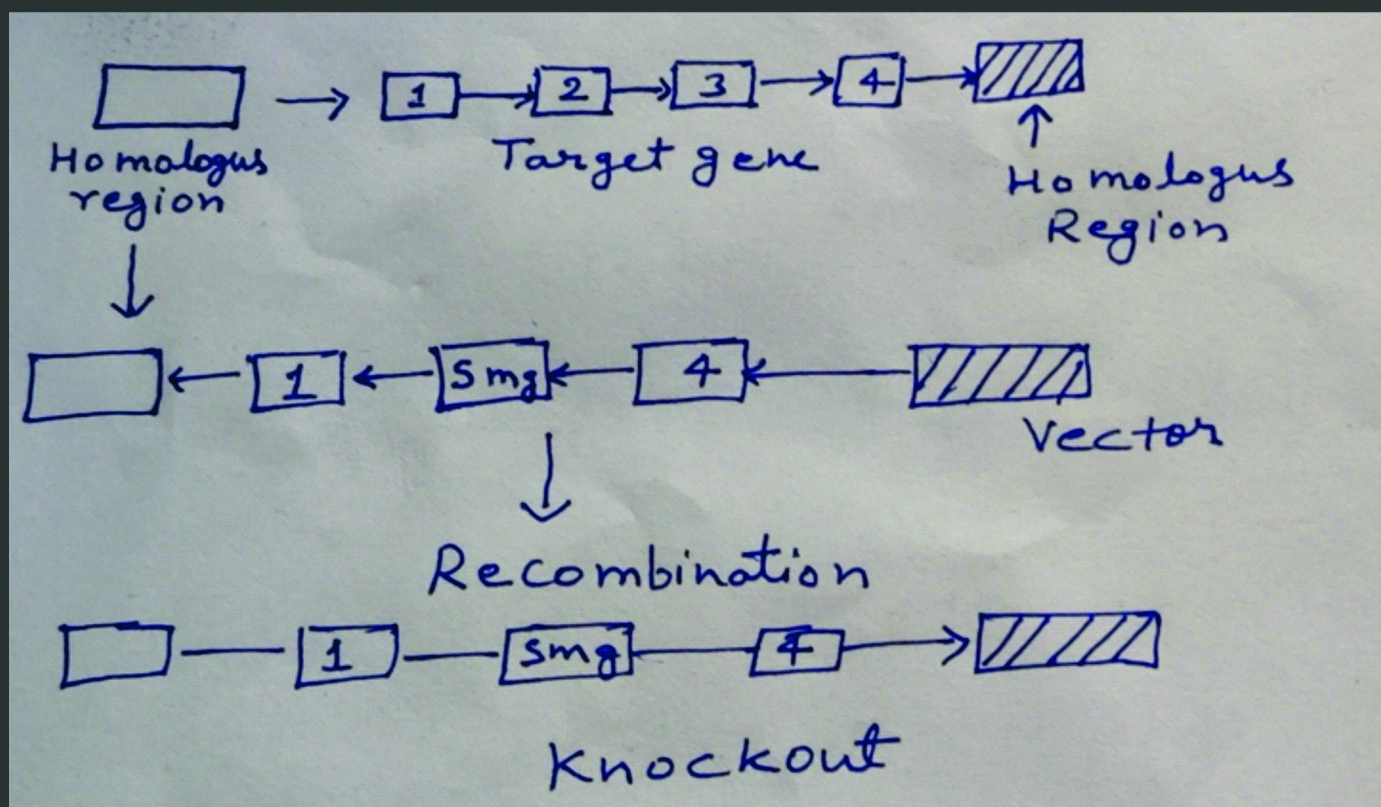




Basics of Industrial Biotechnology

Volume 1



JV'n Dr. Ritu Singh Rajput

JAYOTI VIDYAPEETH WOMEN'S UNIVERSITY, JAIPUR

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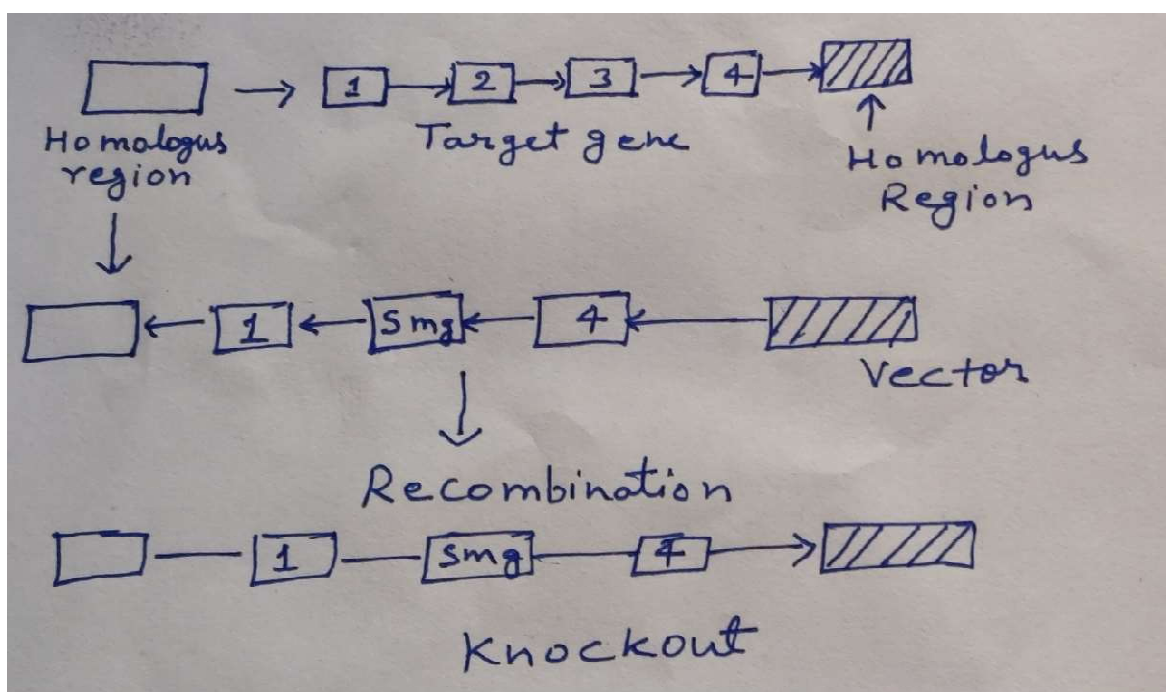
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Basics of Industrial Biotechnology Volume 1

By

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Chapter 1:

Introduction of Industrial Biotechnology

One of the most exciting new approaches to pollution control, resource management, and cost reduction is industrial biotechnology. It is commonly referred to in biotechnology as the third wave. Industrial biotechnology will have a greater effect on the world than health care and agricultural biotechnology if built to its full potential. This provides corporations with a means of lowering costs and developing new opportunities while protecting the environment. It is also a simpler, smoother route to the market since many of its products do not entail the long approval cycles that drug products must undergo. Today, modern manufacturing technologies, compared to up to a decade for drugs, can be taken from laboratory research to commercial application in two to five years.

In addition to transforming the way we produce products, the application of biotechnology to manufacturing processes is also supplying us with new products that were not even conceivable a few years ago. Since industrial biotechnology is so recent, industry, policymakers, or consumers still do not know or understand its advantages well.

Industrial biotechnology has combined product enhancements with pollution reduction from the beginning. Nothing shows this better than the way industrial biotechnology solved the problems of phosphate water contamination caused by the use of phosphates in detergent for laundry in the 1970s. Enzymes that extract stains from clothing better than phosphates have been developed by biotechnology companies, allowing the substitution of a polluting substance with a non-polluting bio-based additive while enhancing the efficiency of the final product. This breakthrough significantly reduced phosphate-related algal blooms in surface waters across the globe, thus allowing customers to clean their clothes with lower temperatures of washing water and concomitant energy savings at the same time.

Currently, primitive industrial biotechnology dates from at least 6000 B.C. When grapes were fermented by Neolithic cultures to make wine, and microbial yeast was used by Babylonians to make beer. Over time, human awareness of fermentation has increased, allowing cheese, yoghurt, vinegar, and other food products to be made. In the 1800s, Louis Pasteur proved that the product of microbial activity was fermentation. Sir Alexander Fleming then extracted penicillin

from the mould in 1928. Large-scale fermentation methods were developed in the 1940s to manufacture industrial amounts of this miracle drug. However, not until after World War II did the revolution in biotechnology begin, giving rise to modern industrial biotechnology.

Industrial biotechnology has since developed enzymes for use in our everyday lives and for the manufacturing sector. Meat tenderizer, for example, is an enzyme, and enzymes are used in certain contact lens cleaning fluids to dissolve sticky protein deposits. The microbial processing of enzymes, which are complex proteins, is primarily involved in industrial biotechnology. In nature, these enzymes have evolved to be super-performing biocatalysts which facilitate and accelerate complex biochemical reactions. What make industrial biotechnology such a strong modern technology are these incredible enzyme catalysts.

In order to maximise and optimise existing biochemical pathways that can be used in manufacturing, industrial biotechnology requires interacting with nature. In three fields of research of detailed knowledge extracted from cells: genomics, proteomics, and bioinformatics, the industrial biotechnology revolution is based on a series of associated innovations. As a result, a large number of microorganisms ranging from bacteria, yeasts, and fungi to marine diatoms and protozoa can be applied by scientists with modern techniques.

To locate and enhance the enzymes of nature, modern biotechnology companies use many advanced techniques. Knowledge on microorganisms from genomic studies helps researchers draw on the abundance of genetic diversity in microbial populations. In the natural world, researchers first search for enzyme-producing microorganisms and then use DNA probes to search for genes producing enzymes with particular biocatalytic capabilities at the molecular level. When isolated, it is possible to classify and characterise certain enzymes for their ability to function in particular industrial processes. They can be enhanced with biotechnology techniques if appropriate. Because of the recent and drastic developments in biotechnology techniques, many biocatalytic instruments are increasingly becoming available for industrial applications. In many cases, biocatalysts or whole-cell processes are so recent that it is not yet clear to many chemical engineers and private sector product development specialists that they are eligible for deployment. This is a clear example of a "technology gap" where there is a difference between a new technology's availability and widespread use. To accelerate progress in creating more economic and sustainable manufacturing processes through the application of biotechnology, this

gap must be resolved. "New Biotech Tools for a Cleaner Environment" offers dramatic examples of what can be achieved with these powerful new tools. The aim of the report is to encourage greater interest in this powerful technology, to help close this technology gap, and to promote progress towards a more sustainable future.

Industrial Biotechnology: An Introduction to Industrial Biotechnology and it's Applications

In the processing of beer, wine, cheese, bread and other fermented goods, the very first expression of the industrial uses of biotechnology was found. Over the years, such applications in the food, chemical and pharmaceutical industries have grown to include a very large range of products. In addition to producing a host of products, genetic engineering and molecular biology have proven invaluable in the implementation of new and more efficient bioprocesses.

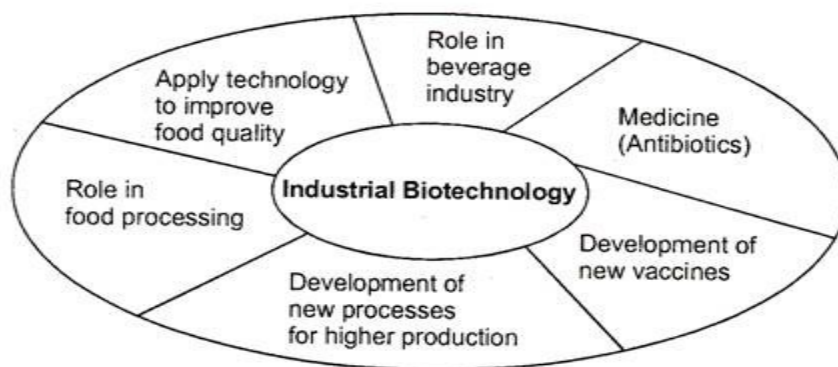


Diagram of Industrial biotech classes/groups

Biotechnology and Medicine:

In the medical sector, the use of biotechnology has opened up a whole new world of possibilities. In turn, this wide variety of applications has brought enormous potential to the medical industry. For example, in the case of oncogenes, different 'genetic markers' have been developed to identify breast, colon, bronchus, oesophagus and prostate malignancies. In light of gene suppression or activation, many psychological conditions that result in memory loss and aberrant behavior are now being understood.

These include dementia, such as schizophrenia and Alzheimer's disease (the latter is incurred by a single aberrant gene). Biotechnology also holds immense fertility control potential. Secure organ transplantation and immune system modulation have also been made possible by the body. Another invention that is precisely tailored to modify whole or sections of individual genes and to inhibit or trigger specific action is the designer drug.

Some of the other applications of biotechnology to medicine are:'

Antibiotics:

The most productive aspect of the pharmaceutical industry is the production of antibiotics. There are already more than a hundred antibiotics in use and many dreaded bacterial infections have been brought under control. Penicillin, tetracycline, cephalosporin and erythromycin are among the main classes of antibiotics.

Penicillin was discovered in 1928 by Fleming, and produced from a fungus called *Penicillium notatum* and later from *Pchrysogenum* by Howard in 1944. The largest amount of penicillin is released by penicillium when the cells stop developing.

For the highest yield, penicillin fermentation takes seven to eight days. The *Cephlosporium* fungus is used for the development of Cephalosporin C, an antibiotic that can destroy even penicillin-resistant bacteria. Streptomycin was discovered and manufactured from the *Streptomyces griseus* filamentous microbe.

Genes, as such, do not encode antibiotics explicitly. After a series of chemical reactions that are catalysed by enzymes, most of them are formed within the cell. The enzymes are assembled from unique gene instructions, and new antibiotics could be generated using cells. Cell fusion facilitates the generation of new gene combinations.

Genes that can instruct the cells to make new antibiotics may be present in the cell itself, but they cannot be expressed. By fusing these cells, this gene may be activated, new enzymes synthesized, and the resulting microbes can manufacture new antibiotics.

Therapeutic Applications:

As cancer cells are recognised as extraneous to the body, monoclonal antibodies produced against a specific type of cancer cell can lead to tumour regression. In order to begin attacking a tumour, monoclonal antibodies will activate the immune system of a patient. It is also possible to deliver anti-cancer drugs that are physiologically attached to monoclonal antibodies targeting particular cancer antigens directly against malignancy.

Autoimmune Disease:

This disease causes a breakdown in the resistance of the body to its own antigens, since both B and T cells respond to their own tissue antigens. In rheumatic fever, the body after an infection is immunized against tissues in the heart and joints. T-cell antigen monoclonal antibodies are also being used to research and treat many autoimmune disorders.

Prediction of Disease Risk:

Particular antigens on the cell surface (like those of human leukocytes) have been associated with the relative risk of occurrence of diseases like rheumatoid arthritis. Thus, early recognition of these antigens using monoclonal antibodies can facilitate suitable preventive measures.

Developing Recombinant Proteins for Medical and Therapeutic Use:

The recombinant proteins are expressed using different expression systems. Such systems of speech may be of yeast, insect, bacteria or viral origin. Prokaryotic expression vectors provide a convenient method for eukaryotic protein synthesis, but many of the immunogenic properties, 3D conformation and other features shown by normal eukaryotic proteins may be missing from the proteins.

Many of these constraints are solved by eukaryotic expression systems, including mammals, amphibians, plants, insects, and yeast. The method of mammalian cell expression presents difficulties in purifying recombinant proteins, including limitations on the size of the recombinant protein expressed and the mechanism of induction of protein expression. Using expression systems from insect and yeast cells, many of these weaknesses can be overcome.

Among the many substances produced using genetically modified bacteria are insulin, interferons, vaccines, blood proteins, and growth factors. Genetic engineering or recombinant DNA technology or genetic modification has allowed genes to be transferred from one organism to another, causing cells to generate materials that would not otherwise be generated, both cheaply and in large quantities. The creation by genetic modification of substances includes the injection into a microbe of the gene that codes for the protein (product) to be produced, which is capable of synthesising the product. The shaped substance can be subsequently stored.

Many essential biomedical substances have been developed and successfully applied with the advent of biotechnology. Initial penicillin G (benzyl penicillin), for example, has a relatively limited range of action against microorganisms and cannot be administered orally.

Semi-synthetic penicillin members are now generated by chemical or biological processes by eliminating and or substituting the side chain at different locations in the molecule. Penicillin differs from penicillin benzyl. On its side chain, it has an additional amino group that confirms a broader antibacterial spectrum and can be given orally. Penicillin acylase is the enzyme used to cleave the side chain, and is extracted from many bacteria, including *E.coli* and *Aspergillus repins*.

New Drug Targets and Production of Vaccines: - Several possible drug targets have been identified already. These include essential metabolic enzymes, growth factors, hormones, oncogene products, transmitter substances, neuropeptides, and different receptor proteins. To completely define them, the power of rDNA technology can be guided at these goals.

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Some of the examples are:

Insulin:

It is an important hormone regulating glucose levels.

Anti-hemophilic Factor:

It is an important material purified from human blood, and used in the treatment of hemophilia. Action has proved difficult because of infection of haemophiliacs with AIDS virus.

Human Serum Albumin:

It is one of the most common blood proteins used in the treatment of shock injuries such as burns.

Engineered Enzymes:

These enzymes are used to treat a range of conditions from cardiac diseases to renal failure, to certain types of inherited enzyme deficiencies.

Rapid advances are continuously being made in the field, and new horizons include the development of enzymes like biosensors or bio electrodes to monitor many physiological processes.

Food and Beverage Industry:

Xylanases:

Biological molecules found in different species are enzymes. A rich source of industrially relevant enzymes has been found to be microorganisms. Xylanase is one such enzyme. Genetic manipulation has described and separated different forms of xylanase. These involve digestive enzymes including wood, pulp and cellulose for natural fibres.

In the improvement of the quality of baked goods, Xylanases play a very positive role. For example, a particular xylanase enzyme from a fungal strain has been identified and generated (*Aspergillus niger* var *awamori*). Molecular manipulations have twenty to forty times increased the amount of development of these enzymes. This enzyme (EXLA) has been developed by Unilever and is currently freely available on the market. Xylanase and cellulase decoction, referred to as Flaxzyme, were found to create a clean fibre when the genes producing knaaf Xylanase were extracted and incorporated into *E.coli*, which is inducted into chick-feed. Xylanase, which breaks down the grain and helps the chick to digest the grain faster, is created by the bacteria, encouraging faster growth.

Another study was conducted to enzymatically produce a new plasma protein-based gel-forming material for optimizing meat products. The TNO Company developed a fresh cold meat binding system called Fibrimex (which is a solution of fibrinogen, thrombin and transglutaminase) with fresh meat pieces, which, in turn, forms a covenant mass of meat.

Emulsifiers: emulsifiers

Due to its emulsifying and stabilisation properties, Acacia gum is mainly used as an emulsifier in the food industry. Emulsifiers are now synthesised from covalently bound carbohydrates such as starch, pectin, sugar, and wheat, milk and soybean proteins using new molecular methods.

Allergy Tests for Peanuts:

Since consuming peanuts, several people have been found to exhibit allergic reactions. It is important to determine the cause of this allergy to combat this problem. A highly sensitive immunological assay has been developed to detect peanut proteins in food by a Netherlands-based company for this purpose. This is the first commercially applicable peanut assay.

Effective Monitoring:

For protection and functionality, scientists are developing flexible gastrointestinal models for thorough monitoring of digestibility, bioconversion and biodegradability of foods and drugs and pollutants. The digestive effects of nutraceutical foods are now being investigated by these models (TIM-TNO-in vitro models).

High Intensity Sweetener:

'Aesulfamek', the high intensity sweetener under the name Sunett™, was created by Hoechst. This substance has been known as an extremely effective sweetener because of its effectiveness and toxicological safety testing.

Calcium Intake:

One of biotechnology's most significant and revolutionary applications is to boost the amount of calcium in our foodstuffs. Researchers have shown that oligo-fructose, a naturally occurring low-digestible oligosaccharide, improves the absorption of calcium by as much as 22%. These studies will open the floodgates for new health application areas and new ingredient groups. These results can be used for the production of new products in the dairy, bakery, confectionery and beverage sectors.

Foods from Microbes:

We are now using genetically pure strains in the process, though brewing and baking have existed for ages. Studies show that every year, nearly 1.5 million tonnes of baker's yeast (*Saccharomyces cerevisiae*) are developed all over the world. Modern plants have also reduced the time taken from months to days in the fermentation process. Similarly, to generate a wide variety of essential enzymes, the fungus *Aspergillus oryzae* is used.

Edible Mushrooms:

Recently, Rank Hons McDougall PLC & ICI (Zeneca) acquired Quorn myco-protein from a *Fusarium graminecerarum* filamentous fungus. Quorn is harvested from mycelia cultivated in large fermenters. The final product obtained has a meat-like texture, and is the most thoroughly tested food recorded. Quorn's annual sales are in the United Kingdom alone at a rate of 15 million pounds.

Industrial Products:

The cellulose enzyme has recently been discovered to be able to replace the pumice stones used to manufacture stonewashed denim in the textile industry. This will help to offset the damage to

the fabric that the pumice stone will cause. As it eliminates the fuzz from the surface of cellulose fibres, the cellulose enzyme can also be used as a bio-polishing agent.

In laundry detergents and starch manufacturing, proteases and hydrolysis are used, respectively. From these complex ones, genetic modification may build simpler molecules, or turn the already established chemical structures into more active compounds.

For example, using the glucose isomerase enzyme, the sweetness of corn syrup can be significantly increased by chemical transformation. In pharmaceutical, food and agricultural fields, these advances can have very broad applications.

Using fermentation technology, many significant industrial products have been developed from fungi. Organic materials can be quickly broken down by fungi, which secrete unique enzymes. Antibiotics were isolated from fungi as well.

Cyclosporine was isolated as an anti-fungal compound from a fungus named *Tolypocladium inflatum*, which turned out to be an immunosuppressive agent. This medicine is often used to prevent human organ transplants from being rejected.

Biopolymers such as poly-saccharides are also a source of fungal species. These strains can aid in obtaining these biopolymers, which are very useful for industry, when grown under specific conditions. A large number of pigments are formed by many fungi, and are thus used to manufacture textile dyes.

Anthraquinone derivatives, which resemble a large group of Vat dyes, are known to be certain fungal pigments. In the textile industry, the use of these fungal dyes eliminates the problems associated with waste disposal of synthetic chemicals.

Cotton plants are particularly vulnerable to insect attacks. Transgenic cotton plants have now been produced to counter this problem. These plants bear a gene from '*Bacillus thuringiensis*' bacteria, which protects the plant from attack by insects.

Scientists are also trying to grow colored transgenic cottons, which may replace the method of bleaching and dyeing. Biotechnology has had an impact on the development of animal fibres as

well. Genetic manipulations can prevent the shearing of wool in sheep that is caused by fry larvae attack.

Several businesses are trying to produce biopolymers that shape fibers. 'Biopol' is one such product produced by Zeneca Bio-products. This chemical compound, polyhydroxybutyrate (PHB), is linear polyester with thermoplastic properties with high molecular weight and can therefore be melted and spun into fibers.

It is also extremely useful for producing surgical instruments due to its biocompatible and biodegradable nature. For example, the enzymes present inside the human body may easily degrade sutures made from PHB. Attempts to clone certain genes are also under way, and then transfer them to plants. This would allow these compounds to be manufactured in much greater quantities, which would consequently reduce their costs as well.

Benefits for the Textile Industry:

Besides cellulose, dyes, and improved cotton plants, the other applications of biotechnology in the textile industry include:

1. Use of improved plant varieties for production of textile fibres and fibre properties.
2. Improvement in fibre derived from animals.
3. Novel fibres from biopolymers and genetically modified microbes.
4. Replacing harsh and energy demanding chemicals by eco-friendly enzymes for textile processing.
5. Development of low energy based detergents.
6. New diagnostic tools for quality control of textile waste management.

Paper Industry:

For the paper industry, fungi causing white rot have proven to be very useful. Some of the chemical steps used in papermaking have been replaced by species like '*Phanerochaete*

chrysosporium' and '*Trametes versicolor*'. This will eliminate the risks of contamination associated with chemicals being used.

The trends of biotechnology are well on their way to heralding a whole new technological revolution. The strength of this revolution would lie in the exploitation of living organisms and the use of molecular resources as powerful alternatives to raw materials based on traditional chemicals. And if recent trends are any indication, in the future, this latest revolution will redefine the industry.

University Library Reference- Industrial Biotechnology by A. H Patel

Online Reference: Anaerobic Breakdown of Molecules

Ancient Indian Literature Reference - The Sfikla Yajurveda describes the formation of two stimulating drinks – Sura– and Parisfrut. Fermentation products, aris.t .a, a– sava and sura– , were considered potent medicinal drugs in Vedic age. In the post Vedic period, two new preparations known as vinegar and liquor from bread were added. The Ka– tyā– yana Sfrautasu– tra also gives a complete description of the preparation of Sura– . References to the medicated liquors and a number of other fermented liquors with their respective medicinal values are found in Caraka and Susfruta-Sam. hita– s. The survey aims to chart out the facts relevant to somarasa drink in Vedic and early historic period.

Competitive questions from today topic (2 questions Minimum)-

Which of the following is the energy of a possible excited state of hydrogen ?

A. +13.6 eV B. +6.8 eV

C. 3.4 eV D. 6.8 eV

Exam Name IITJEE2015

Which of the following compounds is not an antacid ?

A. Cimetidine B. Ranitidine

C. Pfenelxine D. Aluminium hydroxide

Exam Name IITJEE2015

Questions to check understanding level of students-

Explain the industrial biotech in brief?

Reference

Tang, W.L. and Zhao, H., 2009. Industrial biotechnology: tools and applications. *Biotechnology Journal: Healthcare Nutrition Technology*, 4(12), pp.1725-1739.

Chen, G.Q., 2012. New challenges and opportunities for industrial biotechnology. *Microbial Cell Factories*, 11(1), p.111.

Chapter 2 :

Fermentation

Fermentation is an anaerobic process where, even though oxygen is not available, energy can be released from glucose. In yeast cells, fermentation takes place, and a process of fermentation takes place in bacteria and animal muscle cells.

As in other cells, glucose can be metabolised by cellular respiration in yeast cells (the yeast used for baking bread and making alcoholic drinks). However, when oxygen is missing, glucose is still metabolised through glycolysis to pyruvic acid. Pyruvic acid is converted first to ethyl alcohol and then to acetaldehyde. Two molecules are the net gain of ATP to the yeast cell-the two ATP molecules normally formed in glycolysis.

Yeasts can take part in fermentation because they have the requisite enzyme to convert ethyl alcohol to pyruvic acid. This process is important because, during glycolysis, it extracts electrons and hydrogen ions from NADH. The effect is to liberate the NAD so that it can engage in future glycolysis reactions. The net gain of two ATP molecules in the yeast cell allows it to stay alive for some time. However, when the ethyl alcohol percentage exceeds approximately 15 percent, the alcohol kills the cells of the yeast.

In both the processing of bread and alcohol, yeast is used. The mechanism that yields beer, wine, and other spirits is alcohol fermentation. During fermentation, the carbon dioxide given off complements the carbon dioxide given off during the period of Krebs and allows bread to rise.

Another process of fermentation takes place in muscle cells. They quickly use up their oxygen supply when muscle cells contract too regularly (as in strenuous exercise). As a result, the electron transport system and the Krebs cycle are significantly slowing down and the production of ATP is slowing. However, in the absence of oxygen, muscle cells have the ability to produce a small amount of ATP through glycolysis. Glucose is converted by muscle cells to pyruvic acid. The pyruvic acid is then converted into lactic acid by an enzyme in the muscle cells. This reaction frees up the NAD as in the yeast, thus supplying the cells with two glycolysis ATP molecules. However, finally, the buildup of lactic acid induces extreme weakness, and the muscle stops contracting.

Bacteria can break down organic compounds using fermentation in anaerobic conditions. If you've ever eaten fermented foods, like bread, yoghurt or cheese, you've experienced fermented items. Learn the basics of this process now.

Anaerobic Bacterial Metabolism

In order to grow and reproduce, or, in other words, to remain alive, bacteria must absorb energy from food sources. What we are really talking about when we talk about bacteria capturing energy is the ability of bacterial cells to use a chemical substrate (a food) to produce ATP. The high-energy molecule used to store energy and create all the cellular components needed is ATP.

Bacteria can capture energy in anaerobic environments and store it in ATP using either anaerobic respiration or fermentation. Fermentation will be implemented by this lesson. The rich flavours and textures present in these foods are responsible for the process and products of fermentation by bacteria and yeast.

Fermentation Basics

Fermentation is the anaerobic catabolism of a single chemical compound with the aim of producing ATP by phosphorylation at the substrate level using a sequence of redox transformations. That description sounds tricky, so let's break it down a bit.

Catabolism only refers to a chemical compound's breakdown. The breakdown occurs during fermentation through a series of redox transformations, which is just a fancy word for contributing electrons and accepting reactions of electrons. The atoms present in the original substrate molecule operate on all the redox transformations that take place. Some atoms can behave as donors of electrons and some as acceptors of electrons. A series of enzymes that accelerate the reaction and catalyse the development of different intermediate forms of the initial substrate are needed for electron shuffling.

Through these fermentation reactions, the energy present in the chemical bonds of the substrate can be captured to produce ATP by substrate-level phosphorylation. **Substrate-level phosphorylation** is the production of ATP by transferring high energy phosphate groups from an organic molecule to ADP.

Fungal and Yeast Fermentation: Yeast, one of some 1,500 single-celled fungi species, most of which are in the Ascomycota phylum, only a few being Basidiomycota. Yeasts are found in soils

and plant surfaces worldwide and are particularly abundant in sugar mediums such as floral nectar and berries. There are hundreds of economically significant varieties of ascomycete yeast; selected strains of *Saccharomyces cerevisiae* are the forms widely used in the manufacture of bread, beer, and wine. Some yeasts, especially *Candida albicans*, *Histoplasma*, and *Blastomyces*, are mild to dangerous pathogens for humans and other animals.

Yeasts, including fungi, are eukaryotic cells. They usually have a diameter of around 0.075 mm (0.003 inch). By budding, most yeasts replicate asexually: a small bump protrudes, enlarges, matures, and detaches from a parent cell. By fission, a few yeasts replicate, the parent cell splitting into two equal cells. *Torula* is a wild yeast genus that is incomplete and never forms sexual spores.

Yeast is used in food production to cause fermentation and leavening. The fungi feed on sugars, contain alcohol (ethanol) and carbon dioxide; the former is the ideal commodity in beer and wine production, the latter in baking. Some of the carbon dioxide in sparkling wines and beer is preserved in the finished drink. When the dough is baked, the alcohol created in bread making is driven off. Naturally occurring yeasts present in the air also start the fermentation of wine and sourdough breads. One yeast cell can ferment glucose per hour at approximately its own weight.

In commercial development, a solution of molasses, mineral salts and ammonia is fed to selected strains of yeast. The yeast is isolated from the nutrient solution, cleaned, and packed when growth ceases. For baking, yeast is sold in starch-containing compressed cakes or in a dry granular form mixed with cornmeal.

Biochemistry of Fermentation

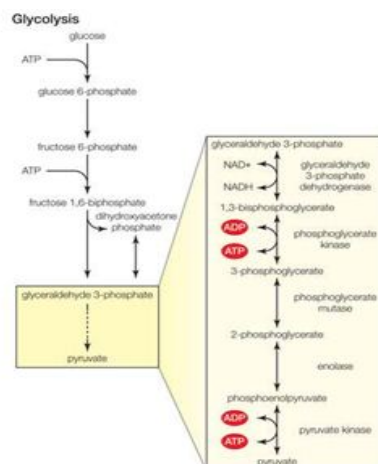


Diagram of Biochemistry of Fermentation

Originally, glycolysis, the breakdown of sugar, was described as the metabolism of sugar into lactate around 1930. It can be further described as the cell-characteristic process of fermentation in which six-carbon sugar glucose is broken down into two molecules of three-carbon organic acid, pyruvic acid (the non-ionized form of pyruvate), in conjunction with the transfer of chemical energy to adenosine triphosphate synthesis (ATP). Pyruvate can then be oxidised, reduced to lactic acid, alcohol, or other products in the presence of oxygen, via the tricarboxylic acid cycle, or in the absence of oxygen. The sequence from glucose to pyruvate is often called the Embden–Meyerhof pathway, named after two German biochemists who in the late 1920s and '30s postulated and analyzed experimentally the critical steps in that series of reactions.

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Online Reference: Anaerobic Breakdown of Molecules

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Competitive questions from today topic (2 questions Minimum)-

Which of the following is an upstream process?

- A. Product recovery
- B. Product purification
- C. Media formulation
- D. Cell lysis

Exam Name GATE 2015

Alcoholic fermentation is carried by yeast known as

- A. Lactobacillus
- B. Bacillus
- C. *Saccharomyces cerevisiae*

D. Escherichia coli
Exam Name GATE 2015

Questions to check understanding level of students-

1. What is Bacterial Fermentation?
2. What is Glycolysis?

Reference

Wenda, S., Illner, S., Mell, A. and Kragl, U., 2011. Industrial biotechnology—the future of green chemistry. *Green Chemistry*, 13(11), pp.3007-3047.

Chapter 3 :

Downstream and Upstream Processing

Upstream processes involve the selection of a microbial strain distinguished by the capacity to synthesise the desired commercial value of a particular commodity. In order to optimise the strain's ability to synthesise economic quantities of the commodity, this strain is then subject to enhancement protocols. The fermentation method itself, generally carried out in large tanks known as fermenters or bioreactors, is included in the upstream process. In addition to the mechanical components that provide adequate conditions inside the tank, such as aeration, cooling, agitation, etc., the tank is also typically fitted with complex sets of monitors and control devices to ensure optimised conditions for microbial growth and product synthesis. Inside the fermenter, the handling of the fermentation reactions can be performed using many engineering technology modifications.

The stirred-tank fermenter that utilises mechanical agitation principles during the fermentation process, primarily using radial-flow impellers, is one of the most commonly used fermenter forms.

Downstream processing requires adequate techniques and methods for recovery, purification, and characterization of the desired fermentation product, the different phases that accompany the fermentation process. A broad variety of downstream processing techniques can be applied, such as centrifugation, filtration, and chromatography. These methods differ according to the chemical and physical nature of the finished product, as well as the grade required.

Upstream Processing Summary

Usually, upstream production deals with three significant stages. The first concerns fermentation media, in particular the collection, along with other essential nutrients, of sufficient cost-effective carbon and energy sources. Media optimization is an important part of process growth to ensure yield maximisation and professional optimization.

The second aspect involves aspects associated with the producer microorganism. They include the strategy for initially obtaining a suitable microorganism, industrial strain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a suitable inoculums

and continuing development of selected strains to increase the economic efficiency of the process.

The third component relates to the fermentation which is usually performed under rigorously controlled conditions developed to optimize the growth of the organism or the production of a target microbial product.

Fermentation medium

The medium used for fermentation may be classified as defined, complex or technical medium. Defined medium consists only of precisely chemically defined substrates. Complex medium is composed of substrates with undefined composition, such as extracts or hydrolysates from waste products, which are cheap substrates commonly used in industrial production. Relatively expensive substrates, such as yeast extract, brain heart infusion, peptone, and tryptone are often used for complex medium. Technical media are used on an industrial scale and are cheaper. The substrate sources can also be derived from industrial waste, and are often highly impure mixtures, requiring pretreatment before they could be used for a fermentation process. Examples are soy meal, whey, fishmeal, malt extract, and sulfite waste liquor. Wastewater from monosodium glutamate production, which contains high levels of chemical oxygen demand (COD), sulphate, and ammoniacal nitrogen at a low pH, has been used as the nitrogen and water source, with sugar beet pulp as the carbon source, for the production of pectinase.

Media sterilization is necessary to ensure that only the desired microorganism is present to carry out the fermentation, that products are made of predicted quality, that the environment is protected from undesirable contamination, and that deterioration (microbial spoilage) of products is prevented. Sterilization by high temperature achieved by direct or indirect steam or electric heating, membrane filtration, microwave irradiation, high voltage pulses and photo semiconductor powders which involve the rupture of the cell membrane by increasing the transmembrane electric field strength beyond a certain threshold.

Inoculation is the transfer of seed material or inoculum into the fermentor. Inoculation of a laboratory fermentor is generally done using presterilized tubing and a peristaltic pump. However, on a larger scale, inoculum transfer is done by applying a positive pressure on the inoculum fermentor and connecting it aseptically to the production fermentor. The connecting lines are sterilized before being used for transfer of inoculum. Heat susceptible substances such

as amino acids and some vitamins must be dissolved in small volumes of water, sterilized by filtration and added separately to the final medium aseptically.

Fermentation systems

A fermentation system is usually operated in one of the following modes: batch, fed batch, or continuous fermentation. The choice of the fermentation mode is dependent on the relation of consumption of substrate to biomass and products. The systems are batch, continuous and fed batch systems that were described earlier in .Today the most common type of upstream processing of proteins utilizes two tools: bioreactors and suspension (or attached) cells transformed with expression vectors genetically engineered to contain one (or more) human genes that produce copious amounts of their protein.

Inoculum

Upstream processing of proteins using bioreactors and cells typically starts with the preparation of the Inoculum, which continues in scale-up steps until the final, clean, media-filled bioreactor is aseptically inoculated by ample Inoculum.

Samples are removed aseptically during the culture cycle, and different parameters, including optical density (OD) and live cell count, are determined by fermentation technicians or operators. Samples are often sent to quality control where other parameters, such as glucose, lactate and ammonia levels, as well as the identity and concentration of the human protein produced by the cells, can be calculated. The initial purification steps, which may involve centrifugation and/or filtration in order to isolate cells from media, are also part of upstream processing. Depending on where the protein was located, the cells or the media will be discarded in the kill tank.

We use glass bioreactors in this course, reflecting three cell types used in the upstream processing of human protein pharmaceuticals: bacterial, animal, and fungal cells. Proteins are secreted into the media in bacteria, such as the workhorse of biotechnology, *Escherichia coli*, *Pichia pastoris*, so the media is preserved for subsequent isolation and purification of the protein of interest in downstream processing. In order to isolate the cells from the media, proteins remain within the cell and the media is discarded in the kill tank. In animal cells, such as cells of the Chinese Hamster Ovary (CHO) and in fungal cells, such as yeast cells,

Overview of Downstream Processing

After fermentation, downstream processing covers all processes. It has the primary objective of recovering the target product to the necessary requirements effectively, reproducibly and safely (biological operation, purity) while optimising recovery yield and minimising costs. Depending on whether it is an intracellular or extracellular product, the target product may be retrieved by processing the cells or the spent medium. The level of purity that needs to be achieved is typically dictated by the product's particular use.

Each stage in the overall recovery procedure is highly dependent on the prior fermentation protocol. The properties of microorganisms, especially morphology, flocculation characteristics, size and cell wall rigidity, include fermentation factors affecting downstream processing. These variables have important effects on the efficacy of filterability, sedimentation and homogenization. The existence of by-products of fermentation, media impurities and additives of fermentation, such as antifoams, which interfere with downstream processing steps and accompanying product analysis.

Fermentation materials are commonly present in complex mixtures of dilute solutions that need to be concentrated and washed. The isolation from the fermentation broth of the substance of interest depends on the accumulation of the component that may be intracellular or extracellular.

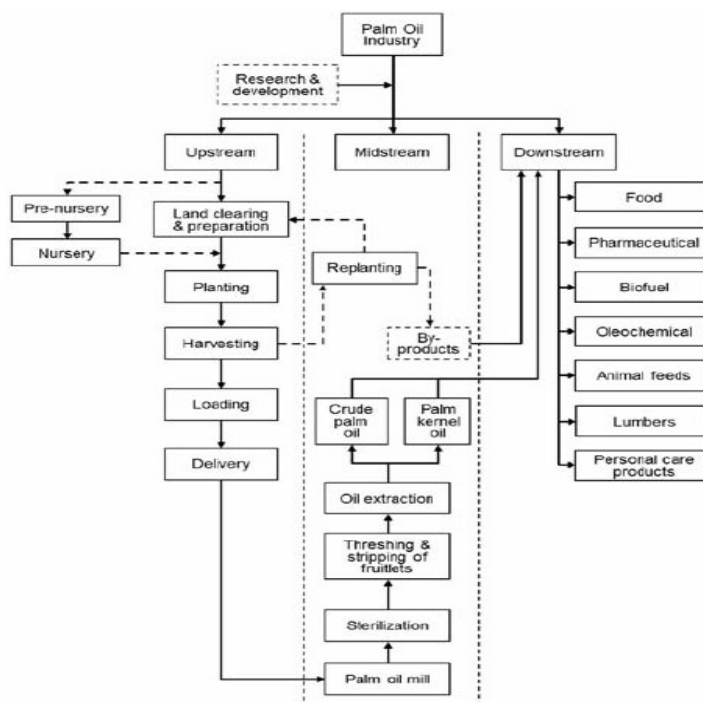


Diagram of Flow sheet of bioprocess

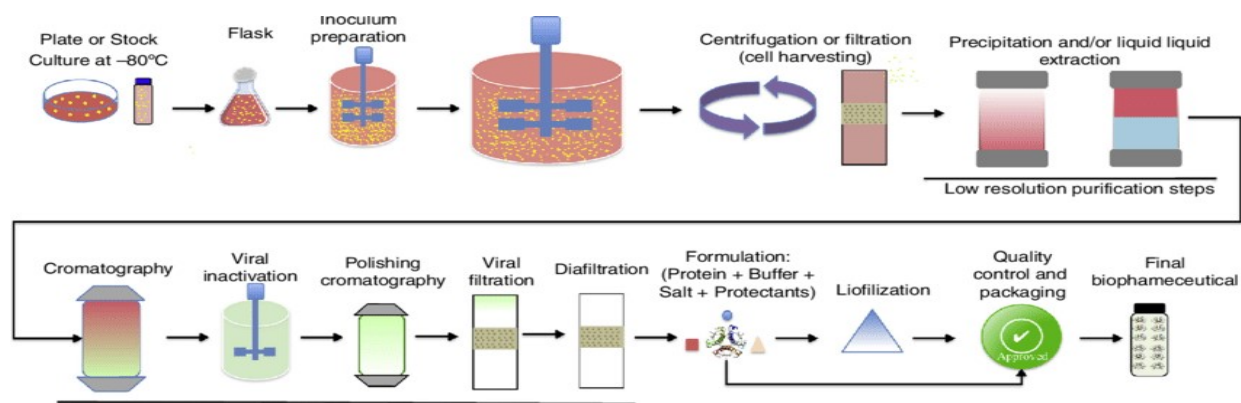
Cell disturbance (high pressure homogenization, wet milling, and lysis) Clarification of extract (centrifugation, filtering, dead end filtration, and cross flow filtration) Enrichment The usual downstream operations and device operations involved in the production of fermentation broth are: (precipitation, batch adsorption, ultra filtration, and partition)

High resolution techniques (ion exchange, affinity, hydrophobic, filtration of gel, chromatography of adsorption and electrophoresis) Concentration (sterile filtration, diafiltration, ultra filtration, freeze drying, spray drying, and precipitation).

High resolution techniques like chromatography techniques

Adsorbed or stuck to beads packed in the column are the molecules of interest. The greater the affinity of the molecule (protein) to the bead, the more at any given times it would be bound to the column. Proteins with a high affinity move through the column steadily so a large amount of the time they are trapped. Higher affinity molecules will not stick as often and will elute more easily. By altering the mobile phase, we will alter the relative affinity of the protein for the column (retention time) and mobile phase (the buffer).

Ion exchange chromatography is the most prevalent method of adsorption chromatography. Affinity, hydrophobic contact and gel filtration are the other ones used in industrial biopharmaceutical processing.



Pictorial diagram of bioprocess

Column chromatography the chemical and physical variations of molecules distinguish molecules

Most common types: exclusion of size (gel filtration): separated by molecular weight, exchange of ions: separated by charge, chromatographic affinity: basic binding Hydrophobic interaction: separated by hydrophobic/hydrophilic properties.

Chromatography of Ion Exchange

Ion Exchange Chromatography depends on interactions between the protein of interest and charges on a resin with charge-charge (bead). It is possible to subdivide ion exchange chromatography into cation exchange chromatography in which a positively charged interest protein binds to a negatively charged resin; and anion exchange chromatography in which a negatively charged interest protein binds to a positively charged resin. **Isoelectric focusing**

The protein of interest would be positively charged if the pH of the buffer is smaller than the pI.

If the buffer's pH is higher than the pI, it will negatively charge the protein of interest.

Affinity chromatography

On the basis of a reversible relationship between it and its antibody coupled to a chromatography bead, affinity chromatography distinguishes the protein of concern With high selectivity, high precision, and high protein potential of interest, purification levels are achievable in the range of several thousand fold.

Hydrophobic interaction chromatography (HIC)

HIC is finding dramatically increased use in production chromatography. Antibodies are quite hydrophobic and therapeutic antibodies are the most important proteins in the biopharmaceutical pipeline. Usually HIC media have high capacity and are economical and stable. Adsorption takes place in high salt and elution in low salt concentrations.

University Library Reference- Industrial Biotechnology by A. H Patel

Online Reference: Upstream Processing and Downstream Processing

Ancient Indian Literature Reference - The *Sfukla Yajurveda (SfY)* describes the formation of two stimulating drinks– *sura*– and *parisfrut*. *Sura*– was supposed to be prepared from

germinated paddy, germinated barley, and parched rice with the help of ferment. Yeast was used most often as the fermenting agent.

Competitive questions from today topic (2 questions Minimum)-

Fermentation is an example of

- A. Aerobic respiration
- B. Anaerobic respiration
- C. Photosynthesis
- D. Protein synthesis

Exam Name CUSAT 2016

In a stirred tank reactor when the agitation rate is increased, the k_L and $k_L a$ values will:

- A. increase and decrease respectively
- B. decrease and increase respectively
- C. both increase
- D. both decrease

Questions to check understanding level of students-

What are Upstream Processing and Downstream Processing?

Reference

Tang, W.L. and Zhao, H., 2009. Industrial biotechnology: tools and applications. *Biotechnology Journal: Healthcare Nutrition Technology*, 4(12), pp.1725-1739.

Chapter 4 :

Production of Primary and Secondary Metabolites

Primary metabolites are involved in the organism's growth, growth, and reproduction. Typically, the primary metabolite is a crucial factor in the preservation of natural physiological processes; it is also often referred to as a core metabolite. As a consequence of energy metabolism, primary metabolites are usually formed during the growth process and are considered necessary for proper growth. Alcohols, such as ethanol, lactic acid, and certain amino acids, are examples of primary metabolites.

Alcohol is one of the most important primary metabolites used for large-scale processing within the field of industrial microbiology. Alcohol is used specifically for processes involving fermentation that produce products such as beer and wine. In addition, primary metabolites such as amino acids are isolated from the mass processing of a single bacterial genus, *Corynebacteria glutamicum*, including L-glutamate and L-lysine, which are widely used as supplements. Citric acid is another example of a primary metabolite widely used in industrial microbiology. One of the most commonly used ingredients in food processing is citric acid, provided by *Aspergillus niger*. It is still used widely in the pharmaceutical and cosmetic industries.

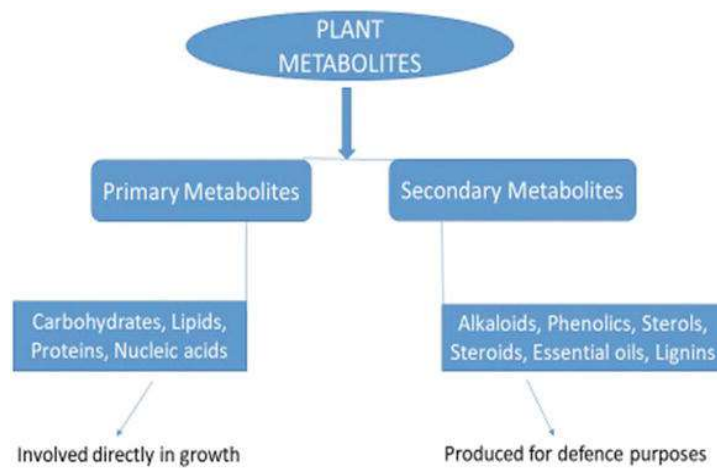


Diagram of Primary Metabolites

Two kinds of metabolites are produced by a plant cell: primary metabolites specifically involved in growth and metabolism, and secondary metabolites known as primary metabolism end products and not involved in metabolic operation. The group of pathways synthesising simpler but important molecules is called primary metabolism for normal physiological growth and

energy demands of plants, and the products are called primary metabolites. In nature, they are commonly dispersed and are often used by man as food.

For plant growth, production, stress adaptation, and protection, primary metabolites such as carbohydrates, organic and amino acids, vitamins, hormones, flavonoids, phenolics, and glucosinolates are needed. These metabolites decide the nutritional content of fruit, colour, flavour, odour, antioxidant, anticarcinogenic, antihypertensive, anti-inflammatory, antimicrobial, immunostimulating and cholesterol-lowering effects, in addition to the significance to the plant itself.

Secondary metabolites of plants

Extremely commercially important compounds are plant secondary metabolites. These are used as chemicals of high importance, such as medications, flavours, fragrances, insecticides, colorings, etc. Plants are abundant in a broad spectrum of secondary metabolites which have been detected to have in vitro antimicrobial effects, such as tannins, terpenoids, alkaloids, and flavonoids. The ability of plants to synthesise aromatic compounds is almost infinite, most of which are phenols or their oxygen-substituted equivalents. Around 25,000 terpenoids are classified as secondary compounds and are derived from isopentenyl diphosphate, a five-carbon precursor (IPP). Overall, about 12,000 identified alkaloids are recognised that have one or two atoms of nitrogen that are biosynthesised from amino acids. The 8000 known phenolic compounds are either synthesised through the pathway of shikimic acid or through the pathway of malonate/acetate.

Production of secondary metabolites from plants

Conventional

The traditional secondary metabolite processing approach focuses on the extraction, not production, of metabolites from plant tissues by various phytochemical methods, such as solvent, steam, and supercritical extraction. In vitro synthesis and the generation of secondary plant metabolites have been enabled by recent advances in biotechnological methods such as plant tissue culture, enzyme and fermentation technology.

Immobilization

Cells or biocatalysts are limited by entrapment, adsorption or covalent association within a matrix. The desired secondary metabolites are synthesised with the inclusion of the required substrate and the provision of optimum physical chemical parameters. Immobilization with an effective bioreactor device offers many benefits, such as continuous process activity, but natural or artificially induced secretion of the accumulated product into the surrounding medium is required for the production of an immobilised plant cell culture process.

In vitro tissue, organ, and cell culture

From explants, such as plant leaves, stems, roots, meristems, etc., both for multiplication and extraction of secondary metabolites, plant cell and tissue cultures can be formed routinely under sterile conditions. To synthesise metabolites of interest, shoot, root, callus, cell suspension, and hairy root culture are used. Via unorganised callus or suspension cultures, metabolites that are dispersed in many tissues may be synthesised. But where the metabolite of interest is restricted to specialised portion or glands in host plant, separated microplant or organ culture is the method of choice. In its roots, ginseng saponins are formed, and in vitro root culture is therefore favoured for saponin synthesis. Similarly, Hypericin and Hyperforin antidepressants are found in *Hypericum perforatum* foliar glands that have not been synthesised from undifferentiated cells.

Through the treatment of plant cells with biotic and/or abiotic elicitors, the quantum of secondary metabolite production in cell cultures can be improved. The widely used emitters are methyl jasmonate, fungal sugars, and yeast extract. Methyl jasmonate is a proven and efficient elicitor used in the manufacture of *Taxus chinensis* taxol and *Panax ginseng* ginsenoside. To increase efficiency, the most recently created and engineered metabolic engineering may be employed.

Much interest has been drawn to the development of metabolites via the hairy root system based on inoculation with *Agrobacterium rhizogenes*. The efficiency and quantity of the secondary metabolite is the same or even greater than the intact host plant root synthesis by hairy root systems.

Furthermore, healthy genetic makeup, immediate growth in the media of plant tissue culture and phytohormones provides additional space for biochemical studies. Infected root tips with *A.*

Rhizogenes are grown on phytohormones deficient in tissue culture media [Murashige and Skoog's (MS) Gamborg's B5 or SH media]. The attempts to adapt bioreactor architecture to hairy root cultures have recently been summarised by Srivastava and Srivastava; stirred tank, airlift, bubble columns, connective flow, turbine blade, revolving drum, as well as different gas phase reactors have all been successfully used. Genetic modification is being studied in the hairy root culture for secondary development of metabolites. For greater metabolite growth and development, the developed roots are screened. Transgenic hairy roots produced by *Agrobacterium rhizogenes* have not only paved the way for the development of plantlets, but also for the synthesis by transgenic hairy root cultures of the desired product.

Secondary metabolites of microorganisms

Low-molecular mass products with peculiar shapes are microbial secondary metabolites. A number of biological functions are demonstrated through structurally complex metabolites, such as antimicrobial agents, enzyme and antitumor inhibitors, immune suppressants and antiparasitic agents, plant growth stimulators, herbicides, insecticides, antihelmintics, etc. They are manufactured during the microorganisms' late growth process. Special regulatory mechanisms in microorganisms regulate the production of secondary metabolites, as their production is typically repressed in the logarithmic phase and depressed in the stationary phases of formation. There is a distinctive molecular skeleton of the microbial secondary metabolites that is not present in chemical repositories, and about 40% of the microbial metabolites may not be chemically synthesised.

Production of Organic Acids

In the broth of the fermenter, a micro-organism develops and creates organic acids during its trophophase of growth. Organic acids are formed by carbohydrate metabolism. They collect in the fermenter's broth, where they are isolated and filtered.

Organic acids are either terminal components of the EMP (glycolysis) pathway, such as lactic acid and propionic acid, or products of incomplete sugar oxidation (citric acid, itaconic acid and gluconic acid). A third form of compound is often obtained in the presence of oxygen, i.e. acetic acid, by the dehydrogenation of alcohol.

Organic acids, since they are developed on a wide scale, provide tremendous potential for future growth. They are sold as pure chemicals or their corresponding salts, in contrast. In 1881, by bacterial fermentation, calcium lactate was developed on a wide scale for the first time. Later on, for acid production, *Penicillium* and *Aspergillus* species were found. In the present sense, citric acid development is defined in detail.

Citric Acid

The isolation and crystallisation of sour products from lemon juice was recorded for the first time by Scheele (1789). However, a limited amount (1 per cent) could add to the demand for citrus fruits. Currently, citric acid available on the market comes from the process of fermentation.

Citric acid has been chemically synthesised from glycerol. Wehmer (1893) reported the large occurrence of citric acid in the metabolites of microbes. In nutritional deficient conditions, Molliard (1922) confirmed the aggregation of citric acid in the cultures of *Aspergillus niger*.

Initially, small quantities of citric acid were recovered; however, it provided the simple notion of processing citric acid under nutrient deficiency conditions. Below optimum nutrient levels, a sufficient amount of biomass is generated. However, it is clear that the production of mycelial mass or sporulation does not lead to the production of cultures of citric acid.

Commercial production methods

There are three methods for the commercial production of citric acid: I Koji Fermentation Process: This method is used in Japan, accounting for approximately one fifth of the annual production of citric acid. Strains from *A. Niger* is used in this process. (ii) Process of fermentation of liquid surface culture: Liquid cultures are inoculated with the spores of *A* in this step. *Niger*, which will germinate in 24 hours. In the whole surface of the solution, Mycelia covers and floats. (iii) Fermentation process of submerged culture: In this case, *A. mycelia*. Japonicum grows in tanks roughly 15 cm deep in solution. Citric acid formed in this manner is inferior to that produced by fermentation of liquid surface culture. In addition, it is manufactured in limited volumes and at high prices. Methods of processing citric acid. For commercial strains of a production. *Niger* is chosen from the hybrids or mutants that are formed through a certain

process. These strains should be capable of producing no less than 80 g of citric acid per 100 g of glucose.

Continuous culture is not acceptable for large scale development. Therefore, for continuous fermentation in any phase in which cell growth and metabolic products occur at different times, a multi tank system is needed.

Production of Industrial enzyme

Micro-organisms (bacteria, yeasts and moulds) and the enzymes they produce have been used by humanity for thousands of years to make bread, cheese, beer and wine. Nowadays, we can remember certain enzymes that, for instance, are responsible for producing beer. Enzymes used for industrial applications are produced in large closed fermentation tanks by regulated and confined fermentation, utilising a well-defined development strain.

Under very particular conditions, these development strains evolve to increase the amount of enzyme that they generate.

When fermentation is complete, by centrifugation/filtration, the production strain cells are inactivated and removed, separating the resulting enzyme from its production strain. Depending on the application in which it may be used, the enzyme concentrate is then purified, standardised and stabilised with diluents, producing liquid or granulated enzyme products.

Enzyme production by fermentation has many benefits. This makes it easier to maintain a constant product consistency and a high output yield. It also helps to acquire precisely targeted enzymes to perform particular activities under the conditions needed, such as detergent enzymes that are active at extremely low temperatures.

Methods of Enzyme Production

Production of enzymes takes place in the following steps:

Microorganism separation, strain production and inoculum storage. Microorganisms are separated using microbiological techniques in culture media. The goal of isolating an effective microorganism is (a) the development of high-quantity enzymes and other low-quantity metabolites, (b) the completion of the fermentation process within a limited period of time, and (c) the use of low-cost microorganisms. If an effective microorganism has been acquired, its capacity to generate enzymes is optimised by improving strains and formulating a culture

medium (pH and temperature). By using mutagens, i.e. mutagenic chemicals and ultraviolet light, strains of microorganisms are created. Procedures for the production of antibiotic-producing strains are listed in *Penicillium* culture collection.

The enzyme strain generating inoculum produced after mutagen treatment is prepared by multiplying its spores and mycelia on the liquid broth.

Medium Formulation and Preparation

The culture medium is designed in such a way that all the nutrients promoting the development of enzymes should be given in large amounts, but not for good microbial growth. The optimal medium must provide an economical supply of carbon, nitrogen, amino acids, growth promoters, trace elements and a limited amount of salt for this reason. To retain pH during fermentation, caution must be taken. The temperature and composition of the culture medium is optimised before inoculation for a particular microbe pH. With the concentration of the culture medium, enzyme production increases. The following typical media components for enzyme fermentation were provided by Aunstrup et al, (1979):

Carbohydrates: Molasses, barley, corn, wheat and starch hydrolysate

Proteins: Meals of soybean, cotton seed, peanut and whey, corn steep liquor and yeast hydrolysate.

Sterilization and medium inoculation, culture management and filtering of fluids

In a large fermenter, the medium is sterilised batch-wise. The continuous sterilisation process is now being popular for this reason. After the medium is sterilised, enough inoculum is inoculated to start the fermentation phase. The fermentation process is the same as defined for the development of antibiotics.

The surface culture technique in which inoculum persists on the upper surface of the broth has been a conventional method of enzyme processing. The process of submerged culture now-a-days is more frequently practised because of less risk of contamination and greater enzyme yield. Some of the fungal enzymes, for example, amylase (from *Aspergillus* sp.), protease (from *Mucor* sp. and *Aspergillus* sp.) and pectinase (from *Mucor* sp. and *Aspergillus* sp.) are also used for the processing of the former technique (from *Penicillium* sp. and *Aspergillus* sp.). Mitra and Wilke

advocated the use of continuous culture techniques for the processing of cellulase through *Trichoderma* (1975).

Development conditions, such as pH, temperature and oxygen, are kept at optimal levels in the fermenter. These variables range from microbe to microbe, even sometimes in the same microbe organisms. To control foaming, as it occurs during fermentation, a small amount of oil is applied to the fermenter. After incubation for 30-150 h, the inoculated microbe releases extracellular enzymes in the culture medium. Most enzymes are released after the exponential growth process is completed, although they are produced after the exponential phase in a few instances. Other metabolites (10-15 percent) are also produced in the fermented broth besides extracellular enzymes. After enzyme purification, these metabolites are extracted.

To prevent pollution, it is preserved at 5 ° C when fermentation is over broth. It is more difficult to extract enzymes from the fermented broth (fluid) of bacteria than from filamentous fungi. After pH change, the fungal broth is directly filtered or centrifuged. Therefore, to precipitate calcium phosphate, the bacterial broth is treated with calcium salts, helping to differentiate bacterial cells and colloids. Then, to remove cell debris, the liquid is drained and centrifuged.

Purification of Enzymes: Purification of enzymes is a dynamic process. Readers are recommended to research elsewhere for detailed explanations. (i) preparation of condensed solution by vacuum evaporation at low temperature or by ultrafiltration; (ii) confirmation of concentrated enzymes by polishing filtration to eliminate other microbes; (iii) incorporation of preservatives or stabilisers such as calcium, protein, flour, sugar, alcohol, sodium chloride (18-20%), sodium benzoate, etc.

University Library Reference- Industrial Biotechnology by A. H Patel

Online Reference: Primary and Secondary Metabolites

Ancient Indian Literature Reference – 'Atharvanaveda' (1200 B.C.). Later, the Ayurvedic concept appeared and developed between 2500 and 500 BC. It has a vast literature in Sanskrit covering all aspect of diseases, pharmacy and therapeutics. The Vedic and Post-Vedic periods roughly from 4500 B.C. to 500 A.D. had celebrated Indian physicians and herbalists. Atreya, Nagarjuna, Vagbhata, Sushruta and the Hindu hippocrates, Charaka were the legendary figures of the traditional Indian medicine. Two memorable treaties are Charak Samhita and Sushruta

Samhita appeared between 400 A.D. and 500 A.D. and said to be golden age of traditional Indian herbal medicine.

Competitive questions from today topic (2 questions Minimum)-

The biochemical reaction involving adding sugars to proteins is called as

- A. Glucogenesis B. Glycolysis
- C. Glycosylation D. Galactolation

Exam Name puducherry ENTRANCE 2016

For an enzyme catalyzed reaction in a batch bioreactor, which one of the following is true under quasi-steady state conditions:

- A. Enzyme-substrate complex concentration remains nearly constant
- B. Substrate concentration remains nearly constant
- C. Product concentration remains nearly constant
- D. Both substrate and product concentration remain nearly constant

Exam Name DBT-2019

Questions to check understanding level of students-

What is Primary Metabolites?

What is Secondary Metabolites?

Reference

Soetaert, W. and Vandamme, E., 2006. The impact of industrial biotechnology. *Biotechnology Journal: Healthcare Nutrition Technology*, 1(7-8), pp.756-769.

Chapter 5 :

Production of Ethanol and vitamins

For centuries, fermentation has been carried out and is the standard method of processing of common alcohol. By fermentation, much of the ethanol in the world is produced using crops such as sugar cane, sugar beet, corn, rice and maize. It is also possible to use urban waste as feedstock, minimize garbage disposal and convert compost into a profitable commodity. Fermentation is a complex series of reactions that transform carbohydrates into ethanol and carbon dioxide, especially sugars and starches. It operates well in the absence of oxygen (anaerobic) at temperatures in the 25 °C to 37 °C range and contains aqueous solutions of up to 14 percent ethanol.

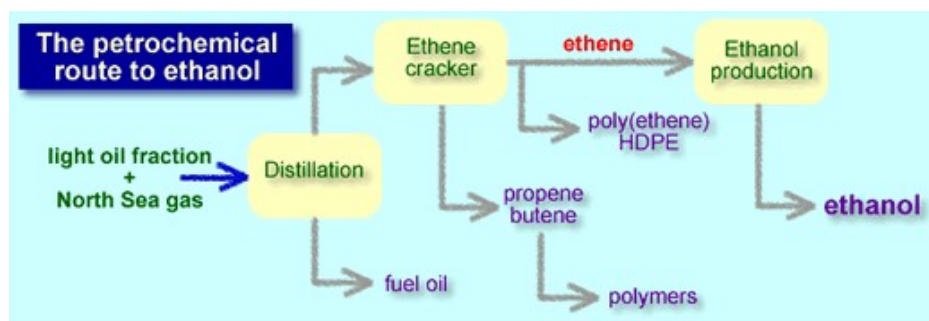
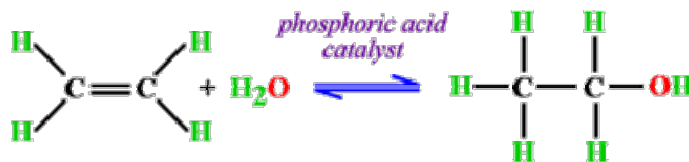


Diagram of Production

Higher alcohol contents require further distillation.

Ethanol is manufactured by the hydration of ethene using steam in the presence of a phosphoric acid catalyst.

chemical equation



Purification

An ethanol and water mixture is formed by the reactions involved in processing synthetic ethanol. A blend of 96 percent ethanol and 4 percent water often results in fractional distillation

(instead of 100 percent pure or absolute ethanol). This is regarded as a boiling mixture that is azeotropic or constant.

Conventionally, either by refluxing with calcium oxide, a dehydrating agent, or by combining with benzene, which breaks up the azeotrope and creates pure ethanol until further purified, the last 4 percent of water is separated from the azeotropic mixture. These two methods increase the expense of producing energy, and benzene is both extremely poisonous and carcinogenic.

New Purification Methods

The use of zeolites with structures with holes that can absorb and thus remove water from the final mixture involves new purification techniques. Several zeolites have an especially strong appeal, acting at normal temperatures and pressures as dehydrating agents and can then be heated and re-used to dry. This generates considerable energy savings and eliminates the need for toxic substances such as benzene to be used.

The zeolites used are known as zeolites of 3\AA (angstrom), as the holes are 3\AA in diameter. The angstrom is a unit, 10^{-8} cm in length. $10\text{\AA} = 1\text{nm}$. Holes are larger than a molecule of water, but significantly smaller than ethanol.

Vitamins

Organic molecules commonly derived from vitamins are coenzymes. Vitamin is a material that can not naturally be synthesised by the body, but is necessary for normal body function in limited amounts. Niacin, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin H and vitamin K will be discussed in this lecture.

Niacin

Nicotinamide adenine dinucleotide (NAD^+) is a coenzyme that is used by enzyme as oxidizing agent for biological oxidations. Since niacin is the portion of coenzyme NAD^+ , it is to be added in the diet so that the body can synthesize NAD^+ (illustrates an example for the enzyme catalyzed oxidation/reduction reactions using NAD^+/NADH).

Vitamin B2

Vitamin B2 is known as riboflavin (flavin plus ribitol). Coenzyme flavin adenine dinucleotide (FAD), which is used as an oxidising agent in oxidation reactions catalysed by enzymes, must be synthesised. For starters, succinate dehydrogenase uses FAD to oxidise succinate to fumarate .

Vitamin B1

Vitamin B1 is recognised as thiamine. The coenzyme thiamine pyrophosphate is needed to form (TPP). Enzymes that catalyse the conversion of a two-carbon fragment from one species to another need TPP. For instance, for the decarboxylation of pyruvate, the pyruvate decarboxylase enzyme requires TPP and transfers the remaining two carbon fragments to a proton to supply acetaldehyde.

Vitamin B12

Coenzyme B12, which is derived from vitamin B12, is essential for enzymes that catalyse such rearrangement reactions. The cyano group of vitamin B12 is co-ordinated with cobalt. This group is substituted by a 5'-deoxyadenosyl group in coenzyme B12. Coenzyme B12 is used by enzymes that catalyse reactions in which a group (Y) bonded to one carbon changes to the adjacent carbon bonded to hydrogen.

Competitive questions from today topic (2 questions Minimum)-

What is the unit of influent flow rate?

A.m³/d B.m²/d

C.m/d D.m d

Exam NameDBT-2016

Compartmental mixing in tall reactors occurs with

A. rushton turbine B. inclined blade turbine

C. propeller D .high efficiency axial flow impellers

Exam NameJNU-MTB-2011

Questions to check understanding level of students-

Explain the Production of Alcohol

Explain the Production of vitamin

Reference

Tang, W.L. and Zhao, H., 2009. Industrial biotechnology: tools and applications. *Biotechnology Journal: Healthcare Nutrition Technology*, 4(12), pp.1725-1739.

Chen, G.Q., 2012. New challenges and opportunities for industrial biotechnology. *Microbial Cell Factories*, 11(1), p.111.



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