNAi
58	Example for organic solvent	Methanol	Butanol	Ethanol	All the above	All the above
59	In SCF super critical _____with a low critical temperature and pressure in commonly used	CO2	O2	H20	H2O2	CO2
60	In floation the cells and other solid particles get on gas bubbles absorbed	Adsorbed	Absorbed	Penetrate	All the above	Adsorbed

Bioethanol and bio diesel production

Biofuel is a form of recycled fuel, produced from biomass or waste generated by the use of organic material which may be any substance including vegetable and animal products like grass, trees, plant parts and plants in the ocean.

Biomass can produce liquid and gaseous fuels, which can do almost everything that fossil fuels do. It can be converted into useful forms of energy like methane gas or transportation fuels. It is a fuel composed of a recently dead biological matter. Biofuel and fossil fuels are different as fossil fuels are made up of long dead organic matter, whereas, biofuels are made up of recently dead matter. Biofuels contain 80% renewable materials.

Plants that photosynthesize produce the organic material making biofuels. The specific biofuel made by corn is Ethanol (ethyl alcohol), biofuel Sériethématique: La stratégie de Lisbonnemade by soybeans is biodiesel and others are classified as biomass.

Biofuels are known as argofuel, also. This type of fuel has been gaining more attention since there has been a sharp depletion in fossil fuel resources, increase in their price and their negative effects in the form of greenhouse gas emissions. Need for energy security further justifies search for biofuels

Most vehicles on the road today are fueled by gasoline and diesel fuels. These fuels are produced from oil, which is a non-renewable fossil fuel. Non-renewable fuels depend on resources that will eventually run out. Renewable resources, in contrast, are constantly replenished and will never run out. Biomass is one type of renewable resource, which includes plants and organic wastes.

What are the general techniques involved in the production of biofuel? (Brief Information)

For ethanol, Brazil and the USA are the largest producers but strong production growth is foreseen in China, India, Thailand and several African countries. Biodiesel production is dominated by the EU but a significant growth is expected in Brazil, Indonesia and Malaysia.

There are two methods currently brought into use to for production of biofuels. In the first one, sugar crops or starch are grown and through the process of fermentation, ethanol is produced. In the second method, plants are grown that naturally produce oil like jatropha and algae. These oils are heated to reduce their viscosity after which they are directly used as fuel for diesel engines. This oil can be further treated to produce biodiesel, which can be used for various purposes.

Biomass can be termed as material, which is derived from recently living organism. Most of the biomass is obtained from plants and animals and include their byproducts. Some agricultural products specially grown for the production of biofuels are switch grass, soybeans and corn (in United States), sugar cane (in Brazil), sugar beet and wheat (in Europe) cassava and sorghum (in China), miscanthus and palm oil (in Southeast Asia) and jatropha (in India).

Bioethanol

Bioethanol is an alcohol made by fermenting the sugar components of biomass. Today, it is made mostly from sugar and starch crops. With advanced technology being developed by the Biomass Program, cellulosic biomass, like trees and grasses, are also used as feed-stocks for ethanol production. Ethanol can be used as a fuel for cars in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions.

Biomass is material that comes from plants. Plants use the light energy from the sun to convert water and carbon dioxide to sugars that can be stored, through a process called photosynthesis. Organic waste is also considered to be biomass, because it began as plant matter.

Bioethanol Production

Two reactions are key to understanding how biomass is converted to bioethanol:

- **Hydrolysis** is the chemical reaction that converts the complex polysaccharides in the raw feedstock to simple sugars. In the biomass-to-bioethanol process, acids and enzymes are used to catalyze this reaction.
- **Fermentation** is a series of chemical reactions that convert sugars to ethanol. The fermentation reaction is caused by yeast or bacteria, which feed on the sugars. Ethanol and carbon dioxide are produced as the sugar is consumed. The simplified fermentation reaction equation for the 6-carbon sugar, glucose, is:

$C_6H_{12}O_6 \longrightarrow 2\ CH_3CH_2OH + 2\ CO_2$

Glucose Ethanol Carbon dioxide

Biodiesel

Biodiesel is a mixture of fatty acid alkyl esters made from vegetable oils, animal fats or recycled greases. Biodiesel can be used as a fuel for vehicles in its pure form, but it is usually used as a petroleum diesel additive to reduce levels of particulates, carbon monoxide, hydrocarbons and air toxics from diesel-powered vehicles.

Biodiesel Feedstocks

In the United States, most biodiesel is made from soybean oil or recycled cooking oils. Animals fats, other vegetable oils, and other recycled oils can also be used to produce biodiesel, depending on their costs and availability. In the future, blends of all kinds of fats and oils may be used to produce biodiesel.

Biodiesel Production

The main reaction for converting oil to biodiesel is called trans-esterification. The trans-esterification process reacts an alcohol (like methanol) with the triglyceride oils contained in vegetable oils, animal fats, or recycled greases, forming fatty acid alkyl esters (biodiesel) and glycerin. The reaction requires heat and a strong base catalyst, such as sodium hydroxide or potassium hydroxide. The simplified trans-esterification reaction is shown below.

Base

Triglycerides + Free Fatty Acids (<4%) + Alcohol ——> Alkyl esters + glycerin

Acid

Triglycerides + Free Fatty Acids (>4%) + Alcohol ——> Alkyl esters + triglycerides

What are the advantages, disadvantages and limitations of these fuels in the society?

Wood has been used since very early times and is one of the major contributors of global warming.

How will biofuel production affect food security and poverty?

Food prices have raised sharply in the past few years, especially for cereals and vegetable oils, in part because they are used both for food and biofuel production. In addition, higher transport costs increased the costs of imported food. While some countries will benefit from higher food prices, the food bill is expected to increase for the least-developed countries, which are net food importers.

Methanogenesis

Methanogenesis or **biomethanation** is the formation of methane by microbes known as methanogens. Organisms capable of producing methane have been identified only from

the domain Archaea, a group phylogenetically distinct from both eukaryotes and bacteria, although many live in close association with anaerobic bacteria. The production of methane is an important and widespread form of microbial metabolism. In anoxic environments, it is the final step in the decomposition of biomass. Methanogenesis is responsible for significant amounts of natural gas accumulations, the remainder being thermogenic

Proposed mechanism

The biochemistry of methanogenesis involves the following coenzymes and cofactors: F420, coenzyme B, coenzyme M, methanofuran, and methanopterin. The mechanism for the conversion of CH_3–S bond into methane involves a ternary complex of methyl coenzyme M and coenzyme B fit into a channel terminated by the axial site on nickel of the cofactor F430. One proposed mechanism invokes electron transfer from Ni(I) (to give Ni(II)), which initiates formation of CH_4. Coupling of the coenzyme Mthiylradical ($RS^{\cdot}$) with HS coenzyme B releases a proton and re-reduces Ni (II) by one-electron, regenerating Ni(I).

Reverse methanogenesis

Some organisms can oxidize methane, functionally reversing the process of methanogenesis, also referred to as the anaerobic oxidation of methane (AOM). Organisms performing AOM have been found in multiple marine and freshwater environments including methane seeps, hydrothermal vents, coastal sediments and sulfate-methane transition zones. These organisms may accomplish reverse methanogenesis using a nickel-containing protein similar to methyl-coenzyme M reductase used by methanogenicarchaea. Reverse methanogenesis occurs according to the reaction:

$$SO_4^{2-} + CH_4 \rightarrow HCO_3^- + HS^- + H_2O$$

Importance in carbon cycle

Methanogenesis is the final step in the decay of organic matter. During the decay process, electron acceptors (such as oxygen, ferric iron, sulfate, and nitrate) become depleted, while hydrogen (H_2) and carbon dioxide accumulate. Light organics produced by fermentation also accumulate. During advanced stages of organic decay, all electron acceptors

become depleted except carbon dioxide. Carbon dioxide is a product of most catabolic processes, so it is not depleted like other potential electron acceptors.

Only methanogenesis and fermentation can occur in the absence of electron acceptors other than carbon. Fermentation only allows the breakdown of larger organic compounds, and produces small organic compounds. Methanogenesis effectively removes the semi-final products of decay: hydrogen, small organics, and carbon dioxide. Without methanogenesis, a great deal of carbon (in the form of fermentation products) would accumulate in anaerobic environments.

Natural occurrence

In ruminants

Testing Australian sheep for exhaled methane production (2001), CSIRO

Enteric fermentation occurs in the gut of some animals, especially ruminants. In the rumen, anaerobic organisms, including methanogens, digest cellulose into forms nutritious to the animal. Without these microorganisms, animals such as cattle would not be able to consume grasses. The useful products of methanogenesis are absorbed by the gut, but methane is released from the animal mainly by belching (eructation). The average cow emits around 250 liters of methane per day. In this way, ruminants contribute about 25% of anthropogenic methane emissions. One method of methane production control in ruminants is by feeding them 3-nitrooxypropanol.

In humans

Some humans produce flatus that contains methane. In one study of the feces of nine adults, five of the samples contained archaea capable of producing methane. Similar results are found in samples of gas obtained from within the rectum. Even among humans whose flatus does contain methane, the amount is in the range of 10% or less of the total amount of gas.

In plants

Many experiments have suggested that leaf tissues of living plants emit methane. Other research has indicated that the plants are not actually generating methane; they are just absorbing methane from the soil and then emitting it through their leaf tissues.

Soils

Methanogens are observed in anoxic soil environments, contributing to the degradation of organic matter. This organic matter may be placed by humans through landfill, buried as sediment on the bottom of lakes or oceans as sediments, and as residual organic matter from sediments that have formed into sedimentary rocks

Biohydrogen

Biohydrogen is H_2 that is produced biologically. Interest is high in this technology because H_2 is a clean fuel and can be readily produced from certain kinds of biomass. Many challenges characterize this technology, including those intrinsic to H_2, such as storage and transportation of a noncondensible gas. Hydrogen producing organisms are poisoned by O_2. Yields of H_2 are often low

Biochemical principles

The main reactions involve fermentation of sugars. Important reactions start with glucose, which is converted to acetic acid:[3]

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3CO_2H + 2\ CO_2 + 4\ H_2$$

A related reaction gives formate instead of carbon dioxide:

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3CO_2H + 2\ HCO_2H + 2\ H_2$$

These reactions are exergonic by 216 and 209 kcal/mol, respectively.

H_2 production is catalyzed by two hydrogenases. One is called [FeFe]-hydrogenase; the other is called [NiFe]-hydrogenase. Many organisms express these enzymes. Notable examples are members of the genera Clostridium, Desulfovibrio, Ralstonia, and the pathogen *Helicobacter*. *E. coli* is the workhorse for genetic engineering of hydrogenases.

It has been estimated that 99% of all organisms utilize dihydrogen (H_2). Most of these species are microbes and their ability to use H_2 as a metabolite arises from the expression of H_2 metalloenzymes known as hydrogenases. Hydrogenases are sub-classified into three different

types based on the active site metal content: iron-iron hydrogenase, nickel-iron hydrogenase, and iron hydrogenase

Production by algae

The **biological hydrogen production** with algae is a method of photobiological water splitting which is done in a closed photobioreactor based on the production of hydrogen as a solar fuel by algae. Algae produce hydrogen under certain conditions. In 2000 it was discovered that if *C. reinhardtii* algae are deprived of sulfur they will switch from the production of oxygen, as in normal photosynthesis, to the production of hydrogen.

Photosynthesis

Photosynthesis in cyanobacteria and green algae splits water into hydrogen ions and electrons. The electrons are transported over ferredoxins. Fe-Fe-hydrogenases (enzymes) combine them into hydrogen gas. In *Chlamydomonas reinhardtii* Photosystem II produces in direct conversion of sunlight 80% of the electrons that end up in the hydrogen gas. Light-harvesting complex photosystem II light-harvesting protein LHCBM9 promotes efficient light energy dissipation. The Fe-Fe-hydrogenases need an anaerobic environment as they are inactivated by oxygen. Fourier transform infrared spectroscopy is used to examine metabolic pathways.

Specialized chlorophyll

The chlorophyll (Chl) antenna size in green algae is minimized, or truncated, to maximize photobiological solar conversion efficiency and H_2 production. The truncated Chl antenna size minimizes absorption and wasteful dissipation of sunlight by individual cells, resulting in better light utilization efficiency and greater photosynthetic productivity by the green alga mass culture.

Economics

It would take about 25,000 square kilometre algal farming to produce biohydrogen equivalent to the energy provided by gasoline in the US alone. This area represents approximately 10% of the area devoted to growing soya in the US.

Bioreactor design issues

- Restriction of photosynthetic hydrogen production by accumulation of a proton gradient.
- Competitive inhibition of photosynthetic hydrogen production by carbon dioxide.
- Requirement for bicarbonate binding at photosystem II (PSII) for efficient photosynthetic activity.
- Competitive drainage of electrons by oxygen in algal hydrogen production.
- Economics must reach competitive price to other sources of energy and the economics are dependent on several parameters.
- A major technical obstacle is the efficiency in converting solar energy into chemical energy stored in molecular hydrogen.

Attempts are in progress to solve these problems via bioengineering.

Industrial hydrogen

Competing for biohydrogen, at least for commercial applications, are many mature industrial processes. Hydrogen is usually derived from fossil fuels by steam reforming of natural gas - sometimes referred to as steam methane reforming (SMR) - is the most common method of producing bulk hydrogen at about 95% of the world production.

$CH_4 + H_2O \rightleftharpoons CO + 3\ H_2$

Bioremediation refers to the use of microorganisms to degrade contaminants that pose environmental and human risks. Bioremediation processes typically involve the actions of many different microbes acting in parallel or sequence to complete the degradation process. Both in situ (in place) and ex situ (removal and treatment in another place) remediation approaches are used. The versatility of microbes to degrade a vast array of pollutants makes bioremediation a

technology that can be applied in different soil conditions. Though it can be inexpensive and in situ approaches can reduce disruptive engineering practices, bioremediation is still not a common practice.

A widely used approach to bioremediation involves stimulating naturally occurring microbial communities, providing them with nutrients and other needs, to break down a contaminant. This is termed **biostimulation.** Biostimulation can be achieved through changes in pH, moisture, aeration, or additions of electron donors, electron acceptors or nutrients. Another bioremediation approach is termed **bioaugmentation**, where organisms selected for high degradation abilities are used to inoculate the contaminated site. These two approaches are not mutually exclusive- they can be used simultaneously.

Recent awareness of the dangers of many chemicals used in society has led to research on formulation of products that are more easily degraded in the environment.

From an ecological point of view, bioremediation depends on the various interactions between three factors: substrate (pollutant), organisms, and environment, as shown in the figure at right. The interactions of these factors affect biodegradability, bioavailability, and physiological requirements, which are important in assessing the feasibility of bioremediation **Biodegradability**, or whether a chemical can be degraded or not, is determined by the presence or absence of organisms that are able to degrade a chemical of interest and how widespread these organisms are in the site. The substrate (pollutant) can interact with its surrounding environment to change its **bioavailability**, or availability to organisms that are capable of degrading it; for example, substrate has low bioavailability if it is tightly bound to soil organic matter or trapped inside aggregates. **Physiological requirements**, or set of conditions required by organisms to carry out bioremediation in the environment, include nutrient availability, optimal pH, and availability of electron acceptors, such as oxygen and nitrate. Also, the environment needs to be habitable for organisms involved in bioremediation.

As stated previously, bioremediation involves various microorganisms that are able to degrade and reduce toxicity of environmental pollutants. Therefore, the interactions of microbes with the environment and pollutants are significant in determining effectiveness of

bioremediation. Those microbes can be either naturally present in the site of bioremediation or isolated from other sites and inoculated artificially. Biodegradation often occurs as part of microbial metabolism and in some cases, microbes are able to directly harvest carbon and energy by breaking down pollutants. Sections below go over bacteria and fungi, the commonly used organisms in bioremediation, and archaea, the more recently discovered group of organisms with unique potential in bioremediation.

Bacteria

Bacteria are widely diverse organisms, and thus make excellent players in biodegradation and bioremediation. There are few universal toxins to bacteria, so there is likely an organism able to break down any given substrate, when provided with the right conditions (anaerobic versus aerobic environment, sufficient electron donors or acceptors, etc.). Below are several specific bacteria species known to participate in bioremediation.

Pseudomonas putida

Pseudomonas putida is a gram-negative soil bacterium that is involved in the bioremediation of toluene, a component of paint thinner. It is also capable of degrading naphthalene, a product of petroleum refining, in contaminated soils.

Dechloromonasaromatica

Dechloromonasaromatica is a rod-shaped bacterium which can oxidize aromatics including benzoate, chlorobenzoate, and toluene, coupling the reaction with the reduction of oxygen, chlorate, or nitrate. It is the only organism able to oxidize benzene anaerobically. Due to the high propensity of benzene contamination, especially in ground and surface water, D. aromatic is especially useful for in situ bioremediation of this substance.

Nitrifiers and Denitrifiers

Industrial bioremediation is used to clean wastewater. Most treatment systems rely on microbial activity to remove unwanted mineral nitrogen compounds (i.e. ammonia, nitrite, and nitrate). The removal of nitrogen is a two stage process that involves nitrification and denitrification. During nitrification, ammonium is oxidized to nitrite by organisms like

Nitrosomonaseuropaea. Then, nitrite is further oxidized to nitrate by microbes like Nitrobacterhamburgensis.

In anaerobic conditions, nitrate produced during ammonium oxidation is used as a terminal electron acceptor by microbes like Paracoccusdenitrificans. The result is N2 gas. Through this process, ammonium and nitrate, two pollutants responsible for eutrophication in natural waters, are remediated.

Deinococcusradiodurans

Deinococcusradiodurans is a radiation-resistant extremophile bacterium that is genetically engineered for the bioremediation of solvents and heavy metals. An engineered strain of Deinococcusradiodurans has been shown to degrade ionic mercury and toluene in radioactive mixed waste environments.

In anaerobic conditions, nitrate produced during ammonium oxidation is used as a terminal electron acceptor by microbes like Paracoccusdenitrificans. The result is dinitrogen gas. Through this process, ammonium and nitrate, two pollutants responsible for eutrophication in natural waters, are remediated.

Methylibiumpetroleiphilum

Methylibiumpetroleiphilum (formally known as PM1 strain) is a bacterium capable of methyl tert-butyl ether (MTBE) bioremediation. PM1 degrades MTBE by using the contaminant as the sole carbon and energy source.

Alcanivoraxborkumensis

Alcanivoraxborkumensis is a marine rod-shaped bacterium which consumes hydrocarbons, such as the ones found in fuel, and produces carbon dioxide. It grows rapidly in environments damaged by oil, and has been used to aid in cleaning the more than 830,000 gallons of oil from the Deepwater Horizon oil spill in the Gulf of Mexico.

Fungi (Mycoremediation)

Current bioremediation applications primarily utilize bacteria, with comparatively few attempts to use fungi. Fungi have fundamentally important roles because of their participation in the cycling of elements through decomposition and transformation of organic and inorganic materials. These characteristics can be translated into applications for bioremediation which could break down organic compounds and reduce the risks of metals. In some cases, fungi have an advantage over bacteria not just in metabolic versatility but also their environmental resilience. They are able to oxidize a diverse amount of chemicals and survive in harsh environmental conditions such as low moisture and high concentrations of pollutants. Therefore, fungi are potentially an extremely powerful tool in soil bioremediation and some versatile species such as White Rot Fungi have been a hot topic of research.

Biodegradation Capacities of White rot fungi

Using fungi as potential treatment of contaminants began in 1985 when the white rot species Phanerochaetechrysosporium was discovered to metabolize multiple key environmental pollutants. The most important feature of these fungi is their enzymatic functional ability to metabolize complex chemicals such as lignin. Similar abilities were later discovered in other white rot fungal species. In addition, white rot fungi are highly advantageous because they degrade lignin extracellularly through its hyphal extension. This allows them to access soil contaminants that other organisms are incapable of and maximize surface area for enzymatic interaction. These inexpensive fungi can tolerate extreme environmental conditions, such as pH, temperature, and moisture content. While many microbial organisms that are used for bioremediation require pre-conditioning of the environment for them to survive in, white rot fungi can directly be applied into most systems because they degrade based upon nutrient deprivation.

Phanerochaete chrysosporium

P. chrysosporium was the first fungi linked to degradation of organic pollutants. Extensive research has showed this it has strong potential for bioremediation in pesticides, PAHs, dioxins, carbon tetrachloride, and many other pollutants. Among fungal systems, P.

chrysosporium has become the model for bioremediation. Other notable species of white rot fungi include Pleurotusostreatus and Trametesversicolor.

Bioremediation of Hydrocarbon Pollutants

Hydrocarbons are stored deep underground but are brought up to the surface to be transformed and utilized, primarily as an energy source known as fossil fuels. The majority of pollution currently comes from these byproducts in the form Polycyclic Aromatic Hydrocarbons (PAHs), which are xenobiotic environmental pollutants that form when carbon materials are incompletely combusted. Some of examples of PAHs include burning wood, fossil fuels, and cigarette smoke. Currently, bioremediation is only effective for soils contaminated with low-molecular weight PAHs because of bacterial commercial use. However, fungi are effective at PAH degradation in comparison to bacteria for a few reasons. Firstly, they are capable degrading PAH's that are high in molecular weight, bacteria in comparison are better at degrading smaller molecules. Secondly, fungi can function well in non-aqueous environments and low oxygen conditions, both are conditions where PAH's can accumulate. Many fungi have evolved mechanisms that allow them to target specific PAHs. Fungi produce extracellular enzymes that degrade lignin, a process called mineralization the produces carbon dioxide as the end product.

Remediating Metals

Toxic metals can enter the environment all life cycle stages of metal compound. For example, metal leaching can occur from the mining process till the disposal of metal wastes. However in nature, the mobility of metals comes from the geological processes that can be released into the soil and aquatic environments. The environmental largest risk from metal contamination comes from the relationship between metals and compounds that are inherently of incapable of being degraded by any natural procedures. The best solution to treating contamination is transporting the metals to location where they cannot produce negative environmental effects. Fungi have various ways of interacting with metals, some of the techniques are increasing or decreasing the mobility of metals, sorption, or even cellular uptake. After the metals have been absorbed the fungus, they can chemically altered to be stored or translocated through the hyphae and into various plants that participate in symbiosis.

Pesticide Degradation

Pesticide accumulation is an issue of great concern among the public, because they are directly associated with food products and water supplies. There are number of technologies used for pesticide clean-up; however, these technologies are generally expensive and inefficient because they require contaminated soil to be excavated and sent to a separate storage location for processing. Bioremediation offers a potential solution that treats contaminated soil and groundwater without needing excavation. Studies show that White Rot Fungi has high promise for soil bioremediation application; however, most tests have been conducted in the lab rather than in the actual environment. This fungi demonstrates the ability to transform and mineralize specific pesticides in soil.

Environmental Applications

Although fungi demonstrate significant biochemical and ecological useful qualities, they are hardly utilized for biotechnological purposes. Instead, bacteria are most commonly used because they usually produce superior results in their numerous advantages ranging from their highly specific biochemical reactions to their capabilities of breaking down pollutants efficiently. Fungi are underused primarily because of the costs that come from providing oxygen to fungi in polluted environments. However, filamentous fungi could be highly valuable in situations where bacteria cannot perform. For example, fungi are useful in situations where contaminants are physically blockaded and bacteria cannot reach or in circumstances of environmental extremes such as high acidity or dryness prevent bacteria from functioning.

Archaea

The role of archaea in bioremediation has not been studied as commonly as that of bacteria. Nevertheless, numbers of researchers have shown their ability to degrade various pollutants and scientists began to discover more about their potential in participating in bioremediation. Below lists some important facts regarding archaea's potential role in bioremediation.

- Biodegradation by extreme halophilicarchaea was not recognized widely in the past, but scientists have found out that extreme halophilicarchaea have greater catabolic diversity than expected.

- Hydrocarbon-contamination is observed in some extreme environments, including hypersaline (high salt concentration), high or low temperature, or extreme pH. Archaea's adaptation to extreme environment gives them the potential to participate in biodegradation and bioremediation in these environments; in fact, microorganisms naturally adapted to the cold environments are known to be important degraders of hydrocarbons in those environments.

- Extreme halophilicarchaea has potential to biodegrade pollutants in hypersaline environment, in which bacteria typically used in bioremediation cannot survive or function properly.

- Some archaea are known to be resistant to variety of antibiotics, including penicillin, cycloheximide, streptomycin, etc, which gives them great advantage in participating in bioremediation in the presence of antibiotics.

Examples of studies of Archaea involved in bioremediation

Four extreme halophilic strains of archaea (belonging to genus Halobacterium, Haloferax, and Halococcus) were studied to evaluate their potential to biodegrade crude oil and hydrocarbons. All four strains could use various kinds of hydrocarbons as their carbon or energy sources. Two strains of Haloferax grew on n-alkanes with different lengths, ranging from C8 to C34, and also benzene, toluene, biphenyl, and naphthalene. The research demonstrated the important fact that archaea have potential to carry out biodegradation at high temperatures, in the range of 40-45 °C, which is advantageous because hydrocarbons have higher solubility and bioavailability at these higher temperature. The four strains studied were resistant to six different antibiotics, including penicillin, streptomycin, cycloheximide and this gave them the potential to carry out biodegradation in conditions unfavorable for bacteria. Research suggests other genera of archaea are also capable of biodegrading in hypersaline environments

Archaeglobusfulgidus, a hyperthermophile which can use sulfate as an electron acceptor, can also break down various aromatic hydrocarbons.

Microbial Processes

Microorganisms use a wide range of processes to transform chemicals in their environment. In some cases, pollutants serve as the carbon and energy source for microbial growth, while in other cases, pollutants serve as the terminal electron acceptor. This manifests itself in the diverse ability of microbes to transform and degrade toxic molecules. Below, several steps and details of the microorganisms' actions are described.

Introduction: General Features of the Microbial Degradation of Xenobiotics

Biodegradation, Biotransformation, and Co-metabolism

More than ten million organic compounds are generated by biosynthetic pathways in animals, plants, and microorganisms, by other natural processes, and by industrial synthesis. Whilst the organic structures found in nature are created by many organisms and processes, microorganisms (bacteria and fungi) perform most of the biodegradation of both natural products and industrial chemicals. Collectively, microorganisms play a key role in the biogeochemical cycles of the Earth. The substances transformed or degraded by microorganisms are used as a source of energy, carbon, nitrogen, or other nutrient, or as final electron acceptor of a respiratory process [see also - Cell thermodynamics and energy metabolism]. 'Biodegradation' involves the breakdown of organic compounds, usually by microorganisms, into biomass and less complex compounds, and ultimately to water, carbon dioxide, and the oxides or mineral salts of other elements present. The complete breakdown of an organic compound into inorganic components is termed 'mineralization', but '(ultimate/complete) biodegradation' and '(complete) mineralization' are often used interchangeably, although 'biodegradation' involves the formation of biomass as well as inorganic compounds. Of course, biomass finally will also undergo mineralization. Degradation of an organic compound to a less complex organic compound is referred to as 'incomplete (partial) biodegradation'. 'Biotransformation' is the metabolic modification of the molecular structure of a compound, resulting in the loss or alteration of some characteristic properties of the original compound, with no (or only minor) loss of molecular complexity. Biotransformation may effect the solubility, mobility in the environment, or toxicity of the organic compound. A microbial population growing on one compound may fortuitously

transform a contaminating chemical that cannot be used as carbon and energy source, a process referred to as 'co-metabolism'. The phenomenon has also been called 'co-oxidation' and 'gratuitous' or 'fortuitous' metabolism. Usually, the primary substrate induces production of (an) enzyme(s) that fortuitously alter(s) the molecular structure of another compound. The organisms do not benefit from the co-metabolic process. Co-metabolic transformation may result in a minor modification of the molecule, or it may lead to incomplete or even complete degradation.

Xenobiotics

Xenobiotics (greekxenos = strange, foreign, foreigner) are chemically synthesized compounds that do not occur in nature and thus are 'foreign to the biosphere'. They have 'unnatural' structural features to which microorganisms have not been exposed to during evolution. Xenobiotics may resist biodegradation, or they undergo incomplete UNESCO – EOLSS SAMPLE CHAPTERS BIOTECHNOLOGY – Vol X -- Biodegradation of Xenobiotics - S. Fetzner ©Encyclopedia of Life Support Systems (EOLSS) biodegradation or just biotransformation. The definition of xenobiotics as compounds 'foreign to life' exhibiting 'unnatural' structural features does not necessarily imply that xenobioticsare toxic compounds, but many xenobiotics indeed are harmful to living organisms. Whereas xenobiotics may persist in the environment for months and years, most biogenic compounds are biodegraded rapidly. Exceptions are lignin, the structural polymer of woody plants, and, above all, the melanin polymers which are constituents of the cell wall of the spores of a number of fungi. Recalcitrance (i.e., the structure immanent stability) of a xenobiotic molecule is mainly due to 'unphysiological' chemical bonds and/or substituent, which block the attack by microbial catabolic enzymes (see Table 1 and Figure 2). Type, number and position of bonds and substituent affect the xenobiotics character. However, it is not always easy to determine which structural moieties indeed are xenobiotic in the sense of 'foreign to life'. Some natural compounds show principally the same unusual structural features as xenobiotics, such as halogen substituent or nitro groups found in some antibiotics, or they contain stable chemical bonds like the ether and carbon-carbon bonds stabilizing lignin. Moreover, microorganisms throughout geological time have also been exposed to a variety of chemicals produced by abiotic natural processes:

Removal of heavy metals

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake. Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents. The major advantages of biosorption over conventional treatment methods include

- **Low cost;**
- **High efficiency;**
- **Minimisation of chemical and biological sludge;**
- **No additional nutrient requirement;**
- **Regeneration of biosorbents; and**
- **Possibility of metal recovery.**

The biosorption process involves a solid phase (sorbent or biosorbents; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbet (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases.

Biosorbent material: Strong bio-sorbent behaviour of certain micro-organisms towards metallic ions is a function of the chemical make-up of the microbial cells. This type of bio-sorbent consists of dead and metabolically inactive cells.

Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms and some have also processed the existing raw biomass to a certain degree to improve their biosorption properties;

Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations. For e.g. the waste mycelia available from fermentation processes, olive mill solid residues

What Is a Patent?

A patent safeguards an original invention for a certain period of time and is granted by the United States Patent and Trademark Office (USPTO). By granting the right to produce a product without fear of competition for the duration of the patent, incentive is provided for companies or individuals to continue developing innovative new products or services**.**

There are three types of patents: utility patents, plant patents and design patents.

Utility Patent

A utility patent covers the creation of a new or improved product, process or machine. Also known as a "patent for invention," it bars other individuals or companies from making, using or selling the creation without consent. Utility patents are good for up to 20 years after the patent application is filed, but require the holder to pay regularly scheduled maintenance fees.

While most people associate patents with machines and appliances, they can also apply to software, business processes and chemical formulations such as in pharmaceutical products.

Plant Patent

A plant patent protects a new and unique plant's key characteristics from being copied, sold or used by others. It is also good for 20 years after the application is filed. The plant must be

asexually reproducible with reproduction being genetically identical to the original and performed through methods such as root cuttings, bulbs, division, or grafting and budding.

Design Patent

A design patent, on the other hand, applies to the unique look of a manufactured item. Take, for example, an automobile with a distinctive hood or headlight shape. These visual elements are part of the car's identity and may add to its value. However, without protecting these components with a patent, competitors could potentially copy them without legal consequences.

Design patents issued since May 2015 last for 15 years from the date the patent is granted and do not require maintenance fees. Patents issued prior to that last for 14 years.

What Is a Trademark?

Unlike patents, a trademark protects words and design elements that identify the source of a product. Brand names and corporate logos are primary examples. A service mark is similar, except that it safeguards the provider of a service instead of a tangible good. The term "trademark" is often used in reference to both designations.

Some examples of trademark infringement are fairly straightforward. You'll probably run into trouble if you try to bottle a beverage and call it Coca-Cola – or even use the famous wave from its logo – since both have been protected for decades.

However, a trademark actually goes a bit further, prohibiting any marks that have a "likelihood of confusion" with an existing one. Therefore, a business can't use a symbol or brand name if it looks similar, sounds similar or has a similar meaning to one that's already on the books – at least if the products or services are related. If the trademark holder believes there's a violation of these rights, it may decide to sue.

What Is a Copyright?

Copyrights protect "works of authorship," such as writings, art, architecture and music. For as long as the copyright is in effect, the copyright owner has the sole right to display, share,

perform or license the material. One notable exception is the "fair use" doctrine, which allows some degree of distribution of copyrighted material for scholarly, educational or news-reporting purposes.

Technically, you don't have to file for a copyright to have the piece of work protected. It's considered yours once your ideas are translated into a tangible form, such as a book, music or published research. However, officially registering with the U.S. Copyright Office before – or within five years of – publishing your work makes it a lot easier to establish that you were the original author if you ever have to go to court.

The duration of a copyright depends on the year it was created, as the laws have changed over the years. Since 1978, most compositions have been copyright-protected for 70 years after the author's death. After that time, individual works enter the public domain and can be reproduced by anyone without permission.

As a general rule, the author retains ownership of copyright privileges, even if the material is published by another company. There is an important exception to this rule, though. Materials you create for your employer as part of your job requirements – for example, contributions to a podcast the company publishes – are usually considered "works for hire." The employer, not you, retains the copyright. If there's a gray area, you can try to negotiate with the publisher over copyright ownership prior to creating the piece – just be sure to get it in writing.

Patenting microorganisms:

A genetically engineered strain of the bacterium Pseudomonas called superbug was the first micro-organism to be patented. Superbug is capable of breaking down the multiple impurity of crude oil. It took almost 10 years of procedural delays, and legal battles to get the superbug patented. Now, many microorganisms are patented.

Patenting multicellular organisms:

A genetically manipulated mouse namely the oncomouse was the first and probably, to date the only animal to be patented in both USA and UK. This transgenic mouse carries a gene that makes it susceptible to tumor formation.

Patenting genes?

The human genome projects (HGPs) have elucidated almost the entire sequence of human genome, besides identifying the genes. Patenting of DNA sequences and genes has become a controversial and debatable issue. Some researchers and institutions in fact have approached the patent offices for grant of patents. So far, patents have not been granted for genes. The most important reason being that the genes are natural, universal and inherent biological functional units of all individuals.

Patenting and cell/tissue donor's rights:

While dealing with human tissues/cells, the involvement of the donor of source material becomes very important. John Moore was a victim of hairy-cell leukemia, a rare form of cancer. His spleen was removed, and Mo cell lines developed. These cell lines were patented by the researchers and their associated organizations. Moore however was not involved in the patenting. Moore filed a suit in the court on the ground that he should also be a party to the profits derived by using his tissue. The court allowed his claim to share the profits.

Plant Breeders' Rights:

Agriculture for the first time was included (in 1994) in the trade-related intellectual property rights (TRIPS). TRIPS is a major concern for developing countries. The plant varieties in many countries (not in India) are protected through plant breeders' rights (PBR) or plant variety rights (PVR).

PVR provides legal protection to the original breeder or owner of the plant variety. It is believed that PBR will encourage innovative research and plant breeding programmes, in view of the expected financial returns.

Plant breeders' rights are comparable to the patent rights. Under TRIPS agreement, plant breeder possesses the exclusive rights over the plant developed. It prevents the third parties from using the plant without the owner's consent.

Although PBR will increase competition and development of good plant varieties, the owner's motto would be profit making. Therefore, PBR will prohibit the free exchange of plant

materials, besides threatening the farmer's rights. It is feared that PBR will benefit rich farmers and developed countries only.

Patenting and Biotechnology Research:

In the earlier years, research used to be mostly for academic interest and worthwhile scientific contributions. And researchers were purely academic-oriented with not much interest in financial gains from what they do. Further, unlike now, the research used to be supported mostly by Governments/Universities.

There used to be no secrecy in research. Academic recognition and outstanding research publications (open to all) were adequate to satisfy the researchers. Watson and Crick who discovered DNA structure never had a thought of patenting!

The situation has now changed. A good proportion of research is either being conducted or financially supported by private companies/ universities. It is quite natural for these companies to expect returns for their investments. There is a lot of change in the attitude of researchers also. For many scientists, financial gains have become more important than academic recognition. Consequently, some people carry out research secretly and opt for patenting of their discoveries rather than publishing.

We have to accept the fact that the progress of research is usually much faster in private companies compared to many government organizations. This may be due to the higher financial returns and efficient administration. Probably, the biotechnological research might not have progressed to the same extent as it is today without the dreams of getting the patents by scientists and research funding organizations.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: III B.Sc MB
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COURSE NAME: MICROBIAL BIOTECHNOLOGY
BATCH-2017-2020

UNIT-5						
S.No	**Questions**	**A**	**B**	**C**	**D**	**ANSWERS**
1	Biofuels are also known as_____	Biodiesel	Argofuels	Biomass	Fossil fuel	Argofuels
2	The biofuel made by corn _____	Ethyl alcohol	Argofuels	oil	Bioethanol	Ethyl alcohol
3	Modern usage of the term IPR began in_____	1987	1977	1967	1955	1967
4	In 1957,which agreement was approved for copy right act_____	TRIPS	PPVER	IPR	PCT	TRIPS
5	In 1994,which act was amended_____	Trade mark	Trade secrete	Patent	Copy right	Copy right
6	Fossil fuels are made of _____	long dead organic matter	recently dead organic matter	resource	Biofuels	long dead organic matter
7	Biofuels contain_____ renewable materials	70%	60%	15%	80%	80%
8	Biofuel is a form of recycled fuel produced from_____	fossil fuel	Biomass	Biodiesel	oil	Biomass
9	Bioethanol is an alcohol made by fermenting the _____ of biomass	plant matter	starch	sugar components	feed-stocks	sugar components
10	The main reaction for converting oil to biodiesel is called_____	Biodiesel production	trans-esterification	fermentation	hydrolysis	trans-esterification

Prepared by R.Dineshkumar, Assistant Professor, Dept of Microbiology, KAHE.

11	_____is the chemical reaction that converts complex polysaccharides into simple sugars	Hydrolysis	fermentation	Biodiesel production	Methanogenesis	Hydrolysis
12	_____is a chemical reaction that converts sugar to ethanol	Hydrolysis	esterification	fermentation	Biodiesel production	fermentation
13	_____is a mixture of fatty acid alkyl esters	Bioethanol	Biodiesel	Biofuels	fossil fuels	Biodiesel
14	Methanogenesis otherwise called as_____	biomethanation	Biodiesel	AOM	Bioethanol	biomethanation
15	Some organisms can oxidise methane, functionally revering the process of methanogenesis,is also called as	AOM	TRIPS	PPFR	PPVR	AOM
16	_____is the final step in the decay of organic matter	Methanogenesis	Biodiesel	Bioethanol	Hydrolysis	Methanogenesis
17	The average cow units around _____ liters of methane per day	250	240	120	100	250
18	Hydrogen producing organisms are poisoned by _____	O2	H2	N2	NH3	O2

19	The _____with algae is a method of photobiological water splitting which is done in photobioreacter	Biological hydrogen production	photosynthesis	Methanogenesis	Biodiesel production	Biological hydrogen production
20	Trade mark uses symbol in _____	Intellectual committee	Intellectual property	Indian committee	Indian property	Intellectual property
21	Trade secrets protects _____ of industries	Trade information	Machinery information	confidential information	Tribunal information	confidential information
22	In which year patent act was published_____	1945	1990	1999	2000	1999
23	copy rights prevents copying and _____	Reproduction	Construction	Development	Tradition	Reproduction
24	_____refers to the use of Mo's to degrade contaminants in environment	Biostimulation	Bioremediation	Bioethanol	Methane	Bioremediation
25	Stimulating naturally occuring microbial communities,to break down a contaminant is by_____	Bioremediation	Biostimulation	Methane	Reproduction	Biostimulation
26	Organisms selected for high degradation abilities to inoculate the contaminated	Biostimulation	Bioagumentation	Biodegradation	Biodiesel	Bioagumentation

	site is termed as_____					
27	_____are widely spread organisms,that make excellent players in biodegradation and bioremediation	Bacteria	virus	yeast	Actinomycetes	Bacteria
28	*pseudomonas putida*are capable of degrading_____	plastic	Diesel	oil	naphthalene	napthalene
29	*pseudomonas putida is a*_____	gram positive	gram negative	Agrobacter	Fungi	gram negative
30	Dechloromonasaromatica is a_____bacterium	rod shape	cocci	vibrio	spherical	rod shape
31	Which of the following are not a nitrogenous compounds,	Ammonia	Nitrates	Nitrites	Acid	Acid
32	_____ degrades MTBE by using the contaminant as the sole carbon and energy source	PMI	PPR	PPVER	PPER	PMI
33	Bioremediation applications primarily utilize fungi is called _____	Mycorem ediation	Biodegradation	Bioremediation	Degradation	Mycoremediation
34	Using fungi as a potential treatment of contaminants began in_____	1975	1969	1983	1985	1985

35	_____was the first fungi linked to degradation of organic pollutants	yeast	molds	Actinomycetes	*p.chrysosporium*	*p.chrysosporium*
36	_____ are xenobiotic environmental pollutants that form when carbon materials are incompletely combusted	PAH	PPR	PPVR	PPFR	PAH
37	Extreme halophilic strains of archae were studied to evaluate their potential to biodegrade_____	crude oil	oil	Biodiesel	Biofuels	crude oil
38	The complete breakdown of an organic into inorganic components is termed as _ _____	mineralization	Biodegradation	Biotransformation	co-metabolism	mineralization
39	_____are chemically synthesized compounds that do not occur in nature	Phenobiotics	Sludge	reproduction	xenobiotics	xenobiotics
40	Trade secrecy applicable rather than patents in _____	Fermentation	drugs	chemicals	invitro-fertilization	Fermentation
41	Not possible to get patents for_____	plastic surgery	modified plants	DNA sequences	modified Mo's	plastic surgery
42	In TRIPS appellations of origin are covered in _____	patents	Trade mark	Geographical indications	Copy rights	Geographical indications

43	WIPO stands for _____	Word intellectual property organization	World intellectual property organization	Word intellectual property origin	World intellectual property origin	World intellectual property organization
44	_____is a milk clotting proteolytic enzymes that hydrolyses the K-casein protein of milk	Chymosin	renin	both a & b	Casein	Chymosin
45	Milk clotting activity found in_____	Chymosin	Lipin	Casein	Protein	Chymosin
46	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
47	_____is an important example for gene piracy	GM *pseudomonas*	*Pentacliplandrabrazzeana*	*clostridium*	*Bacillus*	*Pentacliplandrabrazzeana*
48	Recombinant DNA technology,were known by several phases such as	playing god	manipulation of life	man made evolution	all the above	all the above
49	_ _____process involves a solid and liquid phase	Biosorption	fermentation	bioremediation	purification	Biosorption
50	A patent safeguards an original invention for a	USPTO	WHO	FDA	NIH-RAC	USPTO

	certain period of time and is granted by the _____					
51	A_____ covers the creation of a new or improved product, process or machine	Utility patent	palnt patent	Trade mark	Copy right	Utility patent
52	Utility patent is otherwise called as_____	Patent for invention	Patent for reproduction	Patent for degradation	Patent for fermentation	Patent for invention
53	A genetically engineered strain of the bacterium _ is called superbug	*S.aureus*	*clostridium*	*pseudomonas*	*proteus*	*pseudomonas*
54	Recombinant bovine somatrotropin (BST)which also known as_____	Bovine growth harmone	Bovine releasing harmone	Bovine serum	none of the above	Bovine growth harmone
55	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%
56	_____protects confidental information of the industry with commercial values	Patent rights	patent control term	Trade mark	Trade secrets	Trade secrets
57	Agriculture was included for the first rime in TRIPS in_____	1994	1999	1996	1992	1994

58	____ provides legal protection to the original breeder or owner of the plant variety	PVR	PPER	PGBR	PPR	PVR
59	In 1998 the first patent was given to	Living organism	Hybrid plant	Plant	Fungi	Living organism
60	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