SRINIVASAN COLLEGE OF ARTS & SCIENCE

(Affiliated to Bharathidasan University, Trichy)

PERAMBALUR – 621 212.

DEPARTMENT OF MICROBIOLOGY

Course : M.Sc Year: **II** Semester: **IV**

Course Material on:

BIOPROCESS TECHNOLOGY

Sub. Code : P16MB42

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SRINIVASAN COLLEGE OF ARTS & SCIENCE

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CORE COURSE - X

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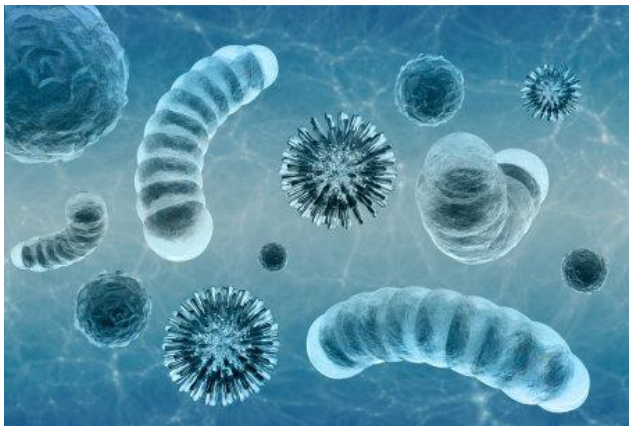

CORE COURSE - X

BIOPROCESS TECHNOLOGY

Subject Code: P16MB42

OBJECTIVES

To learn the process involved in the industrial production of microbial products. Understand the strategies of strain selection and improvement. Understand the process of fermentation. Familiarize with types of fermentors and downstream processing. To learn the role of microbes in food preparation, preservation and spoilage. To understand the quality of food and products.

Unit – I Industrially important microbes and their improvement:

Screening methods for industrial microbes – detection and assay of fermentation products– classification of fermentation types – strain selection and improvement. Mutation and recombinant DNA techniques for strain improvement. Preservation of cultures after strain improvement.

Unit – II Fermenter – types and function:

Fermenters – Basic functions, design and components – asepsis and containment requirements – body construction and temperature control – aeration and agitation systems – sterilization of fermenter, air supply, and medium; aseptic inoculation methods – sampling methods, valve systems – a brief idea on monitoring and control devices and types of fermenters. Photobioreactors.

Unit – III Fermentation process:

Growth of cultures in the fermenter. Importance of media in fermentation, media formulation and modification. Kinetics of growth in batch and continuous culture, specific growth rate, steady state in a chemostat, fed-batch fermentation, yield of biomass, product, calculation for productivity, substrate utilization kinetics. Fermentation process: Inoculum development. Storage of cultures for repeated fermentations, scaling up of process from shake flask to industrial fermentation.

Unit – IV Food microbiology:

Microbiology of fermented milk – starter cultures, butter milk, cream, yoghurt, kafir, kumiss, acidophilus milk and cheese. Microbes as sources of food (*Spirulina*, *Saccharomyces cerviceae*, *Rhizopus* sp.). Food intoxications: *Staphylococcus aureus*, *Clostridium botulinum* and mycotoxins; Food infections: *Bacillus cereus*, *Vibrio parahaemolyticus*, *Escherichia coli*, Salmonellosis, Shigellosis and *Campylobacter jejuni* – spoilage of canned foods – Detection of spoilage and characterization. Food sanitation in food manufacture and in the retail trade; Food control agencies and their regulations.

Unit – V Legal protection and IPR:

GATT and IPR, forms of IPR, IPR in India, WTO, TRIPS Convention on Biodiversity (CBD), Patent Co-operation Treaty (PCT), forms of patents and patentability, process of patenting, Indian and international agencies involved in IPR and patenting, Global scenario of patents and India’s position, patenting of biological materials.

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Unit – I

Industrially important microbes and their improvement:

Screening methods for industrial microbes – detection and assay of fermentation products– classification of fermentation types – strain selection and improvement. Mutation and recombinant DNA techniques for strain improvement. Preservation of cultures after strain improvement.

Screening methods for industrial microbes:

Screening

- ✓ "*The use of highly selective procedures to allow the detection & isolation of only those microorganisms which are of interest from among a large microbial population"*.
- ✓ Screening allows the discarding of many valueless microorganisms; at the same time it allows the easy detection of the useful microorganisms that are present in the population in very less number.

Primary and Secondary Screening of Industrially Important Microbes

Primary screening

- ✓ "*Primary screening allows the detection & isolation of microorganisms that possess potentially interesting industrial application*"
- ✓ Primary screening separate out only a few microorganisms having real commercial value.
- ✓ Primary screening determines which microorganisms are able to produce a compound without providing much idea of the production or yield potential of the organisms.

A. Primary screening of organic acid producing microorganisms

- Incorporation of a pH indicating dye such as neutral red or bromothymol blue into a poorly buffered agar medium.
- Greater buffer capacity of medium screen microbes having capability to produce considerable quantities of the acid.
- Incorporation of calcium carbonate in the medium is also used to screen organic acid producing microbes on the basis cleared zone of dissolved calcium carbonate around the colony.
- These screening approaches do not give idea that which organic acid has been produced.
- Thus the colonies of microorganisms showing the potential to produce any fermentation product should immediately be purified and sub-cultured into appropriate medium to be maintained as stock cultures for further testing.

B. Primary screening of antibiotic producing microorganisms

- The simplest screening technique for antibiotic producers is :Crowded Plate" technique.
- The technique is used to find out the microorganisms that produce an antibiotic without giving much information of sensitivity towards other microorganisms.
- Procedure include dilution and spreading or pouring of soil samples that give 300 or 400 or more colonies per plate.
- Colonies producing antibiotic activity are indicated by an area of agar around the colony
- Such a colony is sub-cultured to a similar medium and purified by streaking, before making stock cultures. The purified culture is then tested to find what types of microorganisms are sensitive in the presence of these the antibiotics i.e. "Microbial Inhibition Spectrum" (MIS).
- The crowded plate procedure also does not necessarily select an antibiotic producing microorganism, because the inhibition area around the colony sometimes can be due to other reason like….

(1) Marked change in the pH of the medium resulted due the metabolism of the colony.

(2) Rapid utilization of critical nutrients in the vicinity of the colony etc.

- Thus further testing is required to confirm the inhibitory activity associated with microorganisms is whether attributed to the presence of an antibiotic or not
- Screening of antibiotic producing microorganisms can be improved by using a “test organism” and Wilkins method

C. Primary screening of extracellular metabolites (Vitamins, Amino acids and Growth factors) producing microorganisms

D. Primary screening of microorganisms utilizing specific Carbon and Nitrogen sources

Secondary screening

Secondary screening allows further sorting out of microorganisms obtained from PS having real value for industrial processes and discarding of those lacking this potential.

1. SS is conducted on agar plates, in flasks or small fermented containing liquid media.
2. SS can be qualitative or quantitative in its approach.
3. Secondary screening should give information about the evaluation of the true potential of the microorganisms for industrial usage.
4. SS should determine whether microorganisms are actually producing new chemical compounds not previously described.
5. SS should reveal whether there is pH, aeration or other critical requirements associated with particular microorganisms, both for the growth of the organism and for the formation of chemical products.
6. SS should also detect gross genetic instability in microbial cultures.
7. SS should show whether certain medium constituents are missing or possibly, are toxic to the growth of the organisms or its ability to accumulate fermentation products.
8. SS should determine whether the product has a simple, complex, or even a macromolecular structure, if this information is not already available.
9. SS should show something of the chemical stability of the product and of the product’s solubility picture on various organic solvents.
10. SS should show whether the product possesses physical properties such as UV light absorption or fluorescence or chemical properties that can be employed to detect the compound during the use of paper chromatography or other analytical methods and which also might be of value in predicting the structure of the compound.
11. In some case, for certain kinds of fermentation product determinations should be made as to whether gross animal, plant or human toxicity can be attributed to the fermentation product, particularly if it is utilized (as are antibiotics) in disease treatment.
12. SS should reveal whether a product resulting from a microbial fermentation occurs in the culture broth in more than one chemical form and whether it is an optically or biologically active material.

13. SS should reveal whether the microorganisms are able to chemically alter or even destroy their own fermentation products.
14. Secondary screening helps in predicting the approaches to be utilized in conducting further research on the microorganisms and its fermentation processes.

Strategies for isolation of industrially important microbes:

- The diversity of microorganisms may be exploited still by searching for strains from the neutral environment able to produce products of commercial value.
- The first stage in the screening of microorganisms of potential industrial is their "isolation".
- Isolation involves obtaining either pure or mixed cultures followed by their assessment to determine which carry out the desired reaction or produce the desired product.
- In some cases it is possible to design the isolation procedure in such a way that the growth of producers is encouraged or that they may be recognized at the isolation stage, whereas in other cases organisms must be isolated and producers recognized at a subsequent stage.
- It should be remembered that the isolate must carry out the process economically and therefore the selection of the culture to be used is a compromise between the productivity of the organism and the economic constraints of the process.

Criteria used for choice of organisms:

- The nutritional characteristics of the organism: Organism should be capable to utilize the ingredients present in the medium to produce interested product.
- The optimum temperature of the organisms: For instance, the use of an organism having an optimistic temperature above 40^{o} C considerably reduces the cooling costs of a large-scale fermentation, and therefore, the use of such a temperature in the isolation procedure may be beneficial.
- The reaction of the organism with the equipment to be employed.
- The stability of the organism and its amenability to genetic manipulation.
- The productivity of the organism, measured in its ability to convert substrate into product and to give a high yield of product per unit time.
- The easy product recovery from the cultures.
- It should be a high yielding strain.
- It should have stable biochemical characteristics.
- It should not produce undesirable substances.
- It should be easily cultivated on a large scale.
- The ideal isolation procedure commences with an environmental source (frequently soil), which is highly profitable to be rich in the desired types.

- Selective pressure may be used in the isolation of organism that will grow on particular substrates in the presence of certain compounds or under agricultural conditions adverse in their types.
- If it is not possible to apply selective pressure for the desired character it may be possible to design a procedure to select for a microbial taxon which is known to show the characteristics at a relatively high frequency. E.g. the production of antibiotic by Streptomycin.
- Alternately, the isolation procedure may be designed to exclude certain microbial "weeds" and to encourage the growth of more novel types.
- The advantages in the taxonomic description of taxa have allowed the rational design of procedures for the isolation of strains that may have a high probability of being productive or are representatives of unusual groups.
- The advances in pharmacology and molecular biology have also enabled the design of more effective screening tests to identify productive strains amongst the isolated organisms.

Detection and assay of fermentation products:

Physical and Chemical Assay of Fermentation Products:

Introduction:

- Screening of Fermentation products require good detection and assay technique. This also is true for most of the fermentation studies at all points of development.
- These procedures must be simple, quick, reliable, and accurate.
- It must detect only the compound of interest in relatively greater concentration of various chemical contaminants from the growth medium.
- Sometimes, more than one assay procedure is possible so one can choose the best assay procedure with an alternate assay used periodically to test the validity of the assay of choice.

Physical-chemical Assays:

The choice of the particular assay techniques from various available technique is depends on the selectivity of the chemical reaction or chemical reactions or chemical analysis involved since the fermentation broth contains many compounds in addition to those to be determined. In fact, in some instances at least a partial purification, of the fermentation product may be necessary before carrying out the assay. Let us see different types of Assay.

Types of Physical and Chemical Assays

1. Titration, and Gravimetric Analysis assay
2. Turbidity analysis, and Cell Yield Determination assay
3. Spectrophotometric assay
4. Chromatographic partition assay
5. Gas Chromatographic assay

6. Other techniques, like Infrared spectroscopy, Nuclear Magnetic Resonance and mass spectroscopy

Titration and Gravimetric Analysis Assay:

- Titration Assay is use for the fermentation product if the product is Acid.
- Volatile small molecular weight organic acids distilled directly from the acidified broth now this distillate used for the analysis by titration.
- Organic acid of higher molecular weight separated from the fermentation medium by adsorption and elution from a suitable anion exchange resins.
- Once organic acid separate from the medium, it is analyzed by titration using pH indicator dye such as Bromothymol Blue to a sample followed by titration with alkali of known strength.
- If electrometric titrations are, used dye is not used.
- If the product is insoluble, then it is precipitated, washed, dried, and weighed. (That is, Gravimetric Analysis)

Turbidity analysis and Cell Yield Determination assay:

- The determination of cell in the given fermentation broth is done by cell yield determination assay if the cells are only insoluble products present in the medium and grow as pellets.
- Cells along with the medium centrifuged in graduated tubes and the volume of sedimented cells measured in cubic centimeters.
- Turbidity is another technique to study the cells in the fermentation broth as product.
- This technique is useful for the organisms growing throughout the medium. The cells suspended in their growth medium diluted to a turbidity range that measures quantitatively as optical density with nephlometer or colorimeter, by the deflection of light that caused by the microbial cells when suspended in the light path of these instruments.
- Turbidity measurements of cell numbers usually standardized against some other techniques, such as plate counts for determining number of cells.
- Standard curve is prepared relating optical density to plate count from each series of dilutions of the cell suspension.
- Once this standard graph is prepared then one can count number of cells from the optical density.

Spectrophotometric Assay:

- Spectrophotometric assays utilizes various types of spectrophotometers to measure amount of visible light absorbed by colored solution at specific wavelength, amount of ultra violet light absorbed by compound or the intensity of the fluorescence emitted by the compound when exposed to ultraviolet light.
- Fermentation products, which are in themselves colored but form different hue than that of the medium measured directly in the colorimeter or possibly after a simple purification step.

- If fermentation product not colored then it is reacted with chemical to form colored compound and then measured by colorimeter.
- If the fermentation product is neither colored nor reacting with any chemical reagent, to give visible color can be determined by absorption of ultraviolet radiation or fluoresce under ultraviolet light.
- The principles of analysis are similar to those for visible light determinations and under ultraviolet wavelengths chosen to allow maximum fluorescence or absorption of ultraviolet light.
- The use of spectrophotometric analysis to assay a previously unknown fermentation product is more difficult because the pure compound may not be available for use as a reference standard in preparing standard curve.
- Spectrophotometric analyses that do not yield quantitative results employed to detect the presence of unsuspected compounds in fermentation broth.
- A dilution of fermentation broth tested for ultraviolet absorption at individual wavelengths over entire spectrum of ultraviolet.
- The spectral analysis will show peaks of ultraviolet absorption at certain wavelengths and other wavelengths will show little if any ultraviolet light absorption.

Chromatographic Partition Assay:

- Paper and Thin layer chromatography are forms of partition chromatography.
- The solute or sample partitioned between a stationary phase such as paper or silica gel of thin layer plates and a mobile phase consisting of a mixture of solvents as these solvents migrate across the paper or silica gel layer.
- Paper chromatography applied for water-soluble compounds while thin layer chromatography used for hydrophobic compounds.
- However, water soluble and insoluble compounds separated by both the procedures. Solute or the fermentation product is loaded to paper or silica gel and allowed to separate on either of these stationary phases with the help of solvent that is present in chamber.
- According to the composition of product, there will be formation of different bands on the paper or silica gel, which is to be colored by means of coloring agent and then each band is measure for its movements from the origin that is its Rf value.
- Based on calculation of Rf value of each band it is possible to determine the chemical characteristic of particular product because each band represent chemical entity which can move up to certain extent in given solvent.
- Different compound can be detected with the help of Rf value of standard chemical compound. When single solvent system is not sufficient to separate compound then two solvent systems used this technique known as ‘two dimensional’ chromatography.

- ❖ After loading of sample to chromatogram, (Paper or Silica gel) it allowed to run with the help of first solvent system then chromatogram is turned ninety degree and again allowed to run with the help of second solvent system while doing so compound will move with one of the solvent systems.

Gas chromatography Assay

- ❖ The gas chromatograph utilizes a form of partition chromatography to volatilize and separate certain types of fermentation products.
- ❖ On injection into the gas chromatograph, the fermentation product converted to gaseous state and in this state it pushed by a stream of inert gas through a partitioning column.
- ❖ Compound separated during passage from this column detected electronically with the help of detector system. If product is not volatile, it made volatile with the help of chemical like esters.
- ❖ Quantitative assay obtained by comparison of the areas occurring under peaks on plots of the data with corresponding areas of various quantities of reference compound.

Fermentation:

Fermentation Definition:

- Fermentation refers to the metabolic process by which organic molecules (normally glucose) are converted into acids, gases, or alcohol in the absence of oxygen or any electron transport chain.
- Fermentation pathways regenerate the coenzyme nicotinamide adenine dinucleotide (NAD^+), which is used in glycolysis to release energy in the form of adenosine triphosphate (ATP).
- Fermentation only yields a net of 2 ATP per glucose molecule (through glycolysis), while aerobic respiration yields as many as 32 molecules of ATP per glucose molecule with the aid of the electron transport chain.
- The study of fermentation and its practical uses is named zymology and originated in 1856 when French chemist Louis Pasteur demonstrated that fermentation was caused by yeast.
- Fermentation occurs in certain types of bacteria and fungi that require an oxygen-free environment to live (known as obligate anaerobes), in facultative anaerobes such as yeast, and also in muscle cells when oxygen is in short supply (as in strenuous exercise).
- The processes of fermentation are valuable to the food and beverage industries, with the conversion of sugars into ethanol used to produce alcoholic beverages, the release of CO_2 by yeast used in the leavening of bread, and with the production of organic acids to preserve and flavor vegetables and dairy products.

Function of Fermentation:

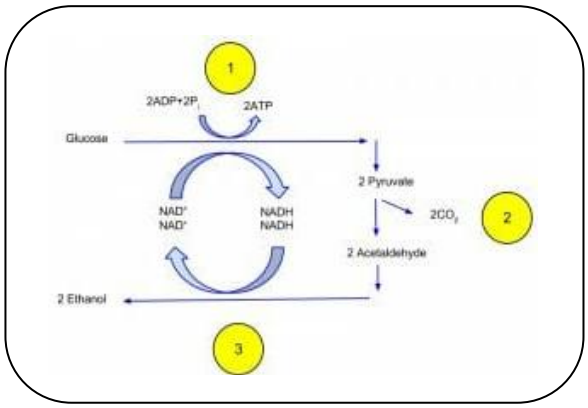
The main function of fermentation is to convert NADH back into the coenzyme NAD^+ so that it can be used again for glycolysis. During fermentation, an organic electron acceptor (such as pyruvate or

acetaldehyde) reacts with NADH to form NAD^+, generating products such as carbon dioxide and ethanol (ethanol fermentation) or lactate (lactic acid fermentation) in the process.

Types of Fermentation:

There are many types of fermentation that are distinguished by the end products formed from pyruvate or its derivatives. The two fermentations most commonly used by humans to produce commercial foods are ethanol fermentation (used in beer and bread) and lactic acid fermentation (used to flavor and preserve dairy and vegetables).

Ethanol Fermentation:

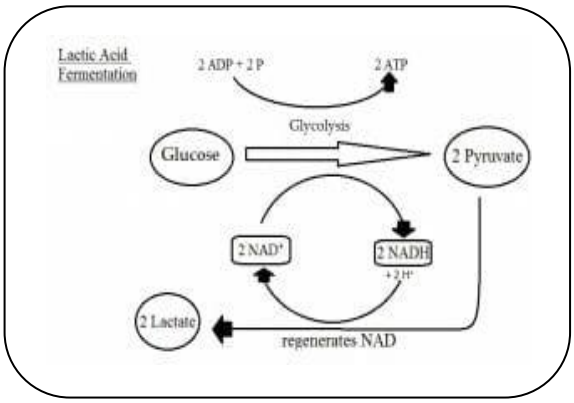


This figure depicts the processes of glycolysis and ethanol fermentation.

In ethanol fermentation, the pyruvate produced through glycolysis is converted to ethanol and carbon dioxide in two steps. First, the pyruvate releases carbon dioxide to form a two-carbon compound called acetaldehyde. Next, acetaldehyde is reduced by NADH to ethanol, thereby regenerating the NAD^+ for use in glycolysis. Overall, one molecule of glucose is converted into two molecules of carbon dioxide and two molecules of ethanol.

Ethanol fermentation is typically performed by yeast, which is a unicellular fungus.

Lactic Acid Fermentation:



This figure depicts the processes of glycolysis and homolactic fermentation.

There are two main types of lactic acid fermentation: homolactic and heterolactic. In homolactic acid fermentation, NADH reduces pyruvate directly to form lactate. This process does not release gas.

Overall, one molecule of glucose is converted into two molecules of lactate. In heterolactic fermentation, some lactate is further metabolized, resulting in ethanol and carbon dioxide via the phosphoketolase pathway.

Lactic acid fermentation is primarily performed by certain types of bacteria and fungi. However, this type of fermentation also occurs in muscle cells to produce ATP when the oxygen supply has been depleted during strenuous exercise and aerobic respiration is not possible.

Fermentation Equation

Ethanol Fermentation

glucose → 2 ethanol + 2 carbon dioxide

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

Lactic Acid Fermentation

glucose → 2 lactic acid

$$\mathbf{C_6H_{12}O_6 \rightarrow 2\ C_3H_6O_3}$$

Products of Fermentation:

While there are a number of products from fermentation, the most common are ethanol, lactic acid, carbon dioxide, and hydrogen gas (H_2). These products are used commercially in foods, vitamins, pharmaceuticals, or as industrial chemicals. In addition, many less common products still offer commercial value. For example, the production of acetone via the acetone – butanol – ethanol fermentation was first developed by the Jewish chemist Chaim Weizmann and was important to the British war industry during Word War I.

What Is Fermentation?

Fermentation is any metabolic process in which microorganisms' activity creates a desirable change in food and beverages, whether it's increasing flavor, preserving foodstuffs, providing health benefits, or more.

The word "ferment" comes from the Latin verb "fervere," which means "to boil." Ironically, fermentation is possible without heat.

How Does Fermentation Work?

To master fermentation, you need to understand the science behind the chemical process.

- Microorganisms survive using carbohydrates (sugars, such as glucose) for energy and fuel.
- Organic chemicals like adenosine triphosphate (ATP) deliver that energy to every part of a cell when needed.
- Microbes generate ATP using respiration. Aerobic respiration, which requires oxygen, is the most efficient way to do that. Aerobic respiration begins with glycolysis, where glucose is converted into pyruvic acid. When there's enough oxygen present, aerobic respiration occurs.

- Fermentation is similar to anaerobic respiration—the kind that takes place when there isn't enough oxygen present. However, fermentation leads to the production of different organic molecules like lactic acid, which also leads to ATP, unlike respiration, which uses pyruvic acid.
- Depending upon environmental conditions, individual cells and microbes have the ability to switch between the two different modes of energy production.
- Organisms commonly obtain energy anaerobically through fermentation, but some systems use sulfate as the final electron acceptor in the electron transport chain.

What Happens During the Fermentation Process?

Fermentation occurs in the absence of oxygen (anaerobic conditions), and in the presence of beneficial microorganisms (yeasts, molds, and bacteria) that obtain their energy through fermentation. If enough sugar is available, some yeast cells, such as *Saccharomyces cerevisiae*, prefer fermentation to aerobic respiration even when oxygen is abundant.

- During the fermentation process, these beneficial microbes break down sugars and starches into alcohols and acids, making food more nutritious and preserving it so people can store it for longer periods of time without it spoiling.
- Fermentation products provide enzymes necessary for digestion. This is important because humans are born with a finite number of enzymes, and they decrease with age. Fermented foods contain the enzymes required to break them down.
- Fermentation also aids in pre-digestion. During the fermentation process, the microbes feed on sugars and starches, breaking down food before anyone's even consumed it.

What Are the Advantages of Fermentation?

Fermented foods are rich in probiotics, beneficial microorganisms that help maintain a healthy gut so it can extract nutrients from food.

- Probiotics aid the immune system because the gut produces antibiotic, anti-tumor, anti-viral, and antifungal substances, and pathogens don't do well in the acidic environment fermented foods create.
- Fermentation also helps neutralize anti-nutrients like phytic acid, which occurs in grains, nuts, seeds, and legumes and can cause mineral deficiencies. Phytates also make starches, proteins, and fats less digestible, so neutralizing them is extremely beneficial.
- Fermentation can increase the vitamins and minerals in food and make them more available for absorption. Fermentation increases B and C vitamins and enhances folic acid, riboflavin, niacin, thiamin, and biotin. The probiotics, enzymes, and lactic acid in fermented foods facilitate the absorption of these vitamins and minerals into the body.

What Are the 3 Different Types of Fermentation?

Microbes specialized at converting certain substances into others can produce a variety of foodstuffs and beverages. These are three distinct types of fermentation that people use.

1. **Lactic acid fermentation**. Yeast strains and bacteria convert starches or sugars into lactic acid, requiring no heat in preparation. These anaerobic chemical reactions, pyruvic acid uses nicotinamide adenine dinucleotide + hydrogen (NADH) to form lactic acid and NAD+. (Lactic acid fermentation also occurs in human muscle cells. During strenuous activity, muscles can expend adenosine triphosphate (ATP) faster than oxygen can be supplied to muscle cells, resulting in lactic acid buildup and sore muscles. In this scenario, glycolysis, which breaks down a glucose molecule into two pyruvate molecules and doesn't use oxygen, produces ATP.) Lactic acid bacteria are vital to producing and preserving inexpensive, wholesome foods, which is especially important in feeding impoverished populations. This method makes sauerkraut, pickles, kimchi, yogurt, and sourdough bread.
2. **Ethanol fermentation/alcohol fermentation**. Yeasts break pyruvate molecules—the output of the metabolism of glucose ($C_6H_{12}O_6$) known as glycolysis—in starches or sugars down into alcohol and carbon dioxide molecules. Alcoholic fermentation produces wine and beer.
3. **Acetic acid fermentation**. Starches and sugars from grains and fruit ferment into sour tasting vinegar and condiments. Examples include apple cider vinegar, wine vinegar, and kombucha.

What Are the Different Stages of the Fermentation Process?

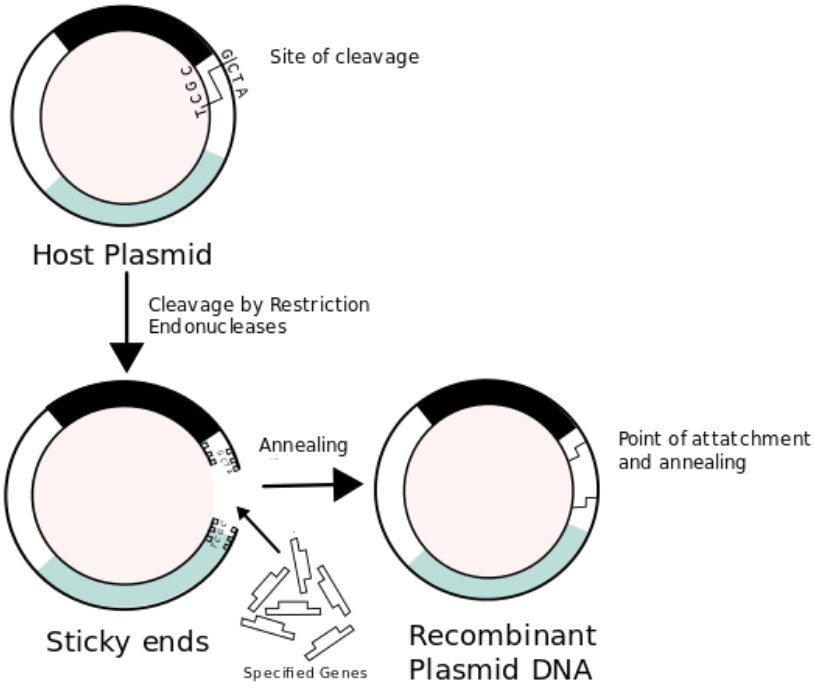
Learn techniques for cooking vegetables and eggs and making pastas from scratch from the award-winning chef and proprietor of The French Laundry.

Depending upon what you're fermenting, the process can have several stages.

- **Primary fermentation**. In this brief phase, microbes begin rapidly working on raw ingredients such as fruit, vegetables, or dairy. The microbes present or in the surrounding liquid (such as brine for fermented vegetables) prevent putrefying bacteria from colonizing the food instead. Yeasts or other microbes convert carbohydrates (sugars) into other substances such as alcohols and acids.
- **Secondary fermentation**. In this longer stage of fermentation, which lasts several days or even weeks, alcohol levels rise and yeasts and microbes die off and their available food source (the carbohydrates) becomes scarcer. Winemakers and brewers use secondary fermentation to create their alcoholic beverages. The pH of the ferment can differ significantly from when it started out, which affects the chemical reactions taking place between the microbes and their environment. Once alcohol is between 12–15% and it kills the yeast, preventing further fermentation, distillation is needed to remove water, condensing alcohol content to create a higher percentage of alcohol (proof).

Recombinant DNA (**rDNA**):

- ✓ **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 combining 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.
- ✓ 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.



Creation:

- Molecular cloning is the laboratory process used to create recombinant DNA. It is one of two most widely used methods, along with polymerase chain reaction (PCR), used to direct the replication of any specific DNA sequence chosen by the experimentalist.
- There are two fundamental differences between the methods. One is that molecular cloning involves replication of the DNA within a living cell, while PCR replicates DNA in the test tube, free of living cells.
- The other difference is that cloning involves cutting and pasting DNA sequences, while PCR amplifies by copying an existing sequence.
- Formation of recombinant DNA requires a cloning vector, a DNA molecule that replicates within a living cell.
- Vectors are generally derived from plasmids or viruses, and represent relatively small segments of DNA that contain necessary genetic signals for replication, as well as additional elements for convenience in inserting foreign DNA, identifying cells that contain recombinant DNA and where appropriate, expressing the foreign DNA.
- The choice of vector for molecular cloning depends on the choice of host organism, the size of the DNA to be cloned, and whether and how the foreign DNA is to be expressed.
- The DNA segments can be combined by using a variety of methods, such as restriction enzyme/ligase cloning or Gibson assembly.
- In standard cloning protocols, the cloning of any DNA fragment essentially involves seven steps:
  (1) Choice of host organism and cloning vector,
  (2) Preparation of vector DNA,
  (3) Preparation of DNA to be cloned,

(4) Creation of recombinant DNA,

(5) Introduction of recombinant DNA into the host organism,

(6) Selection of organisms containing recombinant DNA, and

(7) Screening for clones with desired DNA inserts and biological properties. *These steps are described in some detail in a related article (molecular cloning).*

Expression:

- Following transplantation into the host organism, the foreign DNA contained within the recombinant DNA construct may or may not be expressed.
- That is, the DNA may simply be replicated without expression, or it may be transcribed and translated and a recombinant protein is produced.
- Generally speaking, expression of a foreign gene requires restructuring the gene to include sequences that are required for producing an mRNA molecule that can be used by the host's translational apparatus (e.g. promoter, translational initiation signal, and transcriptional terminator).
- Specific changes to the host organism may be made to improve expression of the ectopic gene. In addition, changes may be needed to the coding sequences as well, to optimize translation, make the protein soluble, direct the recombinant protein to the proper cellular or extracellular location, and stabilize the protein from degradation.

Properties of organisms containing recombinant DNA:

- In most cases, organisms containing recombinant DNA have apparently normal phenotypes. That is, their appearance, behavior and metabolism are usually unchanged, and the only way to demonstrate the presence of recombinant sequences is to examine the DNA itself, typically using a polymerase chain reaction (PCR) test.

Significant exceptions exist, and are discussed below.

- If the rDNA sequences encode a gene that is expressed, then the presence of RNA and/or protein products of the recombinant gene can be detected, typically using RT-PCR or western hybridization methods.
- Gross phenotypic changes are not the norm, unless the recombinant gene has been chosen and modified so as to generate biological activity in the host organism.
- Additional phenotypes that are encountered include toxicity to the host organism induced by the recombinant gene product, especially if it is over-expressed or expressed within inappropriate cells or tissues.

- In some cases, recombinant DNA can have deleterious effects even if it is not expressed. One mechanism by which this happens is insertion inactivation, in which the rDNA becomes inserted into a host cell's gene.
- In some cases, researchers use this phenomenon to "knock out" genes to determine their biological function and importance.
- Another mechanism by which rDNA insertion into chromosomal DNA can affect gene expression is by inappropriate activation of previously unexpressed host cell genes.
- This can happen, for example, when a recombinant DNA fragment containing an active promoter becomes located next to a previously silent host cell gene, or when a host cell gene that functions to restrain gene expression undergoes insertional inactivation by recombinant DNA.

Uses:

- ❖ Recombinant DNA is widely used in biotechnology, medicine and research. Today, recombinant proteins and other products that result from the use of DNA technology are found in essentially every western pharmacy, physician or veterinarian office, medical testing laboratory and biological research laboratory.
- ❖ In addition, organisms that have been manipulated using recombinant DNA technology, as well as products derived from those organisms, have found their way into many farms, supermarkets, home medicine cabinets, and even pet shops, such as those that sell GloFish and other genetically modified animals.
- ❖ The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences.
- ❖ Recombinant DNA is used to identify, map and sequence genes, and to determine their function. rDNA probes are employed in analyzing gene expression within individual cells and throughout the tissues of whole organisms.
- ❖ Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms.
- ❖ Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture and bioengineering.

Some specific examples are identified below.

Recombinant chymosin:

- ✓ Found in rennet, chymosin is an enzyme required to manufacture cheese. It was the first genetically engineered food additive used commercially.
- ✓ Traditionally, processors obtained chymosin from rennet, a preparation derived from the fourth stomach of milk-fed calves.

- ✓ Scientists engineered a non-pathogenic strain (K-12) of *E. coli* bacteria for large-scale laboratory production of the enzyme.
- ✓ This microbiologically produced recombinant enzyme, identical structurally to the calf derived enzyme, costs less and is produced in abundant quantities.
- ✓ Today about 60% of U.S. hard cheese is made with genetically engineered chymosin. In 1990, FDA granted chymosin "generally recognized as safe" (GRAS) status based on data showing that the enzyme was safe.

Recombinant human insulin:

- ✓ Almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of insulin-dependent diabetes.
- ✓ A variety of different recombinant insulin preparations are in widespread use.
- ✓ Recombinant insulin is synthesized by inserting the human insulin gene into *E. coli*, or yeast (Saccharomyces cerevisiae) which then produces insulin for human use.

Recombinant human growth hormone (HGH, somatotropin):

- ✓ Administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development?
- ✓ Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers.
- ✓ This unsafe practice led to some patients developing Creutzfeldt–Jakob disease.
- ✓ Recombinant HGH eliminated this problem, and is now used therapeutically.
- ✓ It has also been misused as a performance-enhancing drug by athletes and others. Drug Bank entry

Recombinant blood clotting factor VIII:

- ✓ A blood-clotting protein that is administered to patients with forms of the bleeding disorder hemophilia, who are unable to produce factor VIII in quantities sufficient to support normal blood coagulation.
- ✓ Before the development of recombinant factor VIII, the protein was obtained by processing large quantities of human blood from multiple donors, which carried a very high risk of transmission of blood borne infectious diseases, for example HIV and hepatitis B. DrugBank entry

Recombinant hepatitis B vaccine:

- ✓ Hepatitis B infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells.
- ✓ The development of the recombinant subunit vaccine was an important and necessary development because hepatitis B virus, unlike other common viruses such as polio virus, cannot be grown in vitro. Vaccine information from Hepatitis B Foundation

Diagnosis of infection with HIV:

- ✓ Each of the three widely used methods for diagnosing HIV infection has been developed using recombinant DNA.
- ✓ The antibody test (ELISA or western blot) uses a recombinant HIV protein to test for the presence of antibodies that the body has produced in response to an HIV infection.
- ✓ The DNA test looks for the presence of HIV genetic material using reverse transcription polymerase chain reaction (RT-PCR).
- ✓ Development of the RT-PCR test was made possible by the molecular cloning and sequence analysis of HIV genomes. HIV testing page from US Centers for Disease Control (CDC)

Golden rice:

- ✓ A recombinant variety of rice that has been engineered to express the enzymes responsible for β-carotene biosynthesis.
- ✓ This variety of rice holds substantial promise for reducing the incidence of vitamin A deficiency in the world's population.
- ✓ Golden rice is not currently in use, pending the resolution of regulatory and intellectual property issues.

Herbicide-resistant crops:

- ✓ Commercial varieties of important agricultural crops (including soy, maize/corn, sorghum, canola, alfalfa and cotton) have been developed that incorporate a recombinant gene that results in resistance to the herbicide glyphosate (trade name *Roundup*), and simplifies weed control by glyphosate application. These crops are in common commercial use in several countries.

Insect-resistant crops:

- ✓ *Bacillus thuringeiensis* is a bacterium that naturally produces a protein (Bt toxin) with insecticidal properties.
- ✓ The bacterium has been applied to crops as an insect-control strategy for many years, and this practice has been widely adopted in agriculture and gardening.
- ✓ Recently, plants have been developed that express a recombinant form of the bacterial protein, which may effectively control some insect predators.
- ✓ Environmental issues associated with the use of these transgenic crops have not been fully resolved.

Strain improvement is aTechnology of manipulating and **improving** microbial **strains** in order to enhancemetabolic capabilities. The directed **improvement** of productformation or cellular properties

through modifications of specific biochemicalpathways or by introduction of new pathways using recombinant DNA technology.

Maintenance and Preservation of Pure Cultures:

The following points highlight the top four methods used for maintenance and preservation of pure cultures. The methods are: 1. Refrigeration 2. Paraffin Method 3. Cryopreservation 4. Lyophilisation.

1. Refrigeration:

- Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped.
- Thus their growth continues slowly, nutrients are utilized and waste products released in medium. This results in, finally, the death of the microbes after sometime.

2. Paraffin Method:

- This is a simple and most economical method of maintaining pure cultures of bacteria and fungi. In this method, sterile liquid paraffin in poured over the slant (slope) of culture and stored upright at room temperature.
- The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium. This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture is preserved for several years.

3. Cryopreservation:

- Cryopreservation (i.e., freezing in liquid nitrogen at-196°C) helps survival of pure cultures for long storage times.
- In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol that prevent the formation of ice crystals and promote cell survival.

4. Lyophilisation (Freeze-Drying):

- In this method, the culture is rapidly frozen at a very low temperature (-70°C) and then dehydrated by vacuum.
- Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years.
- Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators. Freeze- drying method is the most frequently used technique by culture collection centres.

Unit – II

Fermenter – types and function:

Fermenters – Basic functions, design and components – asepsis and containment requirements – body construction and temperature control – aeration and agitation systems – sterilization of fermenter, air supply, and medium; aseptic inoculation methods – sampling methods, valve systems – a brief idea on monitoring and control devices and types of fermenters. Photobioreactors.

Fermentation is the process in which a substance breaks down into a simpler substance. Microorganisms like yeast and bacteria usually play a role in the **fermentation** process, creating beer, wine, bread, kimchi, yogurt and other foods.

- ✓ A fermenter is an apparatus that maintains required optimal environmental conditions for the growth of industrially important microorganisms, used in large scale fermentation process and in the commercial production of a range of fermentation products like Antibiotics, Enzymes, Organic acids, Alcoholic beverages etc.
- ✓ To provide a controlled physico-chemical environment for the growth of a pure culture or a well-defined mixed culture of microorganisms is the key function of an ideal fermenter to obtain the desired fermentation products
- ✓ An ideal fermenter maintains optimal environmental conditions throughout the process for the process organisms, added substrates and additives for a quality end product
- ✓ Saving of energy and cost effective operation is very important concern as far as fermentation economics is concern
- ✓ Many times, the terms “Bioreactor” and “Fermenter” are used synonymously. There is a very minor difference between these two
- ✓ The bioreactor is used for the mass culture of plant and animal cells, while fermenter is mainly used for microbial culture

The operational parameters and design engineering of fermenters and bioreactors are identical.

- ✓ Control the pH of the culture throughout the process
- ✓ Have provision of constant monitoring and control of level of dissolved oxygen
- ✓ Allow feeding of nutrient solutions and other supplementary requirements
- ✓ Provide access points for seed culture inoculation and sampling during the process
- ✓ Reduce liquid loss from the vessel during process by cooling system
- ✓ Be capable of being operated aseptically during the tenure of the process thus fulfilling the requirements of containment regulations
- ✓ Ensure that overall process period should have power consumption, as low as possible
- ✓ Be designed in such a way that it require the minimal use of labours during production process and downstream operations (i.e. harvesting, cleaning and maintenance)

- ✓ Should be suitable for a range of processes along with the containment regulations
- ✓ Be constructed in such a way that it ensure even internal surfaces, using welds instead of flange joints whenever possible
- ✓ Should have identical geometry at different operational level (i.e. both smaller and larger vessels in the pilot or scale-up plant)
- ✓ The material from which the fermenter is made up of should be inert and capable to withstand repeated stem sterilization conditions
- ✓ Apart from all these parameters, it is very imperative to have adequate service provisions for individual plants. The important service provisions are listed below.

Fermentor (Bioreactor):

History, Design and Its Construction:

1. Meaning of Fermentor
2. History of Fermentors
3. Design
4. Construction
5. Use of Computer in Fermentor
6. Types.

Meaning of Fermentor:

- ✓ A fermentor (bioreactor) is a closed vessel with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along-with their products.
- ✓ A fermentor is used for commercial production in fermentation industries and is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Fermentors are extensively used for food processing, fermentation, waste treatment, etc.

History of Fermentors:

- ✓ De Beeze and Liebmann (1944) used the first large scale (above 20 litre capacity) fermentor for the production of yeast. But it was during the first world war, a British scientist named Chain Weizmann (1914-1918) developed a fermentor for the production of acetone.
- ✓ Since importance of aseptic conditions was recognised, hence steps were taken to design-and construct piping, joints and valves in which sterile conditions could be achieved and manufactured when required.
- ✓ For the first time, large scale aerobic fermentors were used in central Europe in the year 1930's for the production of compressed yeast (de Becze and Leibmann, 1944).

- ✓ The fermentor consisted of a large cylindrical tank with air introduced at the base via network of perforated pipes.
- ✓ In later modifications, mechanical impellers were used to increase the rate of mixing and to break up and disperse the air bubbles.
- ✓ This process led to the compressed air requirements. Baffles on the walls of the vessels prevented forming a vortex in the liquid. In the year 1934, Strauch and Schmidt patented a system in which the aeration tubes were introduced with water and steam for cleaning and sterilization.
- ✓ The decision to use submerged culture technique for penicillin production, where aseptic conditions, good aeration and agitation were essential, was probably a very important factor in forcing the development of carefully designed and purpose-built fermentation vessels.
- ✓ In 1943, when the British Govt. decided that surface culture was inadequate, none of the fermentation plants were immediately suitable for deep fermentation.
- ✓ The first pilot fermentor was erected in India at Hindustan Antibiotic Ltd., Pimpri, Pune in the year 1950.

3. Design of Fermentors:

All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of bioreactors.

A bioreactor should provide for the following:

(i) Agitation (for mixing of cells and medium),

(ii) Aeration (aerobic fermentors); for O_2 supply,

(iii) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level etc.,

(iv) Sterilization and maintenance of sterility, and

(v) Withdrawal of cells/medium (for continuous fermentors).

Modern fermentors are usually integrated with computers for efficient process monitoring, data acquisition, etc.

Generally, 20-25% of fermentor volume is left unfilled with medium as “head space” to allow for splashing, foaming and aeration. The fermentor design varies greatly depending on the type and the fermentation for which it is used. Bioreactors are so designed that they provide the best possible growth and biosynthesis for industrially important cultures and allow ease of manipulation for all operations.

Size of Fermentors:

The size of fermentors ranges from 1-2 litre laboratory fementors to 5,00,000 litre or, occasionally, even more, fermentors of upto 1.2 million litres have been used. The size of the fermentor used

depends on the process and how it is operated. A summary of fermentor or size of fermentor (litres) Industrial product sizes for some common microbial fermentations is given in Table 39.6.

TABLE 39.6. Fermentor sizes for various microbial fermentations

Size of fermentor (litres)	*Industrial product*
1-20,000	Diagnostic enzymes, substances for molecular biology.
40-80,000	Some enzymes, antibiotics.
100-1,50,000	Penicillium, aminolycoside, antibiotics, amyloses, proteases, amino acids, steriod transformations, wine, beer.
2,00,000-5,00,000	Amino acids(glutamate), wine, beer.

4. Construction of Fermentors:

Industrial fermentors can be divided into two major classes, anaerobic and aerobic. Anaerobic fermentors require little special equipment except for removal of heat generated during the fermentation process, whereas aerobic fermentors require much more elaborate equipment to ensure that mixing and adequate aeration are achieved.

Since most industrial fermentation process are aerobic, the construction of a typical aerobic fermentor (Fig. 39.1) is the following:

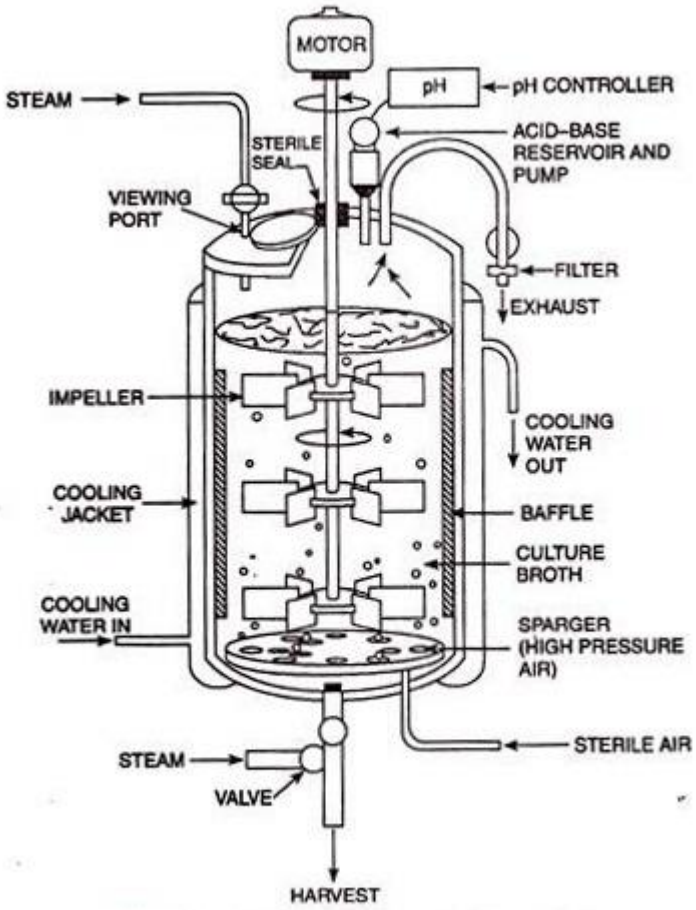


FIG. 39.1. An industrial aerobic fermentor (internal view)

1. Cooling Jacket:

- Large-scale industrial fermentors are almost always constructed of stainless steel. A fermentor is a large cylinder closed at the top and the bottom and various pipes and valves are fitted into it. The fermentor is fitted externally with a cooling jacket through which steam (for sterilization) or cooling water (for cooling) is run.
- Cooling jacket is necessary because sterilization of the nutrient medium and removal of the heat generated are obligatory for successful completion of the fermentation in the fermentor. For very large fermentors, insufficient heat transfer takes place through the jacket and therefore, internal coils are provided through which either steam or cooling water is run.

2. Aeration System:

- Aeration system is one of the most critical part of a fermentor. In a fermentor with a high microbial population density, there is a tremendous oxygen demand by the culture, but oxygen being poorly soluble in water hardly transfers rapidly throughout the growth medium.
- It is necessary, therefore, that elaborate precautions are taken using a good aeration system to ensure proper aeration an oxygen availability throughout the culture. However, two separate aeration devices are used to ensure proper aeration in fermentor. These devices are sparger and impeller.
- The sparger is typically just a series of holes in a metal ring or a nozzle through which filter-sterilized air (or oxygen-enriched air) passes into the fermentor under high pressure.
- The air enters the fermentor as a series of tiny bubbles from which the oxygen passes by diffusion into the liquid culture medium.
- The impeller (also called agitator) is an agitating device necessary for stirring of the fermenter.

The stirring accomplishes two things:

(i) It mixes the gas bubbles through the liquid culture medium and

(ii) It mixes the microbial cells through the liquid culture medium. In this way, the stirring ensures uniform access of microbial cells to the nutrients.

The size and position of the impeller in the fermentor depends upon the size of the fermentor. In tall fermentors, more than one impeller is needed if adequate aeration and agitation is to be obtained. Ideally, the impeller should be 1/3 of the fermentors diameter fitted above the base of the fermentor. The number of impeller may vary from size to size to the fermentor.

3. Baffles:

The baffles are normally incorporated into fermentors of all sizes to prevent a vortex and to improve aeration efficiency. They are metal strips roughly one-tenth of the fermentors diameter and attached radially to the walls.

4. Controlling Devices for Environmental Factors:

- In any microbial fermentation, it is necessary not only to measure growth and product formation but also to control the process by altering environmental parameters as the process proceeds.
- For this purpose, various devices are used in a fermentor. Environmental factors that are frequently controlled includes temperature, oxygen concentration, pH, cells mass, levels of key nutrients, and product concentration.

Use of Computer in Fermentor:

- Computer technology has produced a remarkable impact in fermentation work in recent years and the computers are used to model fermentation processes in industrial fermentors. Integration of computers into fermentation systems is based on the computers capacity for process monitoring, data acquisition, data storage, and error-detection.
- Some typical, on-line data analysis functions include the acquisition measurements, verification of data, filtering, unit conversion, calculations of indirect measurements, differential integration calculations of estimated variables, data reduction, tabulation of results, graphical presentation of results, process stimulation and storage of data.

Types of Fermentor:

The fermentor (bioreactor) types used extensively in industries are the stirred tank fermentor, airlift fermentor, and bubble column fermentor.

(i) Stirred Tank Fermentor:

Stirred tank fermentors consists of a cylindrical vessel with a motor driven central saft that supports one or more impellers.

(ii) Airlift Fermentor:

In airlift fermentor (Fig. 39.2) the liquid culture volume of the vessel is divided into two interconnected zones by means of a baffle or draft tube. Only one of the two zones is sparged with air or other gas and this sparged zone is known as the riser.

The other zone that receives no gas is called down-comer. The bulk density of the gas-liquid dispersion in the gas-sparged riser tends to be lower than the bulk density in the down-comer, consequently the dispersion flows up in the riser zone and down-flow occurs in the down-comer.

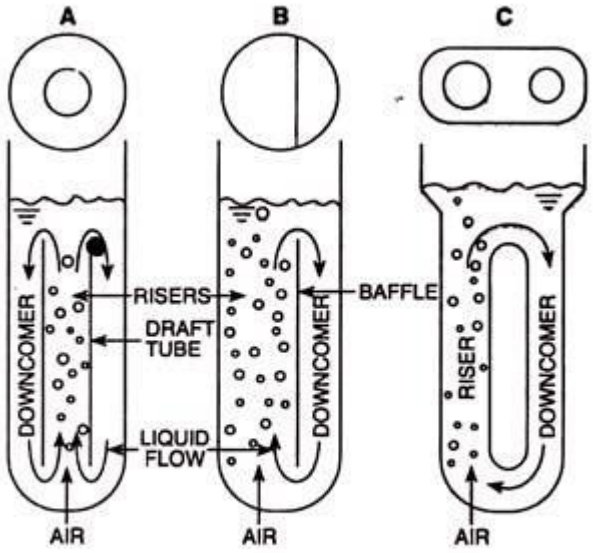


FIG. 39.2. Airlift fermentor. **(A)** Draft-tube internal loop configuration, **(B)** a split cylinder device, and **(C)** an external loop device.

- Airlift fermentors are highly energy-efficient and are often used in large-scale manufacture of biopharmaceutical proteins obtained from fragile animal cells.
- Heat and mass transfer capabilities of airlift reactors are at least as good as those of other systems, and airlift reactors are more effective in suspending solids than are bubble column fermentors.
- All performance characteristics of airlift -fermentor are related ultimately to the gas injection rate and the resulting rate of liquid circulation. Usually, the rate of liquid circulation increases with the square root of the height of the airlift device.
- Because the liquid circulation is driven by the gas hold-up difference between the riser and the down-comer, circulation is enhanced if there is little or no gas in the down-comer.
- All the gas in the down-comer comes from being entrained in with the liquid as it flows into the down-comer from the riser near the top of the reactor.

(iii) Bubble Column Fermentor:

- A bubble column fermentor (Fig. 39.3) is usually cylindrical with an aspect (height-to-diameter) ratio of 4-6. Gas is sparged at the base of the column through perforated pipes, perforated plates, or sintered glass or metal micro-porous spargers.
- O_2transfer, mixing and other performance factors are influenced mainly by the gas flow rate and the rheological properties of the fluid.
- Internal devices such as horizontal perforated plates, vertical baffles and corrugated sheet packing's may be placed in the vessel to improve mass transfer and modify the basic design.
- The column diameter does not affect its behaviour so long as the diameter exceeds 0.1 m. One exception is the axial mixing performance.
- For a given gas flow rate, the mixing improves with increasing vessel diameter. Mass and heat transfer and the prevailing shear rate increase as gas flow rate is increased.

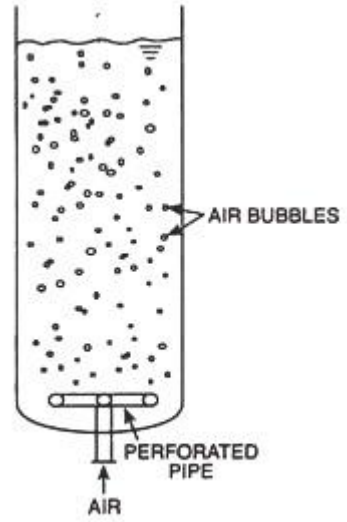


FIG. 39.3. A bubble column fermentor.

Aeration and Agitation:

Introduction:

The main function of aeration is to supply enough oxygen to the microbes in submerge culture technique for proper metabolism, while agitation provides proper mixing of the nutrient so that each and every organisms get proper nutrients.

Each fermentation process requires unique type of aeration and agitation system.

The parts of the fermenter involved in aeration and agitation are:

(a) The agitator (impeller).

(b) The aeration system (sparger).

The agitator (impeller):

The main aim of the agitator is to provide homogenous environment all over the fermenter. It is also used for mixing of different phases, oxygen and heat transport.

The aeration system (sparger):

A sparger is a tool used for introducing air into the fermentation medium.

Three basic types of sparger

1. The porous sparger,
2. The orifice sparger (a perforated pipe) and
3. The nozzle sparger (an open or partially closed pipe).

Porous Sparger :

The porous sparger is mainly used for laboratory scale non agitated fermenter. It is made up of sintered glass, ceramics or metal.

Orifice Sparger:

In small stirred fermenters the perforated pipes were arranged below the impeller in the form of crosses or rings (ring sparger), approximately three-quarters of the impeller diameter.

Nozzle Sparger:

Most modern mechanically stirred fermenter designs from laboratory to industrial scale have a single open or partially closed pipe as a sparger to provide the stream of air bubbles. Ideally the pipe should be positioned centrally below the impeller and as far away as possible from it to ensure that the impeller is not flooded by the air stream (Finn, 1954). The single-nozzle sparger causes a lower pressure loss than any other sparger and normally does not get blocked.

Need For Aeration and Agitation:

- ✓ The majorities of fermentation processes are aerobic and, therefore, require the provision of oxygen. If the stoichiometry of respiration is considered, then the oxidation of glucose may be represented as:

$$C_6H_{12}O_6 + 6O_2 = 6H_2O + 6CO_2$$

- ✓ Thus, 192 grams of oxygen are required for the complete oxidation of 180 grams of glucose. However, both components must be in solution before they are available to a microorganism and oxygen is approximately 6000 times less soluble in water than is glucose (a fermentation medium saturated with oxygen contains approximately 7.6 mg dm-3 of oxygen at 30°C).
- ✓ Thus, it is not possible to provide a microbial culture with all the oxygen it will need for the complete oxidation of the glucose (or any other carbon source) in one addition. Therefore, a microbial culture must be supplied with oxygen during growth at a rate sufficient to satisfy the organisms' demand.
- ✓ The aeration and agitation of the fermentation medium, provides necessary oxygen to the industrial fermentation process.
- ✓ However, the productivity of many fermentations is limited by oxygen availability and, therefore, it is important to consider the factors which affect a fermenter’s efficiency in supplying microbial cells with oxygen.
- ✓ The Oxygen Requirements of Industrial Fermentations Although a consideration of the stoichiometry of respiration gives an appreciation of the problem of oxygen supply, it gives no indication of an organism's true oxygen demand as it does not take into account the carbon that is converted into biomass and products. It has been studied that a culture's demand for oxygen is very much dependent on the source of carbon in the medium.
- ✓ Thus, the more reduced the carbon source the greater will be the oxygen demand. However, it is inadequate to base the provision of oxygen for fermentation simply on an estimation of overall demand, because the metabolism of the culture is affected by the concentration of dissolved oxygen in the broth.
- ✓ It may be seen that the specific oxygen uptake rate increases with increase in the dissolved oxygen concentration up to a certain point (referred to as Ccrit) above which no further increase in oxygen uptake rate occurs.

- ✓ Thus, maximum biomass production may be achieved by satisfying the organism's maximum specific oxygen demand by maintaining the dissolved oxygen concentration greater than the critical level.
- ✓ If the dissolved oxygen concentration were to fall below the critical level then the cells may be metabolically disturbed.
- ✓ However, it must be remembered that it is frequently the objective of the fermentation technologist to produce a product of the micro-organism rather than the organism itself and that metabolic disturbance of the cell by oxygen starvation may be advantageous to the formation of certain products.
- ✓ Equally, provision of a dissolved oxygen concentration greater than the critical level may have no influence on biomass production, but may stimulate product formation.
- ✓ Thus, the aeration conditions necessary for the optimum production of a product may be different from those favoring biomass productions. The oxygen demand of fermentation largely depends on the concentration of the biomass and its respiratory activity, which is related to the growth rate.
- ✓ By limiting the initial concentration of the medium, the biomass in the vessel may be kept at a reasonable level and by supplying some nutrient component as a feed, the rate of growth and hence the respiratory rate, may be controlled.

Oxygen Supply:

Oxygen is normally supplied to microbial culture in the form of air, this being the cheapest available source of the gas.

The method for provision of a culture with a supply of air varies with the scale of the process:

1. Laboratory scale:

Cultures may be aerated by means of the shake-flask method. Flask are shaken on a platform contained in a controlled environment chamber

2. Pilot and Industrial Scale :

Air is provided to the cultures by specific types of fermenter (Bubble fermenter)

Bartholomew et at. (1950) represented the transfer of oxygen from air to the cell, during fermentation, as occurring in a number of steps:

- The transfer of oxygen from an air bubble into solution.
- The transfer of the dissolved oxygen through the fermentation medium to the microbial cell.
- The uptake of the dissolved oxygen by the cell.

The rate of oxygen transfer from air bubble to the liquid phase may be given by the equation: dCL / dt = KLa (C*- Cl) Where CL is the concentration of dissolved oxygen in the fermentation broth (mmole dm-3),

t is time (hours),

dCL/dt is the change in oxygen concentration over a time period, i.e. the oxygen transfer rate (mmole O2 dm-3 h - 1),

KL is the mass transfer coefficient (cm h- 1),

a is the gas/liquid interface area per liquid volume (cm2 cm- 3),

C* is the saturated dissolved oxygen concentration (mmoles dm-3).

Unit – III

Fermentation process:

Growth of cultures in the fermenter. Importance of media in fermentation, media formulation and modification. Kinetics of growth in batch and continuous culture, specific growth rate, steady state in a chemostat, fed-batch fermentation, yield of biomass, product, calculation for productivity, substrate utilization kinetics. Fermentation process: Inoculum development. Storage of cultures for repeated fermentations, scaling up of process from shake flask to industrial fermentation.

Fed-batch culture:

- ✓ It is, in the broadest sense, defined as an operational technique in biotechnological processes where one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the product(s) remain in the bioreactor until the end of the run.
- ✓ An alternative description of the method is that of a culture in which "a base medium supports initial cell culture and a feed medium is added to prevent nutrient depletion".
- ✓ It is also a type of **semi-batch culture**. In some cases, all the nutrients are fed into the bioreactor.
- ✓ The advantage of the fed-batch culture is that one can control concentration of fed-substrate in the culture liquid at arbitrarily desired levels (in many cases, at low levels).
- ✓ Generally speaking, fed-batch culture is superior to conventional batch culture when controlling concentrations of a nutrient (or nutrients) affects the yield or productivity of the desired metabolite.

Types of bioprocesses:

The types of bioprocesses for which fed-batch culture is effective can be summarized as follows:

1. Substrate inhibition:

- ✓ Nutrients such as methanol, ethanol, acetic acid, and aromatic compounds inhibit the growth of microorganisms even at relatively low concentrations.
- ✓ By adding such substrates properly lag-time can be shortened and the inhibition of the cell growth markedly reduced.

2. High cell density (High cell concentration):

- ✓ In a batch culture, to achieve very high cell concentrations, *e.g.* 50-100 g of dry cells/L, high initial concentrations of the nutrients in the medium are needed.
- ✓ At such high concentrations, the nutrients become inhibitory, even though they have no such effect at the normal concentrations used in batch cultures.

3. Glucose effect (Crabtree effect):

- ✓ In the production of baker's yeast from malt wort or molasses it has been recognized since early 1900s that ethanol is produced even in the presence of sufficient dissolved oxygen (DO) if an excess of sugar is present in the culture liquid.
- ✓ Ethanol is a main cause of low cell yield. Aerobic ethanol formation in the presence of glucose concentration is known as glucose effect or Crabtree effect.
- ✓ To reduce this effect, a fed-batch process is generally employed for baker's yeast production.
- ✓ In aerobic cultures of *Escherichia coli* and *Bacillus subtilis*, organic acids such as acetic acid, (and in lesser amounts, lactic acid and formic acid), are produced as byproducts when sugar concentration is high, and these acids inhibit cell growth as well as show deteriorating effect on the metabolic activities.
- ✓ The formation of these acids are called bacterial Crabtree effects.

4. Catabolite repression:

- ✓ When a microorganism is provided with a rapidly metabolizable carbon-energy source such as glucose, the resulting increase in the intracellular concentration of ATP leads to the repression of enzyme(s) biosynthesis, thus causing a slower metabolization of the energy source.
- ✓ This phenomenon is known as catabolite repression. Many enzymes, especially those involved in catabolic pathways, are subject to this repressive regulation.
- ✓ A powerful method of overcoming the catabolite repression in the enzyme biosynthesis is a fed-batch culture in which glucose concentration in the culture liquid is kept low, where growth is restricted, and the enzyme biosynthesis is derepressed.
- ✓ Slow feeding of glucose in penicillin fermentation by *Penicillium chrysogenum* is a classical example in the category.

5. Auxotrophic mutants:

- ✓ In a microbial process employing an auxotrophic mutant (nutritionally requiring mutant), excess supply of the required nutrient results in abundant cell growth with little accumulation of the desired metabolite due to feedback inhibition and /or end-product repression.
- ✓ Starvation of the required nutrient, however, lowers cell growth as well as the overall production of the desired metabolite, as the production rate is usually proportional to the cell concentration.
- ✓ In such a bioprocess, the accumulation of the desired metabolite can be maximized by growing the mutant on a limited amount of the required nutrient.

- ✓ To cultivate the mutant on a low concentration of the required nutrient, it is fed to the batch culture at a controlled rate.
- ✓ This technique is often used in industrial amino acid productions with the auxotrophic mutants.
- ✓ An example is lysine production with homoserine- or threonine/methionine-requiring mutant of *Corynebacterium glutamicum* being lacking for homoserine dehydrogenase gene.

6. Expression control of a gene with a repressible promoter:

- ✓ Transcription of a gene having a repressible promoter upstream of the open reading frame is repressed by combination of the so-called holo-repressor with the operator region on the DNA.
- ✓ When a specified chemical compound exists in the culture liquid, the compound (or its metabolite) in the cells combines as co-repressor with an apo-repressor (a kind of transcription factor) to form the holo-repressor.
- ✓ Keeping the concentration of this compound as low as possible (while still allowing for sufficient cell growth) permits continued expression of the regulated gene. Fed-batch culture is a powerful technique to do so. Examples of the repressible promoter are *trp* promoter and *phoA* promoter.

Types of culturing strategies:

High cell-density culture:

- The fed-batch strategy is typically used in bio-industrial processes to reach a high cell density in the bioreactor.
- Mostly the feed solution is highly concentrated to avoid dilution of the bioreactor. Production of heterologous proteins by fed-batch cultures of recombinant microorganisms have been extensively studied.
- The controlled addition of the nutrient directly affects the growth rate of the culture and helps to avoid overflow metabolism (formation of side metabolites, such as acetate for *Escherichia coli*, lactic acid in mammalian cell cultures, ethanol in *Saccharomyces cerevisiae*), oxygen limitation (anaerobiosis).

Constantly-fed-batch culture:

- The simplest fed-batch culture is the one in which the feed rate of a growth-limiting substrate is constant, *i.e.* the feed rate is invariant during the culture.
- This case is shown in the graph (here the culture volume is variable).
- This type of the fed-batch culture is named constantly-fed-batch culture (CFBC), and is well established mathematically and experimentally.
- In the CFBC, both cases of fixed-volume CFBC and variable-volume CFBC were studied.

Exponential-fed-batch culture:

- Under ideal condition, cells grow exponentially. If the feed rate of the growth-limiting substrate is increased in proportion to the exponential growth rate of the cells, it is possible to maintain the cells' specific growth rate for a long time while keeping the substrate concentration in the culture liquid at a constant level.
- The required feed rate (volumetric or mass) must be increased exponentially with time so that this mode of fed-batch culture is called exponentially-fed-batch culture (EFBC).
- Substrate limitation offers the possibility to control the reaction rates to avoid technological limitations connected to the cooling of the reactor and oxygen transfer. Substrate limitation also allows the metabolic control, to avoid osmotic effects, catabolite repression and overflow metabolism of side products.

Control strategy:

Different strategies can be used to control the growth in a fed-batch process:

Control Parameter	Control Principle
DOT (pO_2)	DOstat (DOT= constant), F~DOT
Oxygen uptake rate (OUR)	OUR=constant, F~OUR
Glucose	on-line measurement of glucose (FIA), glucose=constant
Acetate	on-line measurement of acetate (FIA), acetate=constant
pH (pHstat)	F~pH (acidification is connected to high glucose)
Ammonia	on-line measurement of ammonia (FIA), ammonia=constant
Temperature	*T* adapted according to OUR or pO_2

Importance of media in fermentation:

- ✓ **Industrial fermentation** is the intentional use of fermentation by microorganisms such as bacteria and fungi as well as eukaryotic cells like CHO cells and insect cells, to make products useful to humans.
- ✓ Fermented products have applications as food as well as in general industry. Some commodity chemicals, such as acetic acid, citric acid, and ethanol are made by fermentation.
- ✓ The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature, pH and for aerobic fermentation oxygen. Product recovery frequently involves the concentration of the dilute solution.
- ✓ Nearly all commercially produced enzymes, such as lipase, invertase and rennet, are made by fermentation with genetically modified microbes.

- ✓ In some cases, production of biomass itself is the objective, as in the case of baker's yeast and lactic acid bacteria starter cultures for cheesemaking. In general, fermentations can be divided into four types:

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

These types are not necessarily disjoint from each other, but provide a framework for understanding the differences in approach. The organisms used may be bacteria, yeasts, molds, algae, animal cells, or plant cells. Special considerations are required for the specific organisms used in the fermentation, such as the dissolved oxygen level, nutrient levels, and temperature.

Phases of growth:

- Fermentation begins once the growth medium is inoculated with the organism of interest. Growth of the inoculum does not occur immediately.
- This is the period of adaptation, called the lag phase. Following the lag phase, the rate of growth of the organism steadily increases, for a certain period—this period is the log or exponential phase.
- After a phase of exponential growth, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or a continuously increasing (accumulating) concentrations of toxic substances.
- This phase, where the increase of the rate of growth is checked, is the deceleration phase. After the deceleration phase, growth ceases and the culture enters a stationary phase or a steady state.
- The biomass remains constant, except when certain accumulated chemicals in the culture lyse the cells (chemolysis).
- Unless other micro-organisms contaminate the culture, the chemical constitution remains unchanged. If all of the nutrients in the medium are consumed, or if the concentration of toxins is too great, the cells may become scenescent and begin to die off.
- The total amount of biomass may not decrease, but the number of viable organisms will decrease.

Fermentation medium:

- The microbes or eukaryotic cells used for fermentation grow in (or on) specially designed growth medium which supplies the nutrients required by the organisms or cells.
- A variety of media exist, but invariably contain a carbon source, a nitrogen source, water, salts, and micronutrients.
- In the production of wine, the medium is grape must. In the production of bio-ethanol, the medium may consist mostly of whatever inexpensive carbon source is available.

- Carbon sources are typically sugars or other carbohydrates, although in the case of substrate transformations (such as the production of vinegar) the carbon source may be an alcohol or something else altogether.
- For large scale fermentations, such as those used for the production of ethanol, inexpensive sources of carbohydrates, such as molasses, corn steep liquor, sugar cane juice, or sugar beet juice are used to minimize costs.
- More sensitive fermentations may instead use purified glucose, sucrose, glycerol or other sugars, which reduces variation and helps ensure the purity of the final product.
- Organisms meant to produce enzymes such as beta galactosidase, invertase or other amylases may be fed starch to select for organisms that express the enzymes in large quantity.
- Fixed nitrogen sources are required for most organisms to synthesize proteins, nucleic acids and other cellular components.
- Depending on the enzyme capabilities of the organism, nitrogen may be provided as bulk protein, such as soy meal; as pre-digested polypeptides, such as peptone or tryptone; or as ammonia or nitrate salts.
- Cost is also an important factor in the choice of a nitrogen source. Phosphorus is needed for production of phospholipids in cellular membranes and for the production of nucleic acids.
- The amount of phosphate which must be added depends upon the composition of the broth and the needs of the organism, as well as the objective of the fermentation. For instance, some cultures will not produce secondary metabolites in the presence of phosphate.
- Growth factors and trace nutrients are included in the fermentation broth for organisms incapable of producing all of the vitamins they require.
- Yeast extract is a common source of micronutrients and vitamins for fermentation media.
- Inorganic nutrients, including trace elements such as iron, zinc, copper, manganese, molybdenum and cobalt are typically present in unrefined carbon and nitrogen sources, but may have to be added when purified carbon and nitrogen sources are used.
- Fermentations which produce large amounts of gas (or which require the addition of gas) will tend to form a layer of foam, since fermentation broth typically contains a variety of foam-reinforcing proteins, peptides or starches.
- To prevent this foam from occurring or accumulating, antifoaming agents may be added. Mineral buffering salts, such as carbonates and phosphates, may be used to stabilize pH near optimum.
- When metal ions are present in high concentrations, use of a chelating agent may be necessary.
- Developing an optimal medium for fermentation is a key concept to efficient optimization. One-factor-at-a-time (OFAT) is the preferential choice that researchers use for designing a medium composition.

- This method involves changing only one factor at a time while keeping the other concentrations constant.
- This method can be separated into some sub groups. One is Removal Experiments. In this experiment all the components of the medium are removed one at a time and their effects on the medium are observed.
- Supplementation experiments involve evaluating the effects of nitrogen and carbon supplements on production.
- The final experiment is a replacement experiment. This involves replacing the nitrogen and carbon sources that show an enhancement effect on the intended production.
- Overall OFAT is a major advantage over other optimization methods because of its simplicity.

Production of biomass:

- Microbial cells or biomass is sometimes the intended product of fermentation. Examples include single cell protein, bakers yeast, lactobacillus, E. coli, and others.
- In the case of single-cell protein, algae is grown in large open ponds which allow photosynthesis to occur.
- If the biomass is to be used for inoculation of other fermentations, care must be taken to prevent mutations from occurring.

Production of extracellular metabolites:

- Metabolites can be divided into two groups: those produced during the growth phase of the organism, called primary metabolites and those produced during the stationary phase, called secondary metabolites.
- Some examples of primary metabolites are ethanol, citric acid, glutamic acid, lysine, vitamins and polysaccharides. Some examples of secondary metabolites are penicillin, cyclosporin A, gibberellin, and lovastatin.

Primary metabolites:

- Primary metabolites are compounds made during the ordinary metabolism of the organism during the growth phase.
- A common example is ethanol or lactic acid, produced during glycolysis. Citric acid is produced by some strains of *Aspergillus niger* as part of the citric acid cycle to acidify their environment and prevent competitors from taking over.
- Glutamate is produced by some *Micrococcus* species, and some *Corynebacterium* species produce lysine, threonine, tryptophan and other amino acids.
- All of these compounds are produced during the normal "business" of the cell and released into the environment. There is therefore no need to rupture the cells for product recovery.

Secondary metabolites:

- Secondary metabolites are compounds made in the stationary phase; penicillin, for instance, prevents the growth of bacteria which could compete with *Penicillium* molds for resources.
- Some bacteria, such as *Lactobacillus* species, are able to produce bacteriocins which prevent the growth of bacterial competitors as well.
- These compounds are of obvious value to humans wishing to prevent the growth of bacteria, either as antibiotics or as antiseptics (such as gramicidin S).
- Fungicides, such as griseofulvin are also produced as secondary metabolites.
- Typically secondary metabolites are not produced in the presence of glucose or other carbon sources which would encourage growth, and like primary metabolites are released into the surrounding medium without rupture of the cell membrane.
- In the early days of the biotechnology industry, most biopharmaceutical products were made in *E. coli*; by 2004 more biopharmaceuticals were manufactured in eukaryotic cells, like CHO cells, than in microbes, but used similar bioreactor systems. Insect cell culture systems came into use in the 2000s as well.

Production of intracellular components:

- ✓ Of primary interest among the intracellular components are microbial enzymes: catalase, amylase, protease, pectinase, cellulase, hemicellulase, lipase, lactase, streptokinase and many others.
- ✓ Recombinant proteins, such as insulin, hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase and others are also made this way.
- ✓ The largest difference between this process and the others is that the cells must be ruptured (lysed) at the end of fermentation, and the environment must be manipulated to maximize the amount of the product.
- ✓ Furthermore, the product (typically a protein) must be separated from all of the other cellular proteins in the lysate to be purified.

Transformation of substrate:

Substrate transformation involves the transformation of a specific compound into another, such as in the case of phenylacetylcarbinol, and steroid biotransformation, or the transformation of a raw material into a finished product, in the case of food fermentations and sewage treatment.

Food fermentation:

Ancient fermented food processes, such as making bread, wine, cheese, curds, idli, dosa, etc., can be dated to more than seven thousand years ago.[15] They were developed long before man had any

knowledge of the existence of the microorganisms involved. Some foods such as Marmite are the byproduct of the fermentation process, in this case in the production of beer.

Ethanol fuel:

Fermentation is the main source of ethanol in the production of ethanol fuel. Common crops such as sugar cane, potato, cassava and corn are fermented by yeast to produce ethanol which is further processed to become fuel.

Sewage treatment:

In the process of sewage treatment, sewage is digested by enzymes secreted by bacteria. Solid organic matters are broken down into harmless, soluble substances and carbon dioxide. Liquids that result are disinfected to remove pathogens before being discharged into rivers or the sea or can be used as liquid fertilizers. Digested solids, known also as sludge, is dried and used as fertilizer. Gaseous byproducts such as methane can be utilized as biogas to fuel electrical generators. One advantage of bacterial digestion is that it reduces the bulk and odor of sewage, thus reducing space needed for dumping. The main disadvantage of bacterial digestion in sewage disposal is that it is a very slow process.

Agricultural feed:

A wide variety of agroindustrial waste products can be fermented to use as food for animals, especially ruminants. Fungi have been employed to break down cellulosic wastes to increase protein content and improve *in vitro* digestibility.

Unit – IV

Food microbiology:

Microbiology of fermented milk – starter cultures, butter milk, cream, yoghurt, kafir, kumiss, acidophilus milk and cheese. Microbes as sources of food (*Spirulina*, *Saccharomyces cerviceae*, *Rhizopus* sp.). Food intoxications: *Staphylococcus aureus*, *Clostridium botulinum* and mycotoxins; Food infections: *Bacillus cereus*, *Vibrio parahaemolyticus*, *Escherichia coli*, Salmonellosis, Shigellosis and *Campylobacter jejuni* – spoilage of canned foods – Detection of spoilage and characterization. Food sanitation in food manufacture and in the retail trade; Food control agencies and their regulations.

Introduction:

Food microbiology is the study of the microorganisms that inhibit, create, or contaminate food. This includes the study of microorganisms causing food spoilage; as well as, pathogens that may cause disease especially if food is improperly cooked or stored. Those used to produce fermented foods such as cheese, yogurt, bread, beer, and wine. Then those researchers with other useful roles such as producing probiotics.

Microbiology of fermented milk:

- Fermented milk products or fermented dairy products, also known as cultured dairy foods, cultured dairy products, or cultured milk products, are dairy foods that have been fermented with lactic acid bacteria such as Lactobacillus, Lactococcus, and Leuconostoc.
- The fermentation process increases the shelf life of the product while enhancing its taste and improving the digestibility of its milk.
- There is evidence that fermented milk products have been produced since around 10,000 BC.
- A range of different Lactobacilli strains has been grown in laboratories allowing for many cultured milk products with different flavors and characteristics.

Product:

Many different types of cultured milk products can be found around the world including milk, cheese, yogurt, other cultured dairy foods, ice cream and more.

Starter cultures:

- A fermentation starter (called simply starter within the corresponding context, sometimes called a mother) is a preparation to assist the beginning of the fermentation process in preparation of various foods and alcoholic drinks.
- A starter culture is a microbiological culture which actually performs fermentation. These starters usually consist of a cultivation medium, such as grains, seeds, or nutrient liquids that have been well colonized by the microorganisms used for the fermentation.
- These starters are formed using a specific cultivation medium and a specific mix of fungal and bacterial strains.
- Typical microorganisms used in starters include
  various bacteria and fungi (yeasts and molds): *Rhizopus*, *Aspergillus*, *Mucor*, *Amylomyces*, *Endomy copsis*, *Saccharomyces*, *Hansenula anomala*, *Lactobacillus*, *Acetobacter*, etc. Various national cultures have various active ingredients in starters, and often involve mixed microflora.

Industrial starters include various enzymes, in addition to microflora.

Starter Cultures

Starter cultures are those microorganisms that are used in the production of cultured dairy products such as yogurt and cheese. The natural microflora of the milk is either inefficient, uncontrollable, and unpredictable, or is destroyed altogether by the heat treatments given to the milk. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The primary function of lactic starters is the production of lactic acid from lactose. Other functions of starter cultures may include the following:

- flavour, aroma, and alcohol production
- proteolytic and lipolytic activities
- inhibition of undesirable organisms

There are two groups of lactic starter cultures:

1. simple or defined: single strain, or more than one in which the number is known
2. mixed or compound: more than one strain each providing its own specific characteristics

Starter cultures may be categorized as mesophilic, for example:

- *Lactococcus lactis* subsp. *cremoris*
- *L. delbrueckii* subsp. *lactis*
- *L. lactis* subsp. *lactis* biovar *diacetylactis*
- *Leuconostoc mesenteroides* subsp. *cremoris*

or thermophilic:

- *Streptococcus salivarius* subsp. *thermophilus (S.thermophilus)*
- *Lactobacillus delbrueckii* subsp. *bulgaricus*
- *L. delbrueckii* subsp. *lactis*
- *L. casei*
- *L. helveticus*
- *L. plantarum*

Mixtures of mesophilic and thermophilic microorganisms can also be used as in the production of some cheeses.

Microbes as sources of food:

Spirulina

- ✓ **Spirulina** is a biomass of cyanobacteria (blue-green algae) that can be consumed by humans and animals. The two species are *Arthrospira platensis* and *A. maxima*.
- ✓ Cultivated worldwide, *Arthrospira* is used as a dietary supplement or whole food.[1] It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries.
- ✓ The species *A. maxima* and *A. platensis* were once classified in the genus *Spirulina*. The common name, spirulina, refers to the dried biomass of *A. platensis*, which belongs to photosynthetic bacteria that cover the groups Cyanobacteria and Prochlorophyta.
- ✓ Scientifically, a distinction exists between spirulina and the genus *Arthrospira*. Species of *Arthrospira* have been isolated from alkaline brackish and saline waters in tropical and subtropical regions.
- ✓ Among the various species included in the genus *Arthrospira*, *A. platensis* is the most widely distributed and is mainly found in Africa, but also in Asia. *A. maxima* is believed to be found in California and Mexico.[4] The term *spirulina* remains in use for historical reasons.

- ✓ *Arthrospira* species are free-floating, filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-handed helix.
- ✓ They occur naturally in tropical and subtropical lakes with high pH and high concentrations of carbonate and bicarbonate. *A. platensis* occurs in Africa, Asia, and South America, whereas *A. maxima* is confined to Central America.[2] Most cultivated spirulina is produced in open-channel raceway ponds, with paddle wheels used to agitate the water.
- ✓ Spirulina thrives at a pH around 8.5 and above, which will get more alkaline, and a temperature around 30 °C (86 °F). They are autotrophic, meaning that they are able to make their own food, and do not need a living energy or organic carbon source.

Historical uses:

- ✓ Spirulina was a food source for the Aztecs and other Mesoamericans until the 16th century; the harvest from Lake Texcoco in Mexico and subsequent sale as cakes were described by one of Cortés' soldiers. The Aztecs called it "tecuitlatl".
- ✓ Spirulina was found in abundance at Lake Texcoco by French researchers in the 1960s, but no reference to its use by the Aztecs as a daily food source was made after the 16th century, probably due to the draining of the surrounding lakes for agriculture and urban development.
- ✓ The topic of the Tecuitlatl, which was earlier discovered in 1520, was not mentioned again until 1940, the French phycologist Pierre Dangeard mentioned about a cake called "*dihe*", consumed by Kanembu tribe, who harvest it from Lake Chad in the African nation of Chad. Dangeard studied the *dihe* samples and found it to be a dried puree of the spring form of the blue-green algae from the lake.
- ✓ The *dihe* is used to make broths for meals, and also sold in markets. The spirulina is harvested from small lakes and ponds around Lake Chad.
- ✓ During 1964 and 1965, the botanist Jean Leonard confirmed that *dihe* is made up of spirulina, and later studied a bloom of algae in a sodium hydroxide production facility.
- ✓ As a result, the first systematic and detailed study of the growth requirements and physiology of spirulina was performed as a basis for establishing large-scale production in the 1970s.

Food and Nutrition:

- ✓ As an ecologically sound, nutrient-rich dietary supplement, spirulina is being investigated to address food security and malnutrition, and as dietary support in long-term space flight or Mars missions.
- ✓ Its advantage for food security is that it needs less land and water than livestock to produce protein and energy.
- ✓ Dried spirulina contains 5% water, 24% carbohydrates, 8% fat, and about 60% (51–71%) protein (table).

- ✓ Provided in its typical supplement form as a dried powder, a 100-g amount of spirulina supplies 290 kilocalories (1,200 kJ) and is a rich source (20% or more of the Daily Value, DV) of numerous essential nutrients, particularly protein, B vitamins (thiamin, riboflavin, and niacin, providing 207%, 306%, and 85% DV, respectively), and dietary minerals, such as iron (219% DV) and manganese (90% DV) (table).
- ✓ The lipid content of spirulina is 8% by weight (table) providing the fatty acids, gamma-linolenic acid, alpha-linolenic acid, linoleic acid, stearidonic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid.
- ✓ In contrast to those 2003 estimates (of DHA and EPA each at 2 to 3% of total fatty acids), 2015 research indicated that spirulina products "contained no detectable omega-3 fatty acids" (less than 0.1%, including DHA and EPA).
- ✓ An in vitro study reported that different strains of microalgae produced DHA and EPA in substantial amounts.

Vitamin B_{12}:

- ✓ Spirulina contains no vitamin B_{12} naturally (see table), and spirulina supplements are not considered to be a reliable source of vitamin B_{12}, as they contain predominantly pseudovitamin B_{12} (Coα-[α-(7-adenyl)]-Coβ-cyanocobamide), which is biologically inactive in humans.
- ✓ In a 2009 position paper on vegetarian diets, the American Dietetic Association stated that spirulina is not a reliable source of active vitamin B_{12}. The medical literature similarly advises that spirulina is unsuitable as a source of B_{12}.

Animals and aquaculture:

- ✓ Various studies on spirulina as an alternative feed for animal and aquaculture have been done. Spirulina can be fed up to 10% for poultry and less than 4% for quail.
- ✓ Increase in the spirulina content up to 40 g/kg (0.64 oz/lb) for 16 days in 21-day-old broiler male chicks, resulted in yellow and red coloration of flesh and this may be due to the accumulation of the yellow pigment, zeaxanthin.
- ✓ Pigs and rabbits can receive up to 10% of the feed and increase in the spirulina content in cattle resulted in increase in milk yield and weight.
- ✓ Spirulina as an alternative feedstock and immune booster for big-mouth buffalo, milk fish, cultured striped jack, carp, red sea bream, tilapia, catfish, yellow tail, zebrafish, shrimp, and abalone was established and up to 2% spirulina per day in aquaculture feed can be safely recommended.

Saccharomyces cerevisiae:

- ✓ ***Saccharomyces cerevisiae*** is a species of yeast. It has been instrumental in winemaking, baking, and brewing since ancient times.

- ✓ It is believed to have been originally isolated from the skin of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums; it exists among the waxes of the cuticle).
- ✓ It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model bacterium. It is the microorganism behind the most common type of fermentation. *S. cerevisiae* cells are round to ovoid, 5–10 μm in diameter. It reproduces by budding.
- ✓ Many proteins important in human biology were first discovered by studying their homologs in yeast; these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes.
- ✓ *S. cerevisiae* is currently the only yeast cell known to have Berkeley bodies present, which are involved in particular secretory pathways.
- ✓ Antibodies against *S. cerevisiae* are found in 60–70% of patients with Crohn's disease and 10–15% of patients with ulcerative colitis (and 8% of healthy controls).
- ✓ *S. cerevisiae* has been found to contribute to the smell of bread; the proline and ornithine present in yeast are precursors of the 2-acetyl-l-pyrroline, a roast-smelling odorant, in the bread crust.

Ecology:

- In nature, yeast cells are found primarily on ripe fruits such as grapes (before maturation, grapes are almost free of yeasts). Since *S. cerevisiae* is not airborne, it requires a vector to move.
- Queens of social wasps overwintering as adults (*Vespa crabro* and *Polistes* spp.) can harbor yeast cells from autumn to spring and transmit them to their progeny.
- The intestine of *Polistes dominula*, a social wasp, hosts *S. cerevisiae* strains as well as *S. cerevisiae S. paradoxus* hybrids. Stefanini et al. (2016) showed that the intestine of *Polistes dominula* favors the mating of *S. cerevisiae* strains, both among themselves and with *S. paradoxus* cells by providing environmental conditions prompting cell sporulation and spores germination.
- The optimum temperature for growth of *S. cerevisiae* is 30–35 °C (86–95 °F).

Life cycle:

- Two forms of yeast cells can survive and grow: haploid and diploid. The haploid cells undergo a simple lifecycle of mitosis and growth, and under conditions of high stress will, in general, die. This is the asexual form of the fungus.
- The diploid cells (the preferential 'form' of yeast) similarly undergo a simple lifecycle of mitosis and growth.

- The rate at which the mitotic cell cycle progresses often differs substantially between haploid and diploid cells.
- Under conditions of stress, diploid cells can undergo sporulation, entering meiosis and producing four haploid spores, which can subsequently mate.
- This is the sexual form of the fungus. Under optimal conditions, yeast cells can double their population every 100 minutes.
- However, growth rates vary enormously both between strains and between environments.
- Mean replicative lifespan is about 26 cell divisions.
- In the wild, recessive deleterious mutations accumulate during long periods of asexual reproduction of diploids, and are purged during selfing: this purging has been termed "genome renewal".

Nutritional requirements:

- All strains of *S. cerevisiae* can grow aerobically on glucose, maltose, and trehalose and fail to grow on lactose and cellobiose.
- However, growth on other sugars is variable. Galactose and fructose are shown to be two of the best fermenting sugars.
- The ability of yeasts to use different sugars can differ depending on whether they are grown aerobically or anaerobically. Some strains cannot grow anaerobically on sucrose and trehalose.
- All strains can use ammonia and urea as the sole nitrogen source, but cannot use nitrate, since they lack the ability to reduce them to ammonium ions.
- They can also use most amino acids, small peptides, and nitrogen bases as nitrogen sources. Histidine, glycine, cystine, and lysine are, however, not readily used. *S. cerevisiae* does not excrete proteases, so extracellular protein cannot be metabolized.
- Yeasts also have a requirement for phosphorus, which is assimilated as a dihydrogen phosphate ion, and sulfur, which can be assimilated as a sulfate ion or as organic sulfur compounds such as the amino acids methionine and cysteine. Some metals, like magnesium, iron, calcium, and zinc, are also required for good growth of the yeast.
- Concerning organic requirements, most strains of *S. cerevisiae* require biotin. Indeed, a *S. cerevisiae*-based growth assay laid the foundation for the isolation, crystallisation, and later structural determination of biotin.
- Most strains also require pantothenate for full growth. In general, *S. cerevisiae* is prototrophic for vitamins.

Mating:

- *Saccharomyces cerevisiae* mating type **a** with a cellular bulging called a shmoo in response to **α**-factor

- Yeast has two mating types, **a** and α (*alpha*), which show primitive aspects of sex differentiation. As in many other eukaryotes, mating leads to genetic recombination, i.e. production of novel combinations of chromosomes.
- Two haploid yeast cells of opposite mating type can mate to form diploid cells that can either sporulate to form another generation of haploid cells or continue to exist as diploid cells.
- Mating has been exploited by biologists as a tool to combine genes, plasmids, or proteins at will.
- The mating pathway employs a G protein-coupled receptor, G protein, RGS protein, and three-tiered MAPK signaling cascade that is homologous to those found in humans.
- This feature has been exploited by biologists to investigate basic mechanisms of signal transduction and desensitization.

Cell cycle:

- Growth in yeast is synchronised with the growth of the bud, which reaches the size of the mature cell by the time it separates from the parent cell.
- In well nourished, rapidly growing yeast cultures, all the cells can be seen to have buds, since bud formation occupies the whole cell cycle.
- Both mother and daughter cells can initiate bud formation before cell separation has occurred.
- In yeast cultures growing more slowly, cells lacking buds can be seen, and bud formation only occupies a part of the cell cycle.

Cytokinesis:

- Cytokinesis enables budding yeast *Saccharomyces cerevisiae* to divide into two daughter cells. *S. cerevisiae* forms a bud which can grow throughout its cell cycle and later leaves its mother cell when mitosis has completed.
- *S. cerevisiae* is relevant to cell cycle studies because it divides asymmetrically by using a polarized cell to make two daughters with different fates and sizes. Similarly, stem cells use asymmetric division for self-renewal and differentiation.

Timing:

- For many cells, M phase does not happen until S phase is complete. However, for entry into mitosis in *S. cerevisiae* this is not true.
- Cytokinesis begins with the budding process in late G1 and is not completed until about halfway through the next cycle.

- The assembly of the spindle can happen before S phase has finished duplicating the chromosomes. Additionally, there is a lack of clearly defined G2 in between M and S. Thus, there is a lack of extensive regulation present in higher eukaryotes.
- When the daughter emerges, the daughter is two-thirds the size of the mother.
- Throughout the process, the mother displays little to no change in size.
- The RAM pathway is activated in the daughter cell immediately after cytokinesis is complete. This pathway makes sure that the daughter has separated properly.

Actomyosin ring and primary septum formation:

- Two interdependent events drive cytokinesis in *S. cerevisiae*. The first event is contractile actomyosin ring (AMR) constriction and the second event is formation of the primary septum (PS), a chitinous cell wall structure that can only be formed during cytokinesis.
- The PS resembles in animals the process of extracellular matrix remodeling. When the AMR constricts, the PS begins to grow.
- Disrupting AMR misorients the PS, suggesting that both have a dependent role. Additionally, disrupting the PS also leads to disruptions in the AMR, suggesting both the actomyosin ring and primary septum have an interdependent relationship.
- The AMR, which is attached to the cell membrane facing the cytosol, consists of actin and myosin II molecules that coordinate the cells to split.
- The ring is thought to play an important role in ingression of the plasma membrane as a contractile force.
- Proper coordination and correct positional assembly of the contractile ring depends on septins, which is the precursor to the septum ring.
- These GTPases assemble complexes with other proteins. The septins form a ring at the site where the bud will be created during late G1. They help promote the formation of the actin-myosin ring, although this mechanism is unknown.
- It is suggested they help provide structural support for other necessary cytokinesis processes. After a bud emerges, the septin ring forms an hourglass. The septin hourglass and the myosin ring together are the beginning of the future division site.
- The septin and AMR complex progress to form the primary septum consisting of glucans and other chitinous molecules sent by vesicles from the Golgi body.
- After AMR constriction is complete, two secondary septums are formed by glucans. How the AMR ring dissembles remains poorly unknown.
- Microtubules do not play as significant a role in cytokinesis compared to the AMR and septum. Disruption of microtubules did not significantly impair polarized growth.
- Thus, the AMR and septum formation are the major drivers of cytokinesis.

Differences from fission yeast:

- Budding yeast form a bud from the mother cell. This bud grows during the cell cycle and detaches; fission yeast divide by forming a cell wall.
- Cytokinesis begins at G1 for budding yeast, while cytokinesis begins at G2 for fission yeast. Fission yeast "select" the midpoint, whereas budding yeast "select" a bud site.
- During early anaphase the actomyosin ring and septum continues to develop in budding yeast, in fission yeast during metaphase-anaphase the actomyosin ring begins to develop.

Food intoxications:

- ✓ Food poisoning: The illness resulting from eating food or drinking water containing poisonous substances including bacteria, viruses, pesticides, or toxins. Symptoms generally begin within 2 to 6 hours and include abdominal cramping, diarrhea, fever, headache, nausea, vomiting, and weakness.
- ✓ **Foodborne illness** (also **foodborne disease** and colloquially referred to as **food poisoning**) is any illness resulting from the spoilage of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, as well as toxins such as poisonous mushrooms and various species of beans that have not been boiled for at least 10 minutes.
- ✓ Symptoms vary depending on the cause, and are described below in this article. A few broad generalizations can be made.
- ✓ For contaminants requiring an incubation period, symptoms may not manifest for hours to days, depending on the cause and on quantity of consumption.
- ✓ Longer incubation periods tend to cause sufferers to not associate the symptoms with the item consumed, so they may misattribute the symptoms to gastroenteritis, for example.
- ✓ Symptoms often include vomiting, fever, and aches, and may include diarrhea. Bouts of vomiting can be repeated with an extended delay in between, because even if infected food was eliminated from the stomach in the first bout, microbes, like bacteria, (if applicable) can pass through the stomach into the intestine and begin to multiply.
- ✓ Some types of microbes stay in the intestine, some produce a toxin that is absorbed into the bloodstream, and some can directly invade deeper body tissues.

Staphylococcal (Staph) Food Poisoning:

- ✓ Staph food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus* (Staph) bacteria.
- ✓ About 25% of people and animals have Staph on their skin and in their nose. It usually does not cause illness in healthy people, but Staph has the ability to make toxins that can cause food poisoning.

How do people get Staph food poisoning?

- ✓ People who carry Staph can contaminate food if they don't wash their hands before touching it. If food is contaminated with Staph, the bacteria can multiply in the food and produce toxins that can make people ill.
- ✓ Staph bacteria are killed by cooking, but the toxins are not destroyed and will still be able to cause illness.
- ✓ Foods that are not cooked after handling, such as sliced meats, puddings, pastries, and sandwiches, are especially risky if contaminated with Staph.
- ✓ Food contaminated with Staph toxin may not smell bad or look spoiled.

Other Food Safety Links

- Wash Your Hands
- Four Steps to Food Safety
- Food Poisoning Symptoms
- Preventing Food Poisoning
- People at Risk
- Food Safety at a GlanceExternal

What are the symptoms of Staph food poisoning?

- Staph food poisoning is characterized by a sudden start of nausea, vomiting, and stomach cramps. Most people also have diarrhea.
- Symptoms usually develop within 30 minutes to 8 hours after eating or drinking an item containing Staph toxin, and last no longer than 1 day. Severe illness is rare.
- The illness cannot be passed from one person to another.

How do I know if I have Staph food poisoning?

- ✓ You can suspect Staph food poisoning based on the type of symptoms and their fast resolution.
- ✓ Although laboratory tests can detect toxin-producing Staph in stool, vomit, and foods, these tests are usually not ordered except during an outbreak.
- ✓ If you think you might have Staph food poisoning and are experiencing severe symptoms, contact your health care provider.

How is Staph food poisoning treated?

The most important treatment is drinking plenty of fluids. Your healthcare provider may give you medicine to decrease vomiting and nausea. People with severe illness may require intravenous fluids. Antibiotics are not useful in treating this illness because the toxin is not affected by antibiotics.

How can I prevent Staph food poisoning?

The best way to avoid food poisoning by Staph is to prevent food from being held at an unsafe temperature (between 40°F and 140°F) for more than 2 hours.
Bacteria can multiply rapidly if left at room temperature or in the "Danger Zone" between 40°F and 140°F. Never leave perishable food out for more than 2 hours (or 1 hour if it's hotter than 90° F outside).

Remember to always follow these food safety tips:

- Use a food thermometer and cook foods to their safe minimum internal temperatureExternal.
- Keep hot foods hot (140°F or hotter) and cold foods cold (40°F or colder).
- Store cooked food in wide, shallow containers and refrigerate within 2 hours (or 1 hour if it's hotter than 90° F outside).

The following tips that are part of the four steps to food safety – clean, separate, cook, and chill – also can help protect you and your loved ones from food poisoning:

- Wash your hands for 20 seconds with soap and water before, during, and after preparing food, and before eating.
- Do not preparing food if you are ill with diarrhea or vomiting.
- Wear gloves while preparing food if you have wounds or infections on your hands or wrists.

MICROBIAL SPOILAGE OF CANNED FOODS:

- ✓ Canning is one of important method of packaging food for long term storage. Normally food is stored in metallic containers along with heat treatment.
- ✓ The heat treatment varies depending upon type of food. There is always a chance that microorganisms may survive if the heat treatment is not proper thereby leading to spoilage of food. Usually the incidences of food spoilage in cans are low.
- ✓ The spoilage of can could be due to biological or chemical reasons or combination of both.
- ✓ The biological spoilage is primarily due to microbial growth while chemical spoilage is due to hydrogen produced due to reaction of acid in food and iron on can.
- ✓ The degree of swelling can also be increased by high summer temperature and high altitudes.
- ✓ Certain other factors such as overfilling, buckling, denting or closing the can while cool may also be responsible for spoilage of foods in cans.

Causes of Spoilage in Cans

Chemical spoilage

The chemical spoilage in most cases is due to production of hydrogen gas produced in can because of action of acid of food on iron of can. This spoilage is termed as Hydrogen swell. It occurs due to following factors:

a) Increased storage temperature.

b) Increased acidity of food

c) Improper exhaust

d) Presence of soluble sulfur and phosphorous compounds

e) Improper timing and lacquering of can at internal surfaces

Biological spoilage

The cause of biological spoilage is microbial activity. In heat treated cans, the growth of microorganisms occur due to:

Leakage of can

It occurs because of manufacturing defects, punctures or rough handling. Bacteria are introduced into can by either in holes or improper seams. In this type, the microorganisms are not usually heat resistant and wide array of organisms had been found to cause spoilage as it is post processing contamination. Microbes may also get entry into can due to cold water, used to cool cans after heat treatment. Leakage may also be responsible for release of vacuum, which can favor the growth of microorganisms. Presence of low heat resistance organisms usually indicates leakage of can.

Under processing

- It includes sub-optimal heat treatment, faulty retort operations, excessive microbial load and contamination in product, change in consistency of the product.

Stages in Appearance of Can

- A can undergo different transformations from being a normal can to completely spoilt can as it depend upon various factors.

Microbial Spoilage of Canned Foods

- The microbial spoilage of canned food is classified as caused by thermophilic bacteria and mesophilic organisms. Most common spoilages of microbial origin are known as flat sour spoilage, Thermophilic anaerobic (TA) spoilage and putrefaction. These different types are briefly described here.

Spoilage by thermophilic spore forming bacteria

- Spoilage by these types of bacteria is most prevalent in under processed heat treated canned foods. Their spores survive the heat treatment and undergo vegetative cell formation and subsequent growth in canned conditions. Major spoilages by these organisms are:

Flat sour spoilage

- This is caused by souring bacteria. One characteristic of this spoilage is that ends of can remain flat during souring. Because of this condition, the detection of spoilage from outside is not

possible thereby culturing of contents become necessary to detect the type of organisms. Main organisms involved are *Bacillus,* while it occurs more frequently in low acid foods. *Bacillus* spp. has ability to produce acid without gas formation.

TA spoilage

- This type of spoilage is caused by thermophilic anaerobe not producing hydrogen sulfide. *Clostridium thermosaccharolyticum* is the main organism involved. It produces acid and gas in foods. Spoiled food produces sour or cheesy smell.

Sulfur stinker spoilage

- This type of spoilage occurs in low acid foods and primarily *Desulfotomaculum nigricans* is involved. The spores of these organisms are destroyed at optimal heat treatment, thus presence of this organism usually indicates under processing in terms of heat treatment. It produces hydrogen sulfide which produce typical odour.

Spoilage By Mesophillic Spore formers

- *Bacillus and Clostridium* are involved in this type of spoilage which is usually indicative of under spoilage.

Spoilage by Non-Spore Formers

- Presence of non spore formers in cans indicate post processing contamination. The organisms whose vegetative cells are heat resistant are more readily found. Following organisms are more prominent:

Enterococcus	*Streptococcus thermophilus*
Micrococcus	*Lactobacillus*
Leuconostoc	*Microbacterium*

- Presence of these organisms indicates leakage of container. Cooling water is one of the important source of contamination, thus coilforms also gain entry into the can through leakage.

Spoilage by Fungi

Yeasts

- Yeasts and their spores are not thermo tolerant, thus they are not found in suitably heat treated cans. Their presence indicates under processing or post pasteurization contamination through leakage. Fermentative yeasts are more prominent and they produce carbon dioxide, thus causing swelling of cans. Film yeasts too can grow on the surface of the food products.

Molds

- Among molds, *Aspergilus* and *Penicillium* are most spoiling organisms. These can grow at high sugar concentration. Acidification is considered method of preventing growth of molds. Some of the molds are resistant to heat. Molds are more common in home canned foods where heating as well as sealing is not under total aseptic conditions.

ELEMENTS OF A NATIONAL FOOD CONTROL SYSTEM:

Objectives:

The principal objectives of national food control systems are:

- Protecting public health by reducing the risk of foodborne illness;
- Protecting consumers from unsanitary, unwholesome, mislabelled or adulterated food; and
- Contributing to economic development by maintaining consumer confidence in the food system and providing a sound regulatory foundation for domestic and international trade in food.

Scope:

Food control systems should cover all food produced, processed and marketed within the country, including imported food. Such systems should have a statutory basis and be mandatory in nature.

Building Blocks:

While the components and priorities of a food control system will vary from country to country, most systems will typically comprise the following components.

(a) Food Law and Regulations:

- The development of relevant and enforceable food laws and regulations is an essential component of a modern food control system.
- Many countries have inadequate food legislation and this will impact on the effectiveness of all food control activities carried out in the country.
- Food law has traditionally consisted of legal definitions of unsafe food, and the prescription of enforcement tools for removing unsafe food from commerce and punishing responsible parties after the fact.
- It has generally not provided food control agencies with a clear mandate and authority to *prevent* food safety problems.
- The result has been food safety programmes that are reactive and enforcement-oriented rather than preventive and holistic in their approach to reducing the risk of foodborne illness.
- To the extent possible, modern food laws not only contain the necessary legal powers and prescriptions to ensure food safety, but also allow the competent food authority or authorities to build preventive approaches into the system.

- In addition to legislation, governments need updated food standards. In recent years, many highly prescriptive standards have been replaced by horizontal standards that address the broad issues involved in achieving food safety objectives.
- While horizontal standards are a viable approach to delivering food safety goals, they require a food chain that is highly controlled and supplied with good data on food safety risks and risk management strategies and as such may not be feasible for many developing countries.
- Similarly, many standards on food quality issues have been cancelled and replaced by labelling requirements.
- In preparing food regulations and standards, countries should take full advantage of Codex standards and food safety lessons learned in other countries.
- Taking into account the experiences in other countries while tailoring the information, concepts and requirements to the national context is the only sure way to develop a modern regulatory framework that will both satisfy national needs and meet the demands of the SPS Agreement and trading partners.

Food legislation should include the following aspects:

- it must provide a high level of health protection;
- it should include clear definitions to increase consistency and legal security;
- it should be based on high quality, transparent, and independent scientific advice following risk assessment, risk management and risk communication;
- it should include provision for the use of precaution and the adoption of provisional measures where an unacceptable level of risk to health has been identified and where full risk assessment could not be performed;
- it should include provisions for the right of consumers to have access to accurate and sufficient information;
- it should provide for tracing of food products and for their recall in case of problems;
- it should include clear provisions indicating that primary responsibility for food safety and quality rests with producers and processors;
- it should include obligation to ensure that only safe and fairly presented food is placed on the market;
- it should also recognise the country's international obligations particularly in relation to trade; and
- it should ensure transparency in the development of food law and access to information.

Guidelines for the development of food laws are contained in Annex 6.

(b) Food Control Management

- Effective food control systems require policy and operational coordination at the national level.

- While the detail of such functions will be determined by the national legislation, they would include the establishment of a leadership function and administrative structures with clearly defined accountability for issues such as: the development and implementation of an integrated national food control strategy; operation of a national food control programme; securing funds and allocating resources; setting standards and regulations; participation in international food control related activities; developing emergency response procedures; carrying out risk analysis; etc.
- Core responsibilities include the establishment of regulatory measures, monitoring system performance, facilitating continuous improvement, and providing overall policy guidance.

(c) Inspection Services

The administration and implementation of food laws require a qualified, trained, efficient and honest food inspection service. The food inspector is the key functionary who has day-to-day contact with the food industry, trade and often the public. The reputation and integrity of the food control system depends, to a very large extent, on their integrity and skill. The responsibilities of the inspection services include:

- Inspecting premises and processes for compliance with hygienic and other requirements of standards and regulations;
- Evaluating HACCP plans and their implementation;
- Sampling food during harvest, processing, storage, transport, or sale to establish compliance, to contribute data for risk assessments and to identify offenders;
- Recognizing different forms of food decomposition by organoleptic assessment; identifying food which is unfit for human consumption; or food which is otherwise deceptively sold to the consumer; and taking the necessary remedial action;
- Recognizing, collecting and transmitting evidence when breaches of law occur, and appearing in court to assist prosecution;
- Encouraging voluntary compliance in particular by means of quality assurance procedures;
- Carrying out inspection, sampling and certification of food for import/export inspection purposes when so required;
- In establishments working under safety assurance programmes such as HACCP, conduct risk based audits.

✓ Proper training of food inspectors is a prerequisite for an efficient food control system. As current food systems are quite complex, the food inspector must be trained in food science and technology to understand the industrial processes, identify potential safety and quality problems, and have the skill and experience to inspect the premises, collect food samples and carry out an overall evaluation.

- ✓ The inspector must have a good understanding of the relevant food laws and regulations, their powers under those laws, and the obligations such laws impose on the food sector.
- ✓ They should also be conversant with procedures for collecting evidence, writing inspection reports, collecting samples and sending them to a laboratory for analysis.
- ✓ With gradual introduction of HACCP systems in the food industry, the inspector should be trained to handle HACCP audit responsibilities.
- ✓ Clearly, there is a continuing need for training and upgrading the skills of existing inspectional staff and having a policy for human resource development, especially the development of inspectional specialists in specific technical areas.
- ✓ As human resources in some food control agencies in developing countries may be limited, environmental health inspectors are often also asked to work as food inspectors.
- ✓ This is not the ideal situation as they may lack the skills and knowledge to effectively evaluate and inspect food operations.
- ✓ If environmental health inspectors must be used, then they should be carefully supervised and provided with on-the-job training.

(d) Laboratory Services: Food Monitoring and Epidemiological Data

- ✓ Laboratories are an essential component of a food control system. The establishment of laboratories requires considerable capital investment and they are expensive to maintain and operate.
- ✓ Therefore careful planning is necessary to achieve optimum results. The number and location of the laboratories should be determined in relation to the objectives of the system and the volume of work.
- ✓ If more than one laboratory is required, consideration should be given to apportioning the analytical work to achieve the most effective coverage of the food analyses to be performed and also to having a central reference laboratory equipped for sophisticated and reference analyses.
- ✓ All food analysis laboratories may not be under the control of one agency or ministry, and a number could be under the jurisdiction of the states, provinces and local authorities.
- ✓ The Food Control Management should, however, lay down the norms for food control laboratories and monitor their performance.
- ✓ The laboratories should have adequate facilities for physical, microbiological and chemical analyses.
- ✓ In addition to simple routine analysis, the laboratories can be equipped with more sophisticated instruments, apparatus and library facilities as required.
- ✓ It is not only the type of equipment that determines the accuracy and reliability of analytical results but also the qualification and skill of the analyst and the reliability of the method used.

- ✓ The analytical results of a food control laboratory are often used as evidence in a court of law to determine compliance with regulations or standards of the country.
- ✓ It is therefore necessary that utmost care be taken to ensure the efficient and effective performance of the laboratory.
- ✓ The introduction of analytical quality assurance programmes and accreditation of the laboratory by an appropriate accreditation agency within the country or from outside, enables the laboratory to improve its performance and to ensure reliability, accuracy and repeatability of its results. Prescription of official methods of sampling and analysis also support this effort.
- ✓ An important element of a national food control system is its integration in a national food safety system so that links between food contamination and foodborne diseases can be established and analyzed.
- ✓ Access to reliable and current intelligence on the incidence of foodborne illness is critical. The laboratory facilities for this type of activity are generally situated outside the food control agencies.
- ✓ It is essential, however, that effective linkages are established between food control agencies and the public health system including epidemiologists and microbiologists.
- ✓ In this way information on foodborne diseases may be linked with food monitoring data, and lead to appropriate risk-based food control policies.
- ✓ This information includes annual incidence trends, identification of susceptible population groups, identification of hazardous foods, identification and tracing of causes of foodborne diseases, and the development of early warning systems for outbreaks and food contamination.

(e) Information, Education, Communication and Training

- ✓ An increasingly important role for food control systems is the delivery of information, education and advice to stakeholders across the farm-to-table continuum.
- ✓ These activities include the provision of balanced factual information to consumers; the provision of information packages and educational programmes for key officials and workers in the food industry; development of train-the-trainer programmes; and provision of reference literature to extension workers in the agriculture and health sectors.
- ✓ Food control agencies should address the specific training needs of their food inspectors and laboratory analysts as a high priority. These activities provide an important means of building food control expertise and skills in all interested parties, and thereby serve an essential preventive function.

Unit – V

Legal protection and IPR:

GATT and IPR, forms of IPR, IPR in India, WTO, TRIPS Convention on Biodiversity (CBD), Patent Co-operation Treaty (PCT), forms of patents and patentability, process of patenting, Indian and international agencies involved in IPR and patenting, Global scenario of patents and India's position, patenting of biological materials.

General Agreement on Tariffs and Trade (**GATT**)

- ❖ The General Agreement on Tariffs and Trade, or GATT for short, was drafted in 1947 as a provisional agreement to regulate international trade
- ❖ However, the International Trade Organization (ITO), which was supposed to take the place of GATT, was never ratified
- ❖ GATT was enforced from January 1, 1948 until December 31, 1994, when it was finally replaced by the World Trade Organization (WTO) on January 1, 1995.

GATT Members:

There were 23 nations that originally signed the GATT in 1947 in Geneva before it went into effect. The **signatories**, or contracting parties, included:

The original 23 GATT members were

The original 23 GATT members were Australia	Belgium	Brazil	Burma (now Myanmar)		Ceylon	Chile	China	Czechoslovakia (now Czech Republic and Slovakia)	
France	India	Lebanon	Luxembourg	Netherlands	New Zealand	Norway	Pakistan	Southern Rhodesia (Now Zimbabwe)	
South Africa		The United States		Canada	The United Kingdom			Cuba	Syria

The membership increased to 100 countries by 1993.

Purpose

- ✓ The purpose of GATT was to eliminate harmful **trade protectionism**
- ✓ That had sent global trade down 65 percent during the **Great Depression**
- ✓ By removing **tariffs**, GATT boosted **international trade**.

It restored economic health to the world after the devastation of World War II.

Rules of GATT:

- **First rule:** protecting the domestic industry by tariffs only

- **Second rule:** tariffs should be reduced and bound against further increases
- **Third rule:** trade according to the most-favoured-nation clause
- **Fourth rule:** national treatment

Objectives of GATT:

The preamble to the GATT can be linked to its objectives.

1. To raise the standard of living of the people,

2. To ensure full employment and a large and steadily growing volume of real income and effective demand.

3. To tap the use of the resources of the world fully.

4. To expand overall production capacity and international trade.

Principles of GATT:

For the realization of the above mentioned objectives, GATT adopted the following principles.

1. Non Discrimination,
2. Protection through tariffs,
3. A stable basis of trade, and;
4. Consultation

1. Non Discrimination

- ✓ The international trade should be conducted on the basis of nondiscrimination
- ✓ No member country shall discriminate (**Unfavorable)** between the members of GATT in the conduct of international trade
- ✓ On this basis, the principle "**Most favored Nation**" (MFN) was enunciated (**Announced**)
- ✓ This means that "each nation shall be treated as good as the most favored nation"
- ✓ All contracting parties should regard others as most favorable while applying and administering import and export duties and charges
- ✓ As far as quantitative restrictions are concerned, they should be administered without favor.

Exceptions to the principle of non-discrimination: However, certain exceptions to this basic rule are to be allowed. There is no objection to form free trade areas or custom unions. Such integration should facilitate consistent trade between the constituent territories. They should not raise barriers to the trade of other parties. GATT allows its members to follow measures to counter dumping and export subsidies. However, such measures should be applied only to offending countries.

2. Protection through tariffs only

- GATT rules prohibit quantitative restrictions

- Domestic industries should be protected only through customs tariffs
- Restrictions on trade should be limited to the less rigid tariffs

Exceptions: exceptions to this principle are given to the countries which suffer from unfavorable balance of payments position. Developing countries also enjoy this exception. Import restrictions may be applied to agricultural and fishery products if their domestic production is subject to equally restrictive production.

3. A stable basis of trade

GATT seeks to provide a stable and predictable basis for trade. It binds the tariff levels negotiated among the contracting countries. Binding of tariffs prevents the unilateral increase in tariffs, But still there is a provision for renegotiation of bound tariffs. A return to higher tariffs is discouraged by the requirement that any increase is to be compensated for.

4. Consultation

- The member countries should consult one another on trade matters and problems
- The members who feel aggrieved that their rights under GATT are withheld can call for a fair settlement
- Panels of independent experts have been formed under the GATT council
- Panel members are drawn from countries which have no direct interest in the disputes under investigation
- They look into the trade disputes among members. The panel procedure aims at mutually satisfactory settlement among members.

Three Provisions:

GATT had three main provisions.

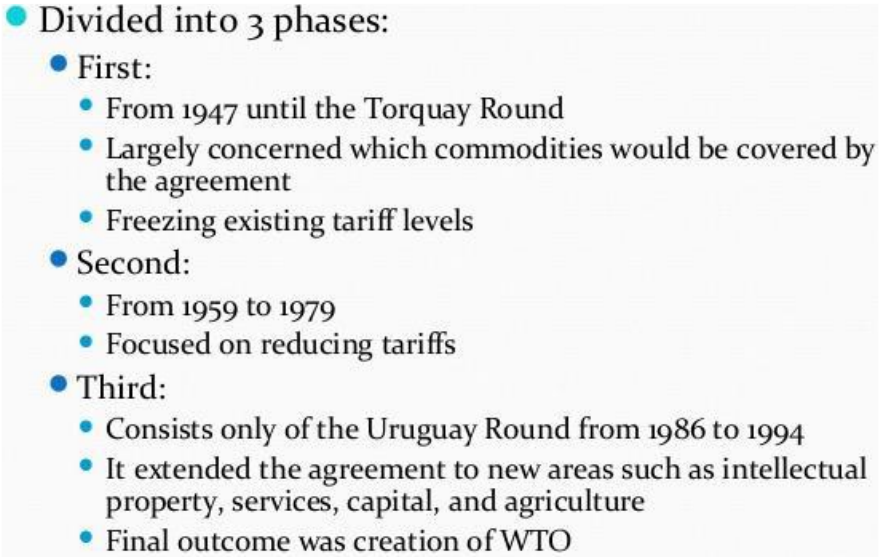
Reason for GATT Creation

- GATT was established in 1948 to regulate world trade.

- It was created as a means to boost economic recovery after the Second World War by reducing or eliminating trade tariffs, quotas and subsidies.

During the Great Depression, a breakdown of international relations and an increase in trade regulation made poor economic conditions worse and contributed to the outbreak of the Second World War. After the war, the Allies believed that a multilateral framework for world trade would loosen the protectionist policies that defined the 1930s and create an economic interdependency that would encourage partnership and reduce the risk of conflict.

The idea was to establish a code of conduct that would progressively liberalize (remove or loosen restrictions on) international trade. Within this code of conduct, consultation on trade issues among member nations could take place and be resolved, and data on world trade characteristics and trends could be collected and shared.



INTELLECTUAL PROPERTY (IPR)

In common sense intellectual property is a product of mind. It is similar to the property (**consisting of movable and immovable thing**) like a house or car where in the property or owner may use his property as his wishes nobody else can use his property without his permission as per Indian law.

Types of Intellectual Property

- Patents
- Copyright
- Trademarks
- Related Rights
- Geographical Indications
- Industrial Designs
- Unfair Competition
- Enforcement of Intellectual Property Rights

- Emerging Issues in Intellectual Property
  1. Biotechnology
  2. Traditional Knowledge

Patents

A patent is a government granted and secured legal right to prevent other forms of making, using or selling the invention covered by the patent. A patent is a personal property which can be licensed or sold by the person/organization like any other property.

Examples:

- Electric lighting- patents held by Edison and Swan
- Plastic- patents held by Baekeland
- Ballpoint pens- patents held by Biro
- Microprocessors- patents held by Intel.
- Telephones-patents held by Bell

Patents for:

- ✓ The drug substance itself:
  - Chemical composition of the API
- ✓ Method of use:
  - Use of the drug to treat a particular condition
- ✓ The formulation:
  - The physical form of a drug and method of administration
- ✓ The process of making it:
  - Manufacturing methods

Copyright

Copyright aims at providing protection to authors (writers, artists, music composers, etc) on their creations. Such creations are usually designated as ‘works”.

The best example of copyright is the authored and edited books, or audio and video cassettes, which cannot be reproduced without the permission of the person (author, editor or publisher), who holds the copyright. In biotechnology, the copyright may cover DNA sequence data which may be published.

Trademarks

A trademark is a sign that is used to identify certain goods and services as those produced or provided by a specific person or enterprises.

E.g. “DELL” is trademark that identifies goods (computers and computer related objects).

E.g. “CITY BANK” is a trademark that relates to services (banking and financial services).

Related Rights

Related rights provide protection to the following persons or organizations:

- **Performers** (actors, musicians, singers, dancers, or generally people who perform), in their performances
- **Producers of sound recordings** (for example, cassette recordings and compact discs) in their recordings and
- **Broadcasting organizations,** in their radio and television programs.

Sometimes, these rights are also referred to as neighboring rights.

Industrial Designs

An industrial design is the ornamental or aesthetic aspect of an article. The design may consist of three-dimensional features, such as the shape of an article, or two-dimensional features, such as patterns, lines or color.

Industrial designs are applied to a wide variety of products of industry and handicrafts such as technical and medical instruments, watches, jewelry, house ware, electrical appliances, vehicles, architectural structures, textile designs and other luxury items.

To be protected under most national laws, an industrial design must appeal to the eye. This means that an industrial design is primarily of an aesthetic nature, and does not protect any technical features of the article to which it is applied.

Unfair Competition

Unfair competition is generally understood as any act of competition that is contrary to honest practices in industrial or commercial matters.
A dishonest practice is not something that can be defined with precision.

The standard of fairness or honesty may change from country to country, as well as evolve with time. It is, therefore, difficult to attempt to encompass all existing acts of unfair competition in one definition.

Enforcement (a law) of Intellectual Property Rights

A publisher may own copyright in a book, which has been reproduced and sold without his or her consent, at a cut price.

A sound producer, who has invested large amounts of money, in terms of talent and technical skill, in producing records, sees that copies of it are sold on the market, at cheap prices, without his authorization.

Someone else's trade mark may have been used by a company on similar or identical goods of lesser quality, harming thus the reputation of the legitimate owner, and inflicting on him or her serious financial loss, let alone exposing customer's health to danger.

Somebody may be using the geographical denomination of "Roquefort" on cheese manufactured elsewhere than in the region of Roquefort in France, thus deceiving the consumers as well as taking away business from legitimate producers.

In all such cases intellectual property rights (i.e. copyright, related rights, trademarks and geographical indications) have been infringed. It is important that in such cases enforcement mechanisms be called into play to protect not only the legitimate interests of the rights of the owners, but also of the public.

Emerging Issues in Intellectual Property

Intellectual property plays an important role in an increasingly broad range of areas, ranging from the internet to health care, to nearly all aspects of science and technology, literature and the arts.

The following two topics, ***Biotechnology*** and ***Traditional Knowledge***, are now being discussed at length at the international arena.

❖ Biotechnology

Biotechnology is a field of technology of growing importance in which inventions may have a significant effect on our future, particularly in medicine, food, agriculture, energy and protection of the environment.

The science of biotechnology concerns living organisms, such as plants, animals, seeds and microorganisms, as well as biological material, such as enzymes, proteins and plasmids (which are used in "genetic engineering")

❖ Traditional Knowledge□

Traditional knowledge-used here broadly to refer to tradition-based innovations and creations resulting from intellectual activity in the industrial, scientific, literary or artistic fields-had been largely over-looked in the IP community until quite recently.

It is now increasingly recognized that the economic value of traditional knowledge assets could be further enhanced by the use of IP.

IPR in India:

Patent Administration in India

The Head Office is in **Kolkata**

Four branches:

1. Kolkata

2. Mumbai

3. Delhi

4. Chennai

The appropriate office of the patent office shall be the head office of the patent office or the ranch office as the case may be within whose territorial limits …

- Residence of applicant or domicile; or
- His the place of business; or
- The place where the invention actually originated.

If the applicant has no business or domicile in India, the address for service in India is given by such applicant

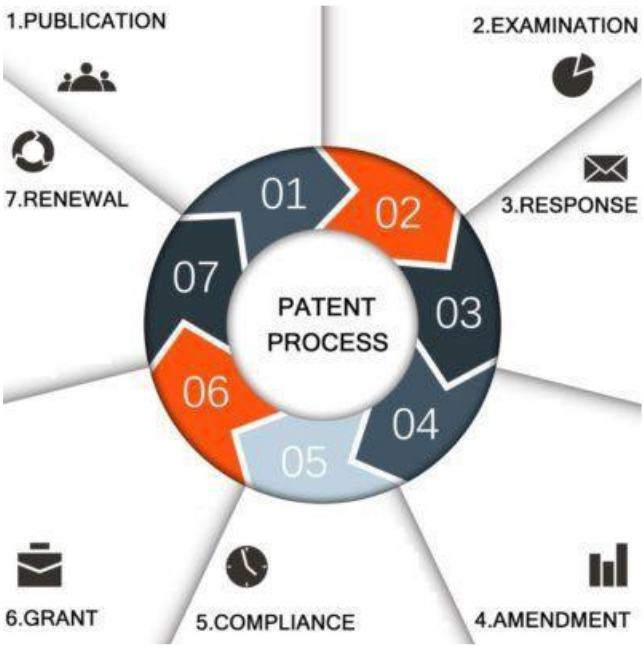
Territorial Jurisdiction Patent Office Branch,

- **Mumbai:** The States of Maharashtra, Gujrat Madhya Pradesh and Goa, Daman & Diu & Dadar & Nagar Haveli
- **Chennai:** The States of Andhra Pradesh, Kerala Tamil Nadu, Mysore and Pondicherry, Laccadive, Minicoy and Aminidivi Islands.
- **New Delhi:** States of Haryana, Himachal Pradesh, Jammu & Kashmir, Punjab, Rajasthan and Uttar Pradesh, Chandigarh and Delhi.
- **Kolkata:** The rest of India.e from the act

Who can file Patent Application in India

Either alone or jointly:

- ✓ By any person claiming to be true and first inventor(s)
- ✓ By any person being the assignee of person claiming to be true and first inventor(s)
- ✓ By the legal representative of any deceased person who can immediately after his death is entitled to make such application



Documents Required / Patent Forms***

✔Application Form (Form 1)

✔Request for Early Publication (Form 9)

✔Proof of Right to Apply

✔Request for Examination (Form 18)

(Paragraph 9 of Form 1)

✔ Provisional or complete Specification (Form 2)

✔ Power of Attorney, if required (Form 26)

✔Certified Copy of Convention Application, if required

✔Statement of Foreign Filing (Form 3)

✔Abstract of Invention

✔ Declaration as to Inventor ship (Form 5)

✔ Drawing(s), if any

History of Indian patent system	
1856	The act vi of 1856 on protection of inventions based on the British patent law of 1852. Certain exclusive privileges granted to inventors of new manufacturers for a period of 14 years.
1859	The act modified as act xv; patent monopolies called exclusive privileges (making. selling and using inventions in India and authorizing others to do so for 14 years from date of filing specification).
1872	The patents & designs protection act.
1883	The protection of inventions act.
1888	Consolidated as the inventions & designs act.
1911	The Indian patents & designs act.
1972	The patents act (act 39 of 1970) came into force on 20th April 1972.
1999	On march 26, 1999 patents (amendment) act, (1999) came into force from 01-01-1995.
2002	the patents (amendment) act 2002 came into force from 2oth may 2003

2005	the patents (amendment) act 2005 effective from Ist January 2005

Provisional Specification

- ✓ It should describe the nature of invention & contain the description of essential features of the invention.
- ✓ No need to include claims & details of the manner in which it to be performed

Advantages of Provisional Specification

- Priority for invention
- No risk of losing priority
- Liberty to develop
- Disclose to interested person to obtain financial support
- Virtually extend the term
- Utilize for exploring commercial feasibility
- Avoid incurring further expenses, if no commercial possibility

Contents of Complete Specification

- Title of the invention
- Field & background of the invention
- Use of the invention
- Prior art in the said field of invention & its drawback(s)
- Comparison between prior art & present invention
- Object (aim) of the present invention
- Summary of the present invention
- Brief description of drawings, if any
- Statement of the invention
- Detailed description of the invention w.r.t. drawings, if any
- Working examples for best method of the invention
- Claims for legal monopoly

Request for early Publication (Form 9)

Applicant may in Form-9 request the controller to publish the application at any time before the expiry of 18 months and such application will be published within 1 month of such request (Sec. 11A).

Publication of Patent Application

[Under Section 11A]

- Every application for patent shall be published in Patent Office Official Journal on expiry of period of 18 months from the date of filing or date of priority, whichever is earlier & shall be open for public inspection (Rule 27)

- Except in the case, where the application –
  (a) in which secrecy direction is imposed U/s. 35
  (b) has been abandoned U/s. 9(1)
  (c) has been withdrawn 3 months prior to 18 months

Importance of Publication

- On and from the date of publication of the application for patent and until the date of grant of a patent in respect of such application, the applicant shall have the like privileges and rights as if a patent for the invention has been granted on the date of publication of the application.
- The applicant shall not be entitled to institute any proceedings for infringement until the patent has been granted
- Right of the patentee in the case of applications filed u/s 5(2) shall accrue from the date of grant of the patent

Examination of Application [Under Section 12 & 13]

Every application after request for examination (U/s. 11B) shall be examined for -

(a) Whether application is in accordance with the requirements of the Act or the rules made there under

(b) Whether there is any lawful ground of objection to the grant of patent

(c) Whether the novelty & inventive step is anticipated by prior claiming in India or publication anywhere in the world

(d) Any other matter which may be prescribed under the Act

Grant or Refuse or Abandonee (Remove) [Under Section 43, 15, & 21]

If, within 12 months from the date FER -

(a) all requirements met, patent will be granted immediately with seal of the Patent Office (Letters Patent) & grant publication U/s. 43(2);

(b) requirement(s) not met, patent application will abandoned U/s. 21(1); or

(c) requirement(s) met or not met even after hearing U/s. 15, patent will be granted or refused, as the case may be.

• Term of patent will be 20 years from the date of filing (sec. 53)

What rights a Patent confers on the patentee

If the patent is for a product:-

- The right to prevent others from

- making
- using
- offering for sale
- selling
- importing the patented product

If the patent is for a process:-

- the right to prevent others from
- using the process
- offering for sale the product using the process
- selling the product using the process
- importing the product using the process

Post-Grant Opposition [Under Section 25(2)]

- Any time after the grant or before expiry of 1 year from the date of publication of grant U/s. 43(2), any interested person may give notice of opposition on any of the grounds (a) to (k) of Section 25(2)
- Opposition board [constituted U/s. 25(3) (b)] after completion of proceeding & maturation of case for hearing will examined the matter & submit its report
- On receipt of report & after hearing the patentee & opponent, the patent will be either maintained or amended or revoked by order U/s. 25(4)

WTO (*World Trade Organization*)

- ✓ The *World Trade Organization* (*WTO*) deals with the global rules of trade between nations
- ✓ Its main function is to ensure that trade flows smoothly, predictably and freely as possible

They deal with: agriculture, textiles and clothing, banking, telecommunications, government purchases, industrial standards and product safety, food sanitation regulations, intellectual property, and much more. The WTO agreements are lengthy and complex because they are legal texts covering a wide range of activities.

Fact files of WTO:

Headquarters:	Geneva, Switzerland
Purpose:	Regulate international trade
Founded:	1 January 1995
Membership:	164 member states
Formation:	1 January 1995; 22 years ago
Official language:	English, French, Spanish
Secretariat staff:	625

Current WTO members: 153 members, Observers (31)

History:

- The WTO was officially created in January of 1995 and essentially replaced the General Agreement on Tariffs and Trade (GATT), which had been in force since 1948, a few years after the Second World War
- Before the WTO was created, an initiative to start something similar known as the International Trade Organization (ITO) took place
- Unfortunately, the ITO treaty was not approved by the U.S. and a few other countries and ultimately never went into effect
- It was the outcome of the lengthy (1986-1994) Uruguay round of GATT negotiations. The WTO was essentially an extension of GATT
- It extended GATT in two major ways.
    - First GATT became only one of the three major trade agreements that went into the WTO (the other two being the General Agreement on Trade in Services (GATS) and the agreements on Trade Related Aspects of Intellectual Property Rights (TRIPS))
    - Second the WTO was put on a much sounder institutional footing than GATT. With GATT the support services that helped maintain the agreement had come into being in an ad hoc manner as the need arose. The WTO by contrast is a fully fledged institution (GATT also was, at least formally, only an agreement between contracting parties and had no independent existence of its own while the WTO is a corporate body recognized under international law).

Objectives:

The important objectives of WTO are:

1. To improve the standard of living of people in the member countries
2. To ensure full employment and broad increase in effective demand
3. To enlarge production and trade of goods
4. To increase the trade of services
5. To ensure optimum utilization of world resources
6. To protect the environment
7. To accept the concept of sustainable development

PRINCIPLES OF WTO

The basic principles of the WTO (according to the WTO):

• **Trade without Discrimination**

1. Most-favoured-nation (MFN): treating other people equally Under the WTO agreements, countries cannot normally discriminate between their trading partners. Grant someone a special favour (such as a lower customs duty rate for one of their products) and you have to do the same for all other WTO members.
2. National treatment: Treating foreigners and locals equally Imported and locally-produced goods should be treated equally — at least after the foreign goods have entered the market. The same should apply to foreign and domestic services, and to foreign and local trademarks, copyrights and patents.

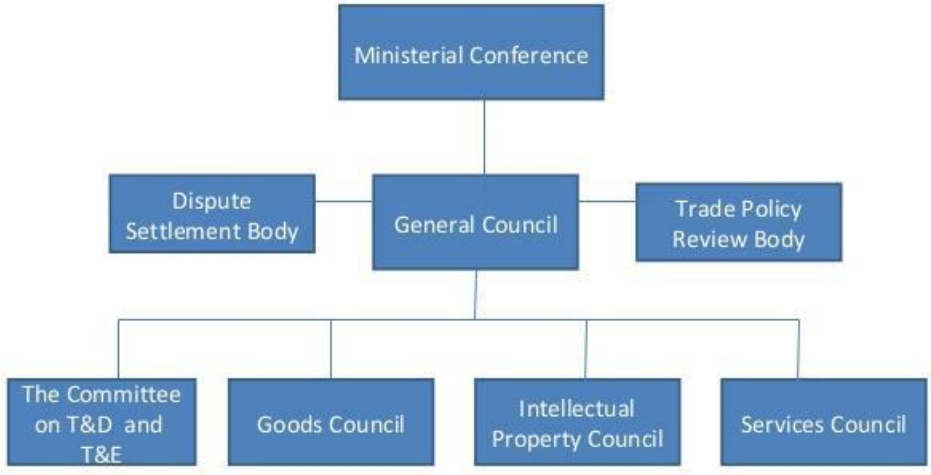
• **Freer trade**: gradually, through negotiation Lowering trade barriers is one of the most obvious means of encouraging trade. The barriers concerned include customs duties (or tariffs) and measures such as import bans or quotas that restrict quantities selectively

• **Predictability:** through binding and transparency Sometimes, promising not to raise a trade barrier can be as important as lowering one, because the promise gives businesses a clearer view of their future opportunities. With stability and predictability, investment is encouraged, jobs are created and consumers can fully enjoy the benefits of competition — choice and lower prices. The multilateral trading system is an attempt by governments to make the business environment stable and predictable.

• **Promoting fair competition:** The WTO is sometimes described as a "free trade" institution, but that is not entirely accurate. The system does allow tariffs and, in limited circumstances, other forms of protection. More accurately, it is a system of rules dedicated to open, fair and undistorted competition.

• **Encouraging development and economic reform:** The WTO system contributes to development. On the other hand, developing countries need flexibility in the time they take to implement the system's agreements. And the agreements themselves inherit the earlier provisions of GATT that allow for special assistance and trade concessions for developing countries.

STRUCTURES OF WTO



Functions:

- Administering WTO trade agreements
- Forum for trade negotiations
- Handling trade disputes
- Monitoring national trade policies
- Technical assistance and training for developing countries
- Cooperation with other international organizations

WTO – Why?

- ✓ To arrange the implementation, administration and operations of multilateral (involving three or more participants) and Plurilateral trade agreements (power which shared between different countries)
- ✓ To arrange the forum for deliberations for the member nations in regard to their multilateral trade relations in issues deal with under the agreements
- ✓ To provide a framework for implementing of the results arising out of the deliberations (long and care full agreements/consideration) which taken place at ministerial conference level
- ✓ To manage the created understanding on rules and procedure governing the settlement of disputes
- ✓ To manage effectively and efficiency the trade policy review mechanism (TRIM)
- ✓ To create more together relationship with all nations in respect of global economic policy-making, it would cooperate with the IMF and the World Bank & its affiliated Organizations.

WTO Ministerial Conference:

Conference	Year	Place
I	9-13 Dec., 1996	Singapore
II	18-20 May 1998	Geneva (Switzerland)
III	30 Nov.-3 Dec., 1999	Seattle (USA)
IV	9-14 Nov., 2001	Doha (Qatar)
V	10-14 Sep., 2003	Cancun (Mexico)
VI	13-18 Dec.. 2005	Hong Kong
VII	30 Nov-2Dec., 2009	Geneva (Switzerland

WTO Vs GATT

GATT	WTO
• It was ad hoc & provisional.	• It is permanent.
• It had no provision for creating an organization.	• It has legal basis because member nations have verified the WTO agreements.
• It allowed contradictions in local law & GATT agreements.	• More authority than GATT.
	• It doesn't allow any contradictions in local law .

Convention on Biodiversity

Introduction

The Convention on Biological Diversity (CBD) entered into force on 29 December 1993. It has 3 main objectives:

1. The conservation of biological diversity
2. The sustainable use of the components of biological diversity
3. The fair and equitable sharing of the benefits arising out of the utilization of genetic resources

Biodiversity

Biological diversity (Biodiversity) is the degree of variation of life forms within a given ecosystem, biome, or an entire planet.

It is measured by two parameters:

1. Alpha diversity which represents the no. of species in a specified area
2. Beta diversity which represents the turnover of species across space.

A unified view of the traditional three levels at which biological variety has been identified are:

1. Species Diversity
    - Species diversity which refers to the numbers and kinds of living organisms
2. Genetic Diversity
    - Genetic diversity which refers to the genetic variation within a population of species
3. Ecosystem Diversity

- Ecosystem diversity which is the variety of habitats, biological communities and ecological processes that occur in the biosphere

History of the Convention:

- The Earth's biological resources are vital to humanity's economic and social development
- As a result, there is a growing recognition that biological diversity is a global asset of tremendous value to present and future generations
- At the same time, the threat to species and ecosystems has never been so great as it is today
- Species extinction caused by human activities continues at an alarming rate.

In response, the United Nations Environment Programme (UNEP) convened the Ad Hoc Working Group of Experts on Biological Diversity in November 1988 to explore the need for an. international convention on biological diversity. Soon after, in May 1989, it established the Ad Hoc Working Group of Technical and Legal Experts to prepare an international legal instrument for the conservation and sustainable use of biological diversity. The experts were to take into account "the need to share costs and benefits between developed and developing countries" as well as "ways and means to support innovation by local people".

Biodiversity – the web of life:

Biological diversity – or biodiversity – is the term given to the variety of life on Earth and the natural patterns it forms. The biodiversity we see today is the fruit of billions of years of evolution, shaped by natural processes and, increasingly, by the influence of humans. It forms the web of life of which we are an integral part and upon which we so fully depend.

This diversity is often understood in terms of the wide variety of plants, animals and microorganisms. So far, about 1.75 million species have been identified, mostly small creatures such as insects. Scientists reckon that there are actually about 13 million species, though estimates range from 3 to 100 million.

Biodiversity also includes genetic differences within each species – for example, between varieties of crops and breeds of livestock. Chromosomes, genes, and DNA – the building blocks of life – determine the uniqueness of each individual and each species.

Our personal health, and the health of our economy and human society, depends on the continuous supply of various ecological services that would be extremely costly or impossible to replace. These natural services are so varied as to be almost infinite.

For example, it would be impractical to replace, to any large extent, services such as pest control performed by various creatures feeding on one another, or pollination performed by insects and birds going about their everyday business

"Goods and Services" provided by ecosystems include:

- ✓ Provision of food, fuel and fibre
- ✓ Provision of shelter and building materials
- ✓ Purification of air and water
- ✓ Detoxification and decomposition of wastes
- ✓ Stabilization and moderation of the Earth's climate
- ✓ Moderation of floods, droughts, temperature extremes and the forces of wind
- ✓ Generation and renewal of soil fertility, including nutrient cycling
- ✓ Pollination of plants, including many crops
- ✓ Control of pests and diseases
- ✓ Maintenance of genetic resources as key inputs to crop varieties and livestock breeds, medicines, and other products
- ✓ Cultural and aesthetic benefits
- ✓ Ability to adapt to change

Patent Cooperation Treaty (PCT):

The **Patent Cooperation Treaty** (**PCT**) is an international patent law treaty, concluded in 1970. It provides a unified procedure for filing patent applications to protect inventions in each of its contracting states. A patent application filed under the PCT is called an **international application**, or **PCT application**.

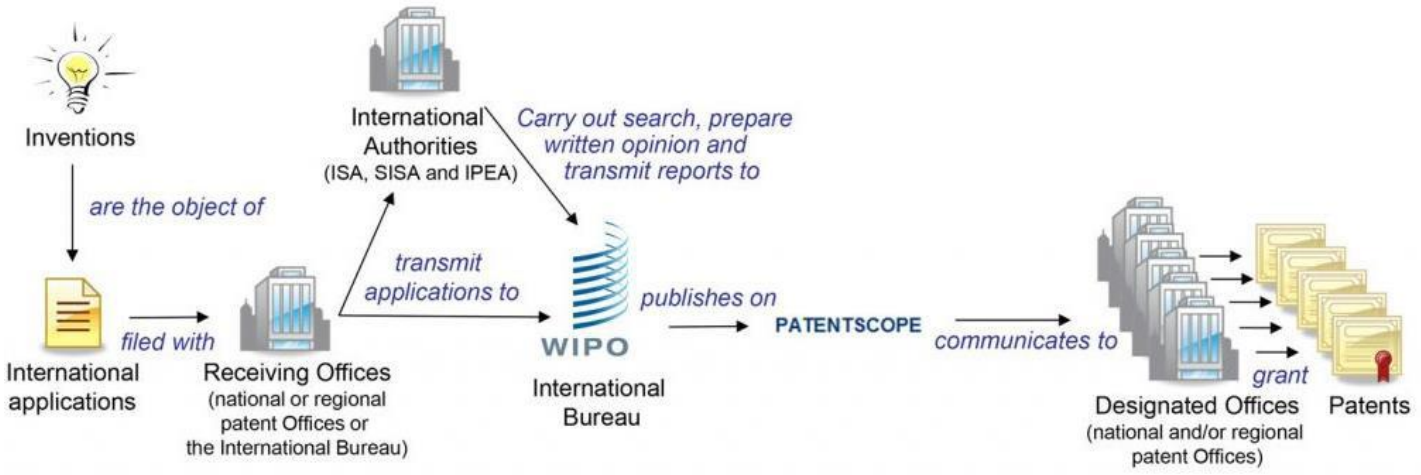
Languages: English; French
Signatories: **36**
Parties: **152**
Effective: 24 January 1978
Location: Washington, United States
Condition: ratification by 8 States, 4 of which have significant patenting activity
Signed: 19 June 1970

Steps involved in PCT:

- ✓ Filing of international application in a Receiving Office (RO)
- ✓ International Search by an International Searching Authority (ISA)
- ✓ International Preliminary Examination by an International Preliminary Examining Authority (IPEA)

Process of filling a PCT-application:

- ***Filing:*** you file an international application with a national or regional patent Office or WIPO (if permitted by your State's national security provisions), complying with the formality requirements, in one language (preferably English), and you pay **one set of fees**.
- ***International Search:*** an "International Searching Authority" (ISA) identifies the published patent documents and technical literature ("prior art"). So it's a check whether your invention is patentable or not.
- ***International Publication:*** after the expiration of 18 months from the earliest filing date, the content of your international application is disclosed to the world.
- ***Supplementary International Search (optional):*** a second authority identifies published documents which may not have been found by the first ISA.
- ***International Preliminary Examination (optional):*** another way to get clarification whether your invention is patentable. A third ISAs carries out an additional patentability analysis, usually on an amended version of the application.
- ***National Phase:*** after the end of the PCT procedure, usually at 30 months from the earliest filing date of your initial application, from which you claim priority, you start to pursue the grant of your patents directly before the national (or regional) patent Offices of the countries in which you want to obtain them.



Advantages of PCT:

- Use of the PCT saves effort—time, work, money—for any person or firm seeking protection for an invention in a number of countries
- Use of the PCT also helps the applicant to make decisions about the prosecution of the application before the various national Patent Offices in the PCT National Phase of processing
- The saving arises primarily from the fact that, under the PCT, the applicant files one application—the PCT international application—in one place, in one language and pays one initial set of fees, and that this PCT international application has the effect of a national or regional application, which, without the PCT, he would have to file separately for each country or region
- The help to the applicant in the PCT National Phase prosecution of the application follows from the "advice" he obtains from the PCT international search report, a report which is established for each PCT international application, according to high, internationally regulated standards, by one of the Patent Offices that are highly experienced in examining patent applications and that have been specially appointed to carry out international searches.

Patent:

Patents protect inventions and new discoveries that are new and non-obvious.

Types of patents:

1. Utility patents
2. Design patents
3. Plant patents

1. Utility Patents:

- A utility patent is the most common type of patent that people seek
- This type of patent covers processes, compositions of matter, machines, and manufactures that are new and useful
- A utility patent can also be obtained for new and useful improvements to existing processes, compositions of matter, machines, and manufactures
- Processes refer to any acts or methods of doing something, usually involving industrial or technical processes. Compositions of matter are basically chemical compositions, which can include a mixture of ingredients or new chemical compounds. Machines include things that are generally defined as a machine, such as a computer, while manufactures are defined as goods that are manufactured or made.

2. **Design Patents**

- In terms of obtaining a design patent, a design is defined as the "surface ornamentation" of an object, which can include the shape or configuration of an object
- In order to obtain this type of patent protection, the design must be inseparable from the object

- While the object and its design must be inseparable, a design patent with only protect the object's appearance
- In order to protect the functional or structural features of an object, a person must also file for a utility patent.

3. Plant Patents

- A plant patent can be obtained to protect new and distinctive plants
- A few requirements to obtain this type of patent are that the plant is not a tuber propagated plant (i.e. an Irish potato), the plant is not found in an uncultivated state, and the plant can be asexually reproduced.
- Asexual reproduction means that instead of being reproduced with seed, the plant is reproduced by grafting or cutting the plant
- Plant patents require asexual reproduction because it's proof that the patent applicant can reproduce the plant.

Patentability

Inventions which are new, involve an inventive step and are susceptible of industrial application are **patentable** even if they concern a product consisting of or containing biological material *. Biological material which is isolated from its natural environment or produced by means of a technical process may also be the subject of an invention.

The following are **not patentable**:

- ✓ plant and animal varieties;
- ✓ Essentially biological processes for the production of plants or animals, such as crossing or selection. This exclusion from patentability does not, however, affect the patentability of inventions which concern a microbiological process
- ✓ The human body and the simple discovery of one of its elements, including the sequence or partial sequence of a gene.

However, an element isolated from the human body or produced by means of a technical process, including the sequence or partial sequence of a gene, may constitute a patentable invention.
The following inventions include those that are unpatentable where their exploitation would be **contrary to public policy or morality**

- processes for cloning human beings;
- processes for modifying the germ-line genetic identity of human beings;
- uses of human embryos for industrial or commercial purposes;

- Processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes.

Patenting process:

An invention is patentable only if it is:

- New and previously undisclosed
- Distinguished by an inventive step not obvious to someone expert in that technology
- Capable of industrial application - that is, it is physically possible to make the invention

Some issues to consider before deciding to patent:

- Do you really need a patent? Would some combination of other forms of IPR protect your idea adequately? And be honest with yourself - are you perhaps motivated more by vanity (the prospect of a patent in your name) than by commercial necessity?
- Have you studied the total cost of patenting (which should include annual renewal fees in every country in which you have protection)? Is your invention likely to earn enough income to justify the cost? Normally, you should not apply for a patent until you have thoroughly researched the commercial and financial potential of your idea.
- Is the time right to apply for a patent? Application starts a sequence of events which cannot be delayed. Do you apply for a patent early on, or wait until the invention is market-ready and more capable of quickly recouping its IPR costs? Later may be better than sooner, but circumstances will vary so you should always seek the advice of a patent attorney.
- Does your invention have a short product life cycle? The patenting process typically takes 3-4 years. If your invention is aimed at a highly competitive market in which products are rapidly replaced or improved, your patent may be worth little by the time it is granted.
- Who will pay to enforce your patent? National IP offices do not enforce patents or monitor them for infringement. These are the responsibilities of the patent owner or a licensee. Until funds are potentially available to enforce your patent - from royalties or sales income - it may offer limited practical protection.
- How strongly might your patent resist legal challenge? You will definitely need a patent attorney's advice on the strength of your claims. This is important because the validity of patent claims is often challenged, usually by competitors who want to copy a successful product. If they succeed, you may be left with a valueless patent and an order to pay the victor's legal costs.

Applying for a patent

Applying for a patent is a legal process governed by strict timescales and usually immovable deadlines. It is not something to rush into! To maximize your chances of a worthwhile patent you should:

- Study the application procedure in detail.
- Aim to apply not in haste, but **strategically** - at a time and for a reason that most benefits your exploitation plans.
- Use a patent attorney! **Do not** do it all yourself - the risk of making mistakes is too great.

Here is only a very brief guide to the application process for a European Patent according to the European Patent Convention (EPC).

Applying for a patent at a national IP office is roughly similar to stages 1-6 below, but an application must be made in the local language.

Making an international application through the Patent Co-operation Treaty (PCT) involves a single procedure for stages 1-4, but 30 months after filing the application goes through stages 5 and 6 in every national or regional IP office where you wish to take up protection.

Choosing your route for a patent application (EPC, PCT, national and regional, or combinations thereof will depend on:

- Your invention.
- Your business plan.
- Your available funds.
- Your intended market.
- The likeliest sources of infringing products.

A patent attorney will be able to advise you on the route that is best **for you and your invention**.

Stage 1: Beginning the process:-

Your patent attorney must provide documentation consisting of:

- A request for a patent.
- Details of the applicant (you).
- A description of the invention.
- Claims.
- Drawings (if any).
- An abstract.

A fee must also be paid. In order to avoid delay, it is vital that all documentation conforms in every detail to official requirements. Your patent attorney will ensure that it does. At the EPO, applications are accepted in English, French or German.

For your patent attorney to prepare all the information about your invention, he or she will obviously need to work closely with you. **Do not assume that you know best because it is your invention**. You must trust the skill and judgment of your patent attorney, as patenting involves a

complex mix of law and technology. The claims in particular need to be drafted with skill, as they are the most important aspect of a patent.

Stage 2: Filing date and initial examination:-

If your documentation appears correct, your application is given a **filing date** - also known as your **priority date**. After filing there is a **formalities examination** to ensure that your documentation is correct and complete.

At any time in the next 12 months you can file for patent protection in other countries and have those later filings treated as if they had been filed on your priority date. In practice, this gives you a year to decide how many countries you wish to include in your patent protection.

Stage 3: Search:-

A **search report** is sent to you, listing and including copies of all prior art documents found by an experienced examiner and regarded as relevant to your invention. The search is based mainly on your claims for novelty, but your description and any drawings will also be taken into account. The report will often include an initial opinion on the patentability of your invention.

Stage 4: Publication:-

Your application is **published** 18 months after the filing date. Your invention will appear in databases accessible to other people around the world. It will act as **prior art** against any future patent applications from other inventors or companies for similar inventions.

You then have six further months to make two decisions:

- Do you want to continue with your application? You indicate ‘yes' by requesting a more thorough (‘substantive') examination.
- Which countries do you want to include (‘designate') in your patent protection? Designation fees must be paid.

After your patent is granted, you may claim damages for infringements originating as far back as the publication date of your application. However, to enjoy this right in some countries it may be necessary to file a translation of your claims with their national IP office and for them to publish the translated claims.

Stage 5: Substantive examination:-

If you request **substantive examination**, the EPO has to decide whether your invention **and** your application meet the requirements of the European Patent Convention. For maximum objectivity there are usually three EPO examiners, one of whom maintains contact with your patent attorney. This stage will often involve dialogue between the examiners and your patent attorney, which may result in

the re-drafting of key parts of your application. Your patent attorney will defend your application, and this is one more reason why it is essential to have professional representation.

Stage 6: Decision to grant a patent:-

If the examiners decide to grant a patent, and all fees have been paid and any claims translations filed, the decision is reported in the European Patent Bulletin. The **decision to grant** takes effect on the date of publication.

Stage 7: Validation:-

What you have now got is a 'bundle' of individual national patents. After the EPO decision to grant is published, your patent has to be **validated** in each designated state within a specific time limit. If this is not done, your patent may not be enforceable in that state. In some states, validation may include having to file (and pay for) a translation of the whole patent, or just a translation of the granted claims.

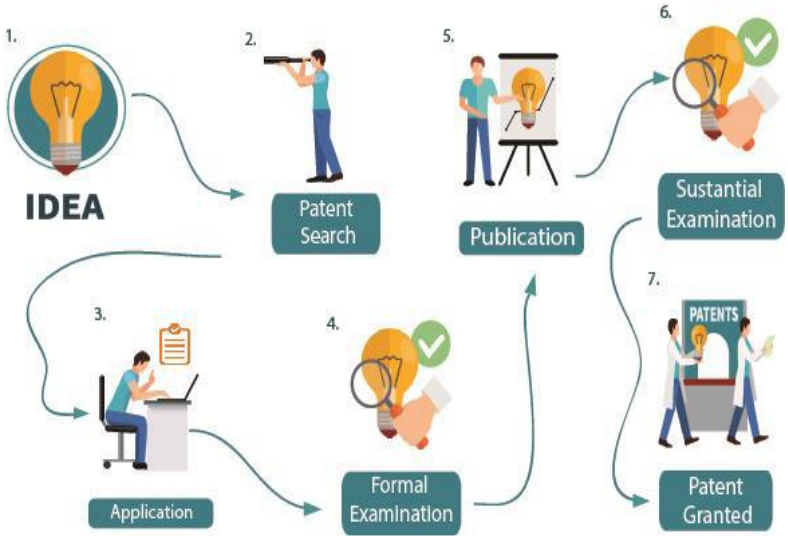
Stage 8: Opposition:-

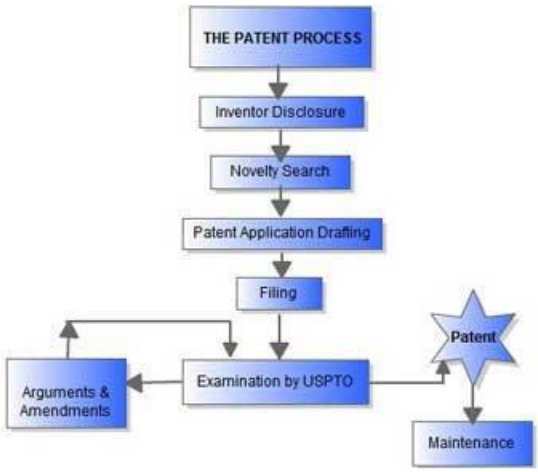
A granted patent may be **opposed** by third parties - usually the applicant's competitors - if they believe it should not have been granted. After the grant is reported in the European Patent Bulletin they have nine months in which to file notice of opposition. The most common charge is that the invention is not novel or lacks an inventive step. The case will be examined by an EPO team, again of three examiners.

Opposition is the last chance to attack a European patent **as a single entity in a single forum**. Later, the patent can only be challenged in national courts and a ruling in one country has no effect on the patents for the same invention in other countries. This gives competitors a strong incentive to challenge an invention during the opposition period, as challenging patents in separate national courts can be much more expensive.

Stage 9: Appeal:-

All EPO decisions are open to appeal. Responsibility for decisions on appeals is taken by independent boards of appeal.





Step by step guide for how to get patent in India

Step 1: Write down the invention (idea or concept) with as much details as possible

Collect all the information about your invention such as:

- Area of invention
- Description of the invention what it does
- How does it work
- Advantages of the invention

Ideally, if you have worked on the invention during research and development phase you should have something call lab record duly signed with date by you and respective authority.

Step 2: include drawings, diagrams or sketches explaining working of invention

The drawings and diagrams should be designed so as to explain the working of the invention in better way with visual illustrations. They play an important role in patent application.

Step 3: check whether the invention is patentable subject matter

All inventions may not be patentable, as per Indian patent act there are certain inventions that are not patentable explained in detail in(inventions not patentable)

Step 4a: Patentability search

The next step would be finding out whether your invention meets all patentability criteria as per Indian patent act? That is,

- Novelty
- Non-obviousness
- Industrial application
- Enabling

The detailed explanation for patentability criteria is given here (what are patentability criteria's). The patentability opinion is provided by the patent professionals up on conducting extensive search and forming patentability report.

Step 4b: Decide whether to go ahead with patent

The patentability report and opinion helps you decide whether to go ahead with the patent or not, chances are what you thought as novel might already been patented or know to public in some form of information. Hence this reports saves lots of time, efforts and cost of the inventor by helping him decide whether to go ahead with the patent filing process or not.

Step 5: Draft (write) patent application

In case you are at very early stage in the research and development for your invention, then you can go for **provisional application**. It gives following benefits:

- Secures filing date
- 12 months of time to file complete specification
- Low cost

After filing provisional application, you secure the filing date which is very crucial in patent world. You get 12 months of time to come up with the complete specification, up on expiry of 12 months your patent application will be abandoned.

When you complete the required documents and your research work is at level where you can have prototype and experimental results to prove your inventive step you can file complete specification with patent application.

Filing the provisional specification is the optional step, if you are at the stage where you have complete information about your invention then you can directly go for complete specification.

Step 6: Publication of the application

Up on filing the complete specification along with application for patent, the application is published after 18 months of first filing.

An early publication request can be made along with prescribed fees if you do not wish to wait till the expiry of 18 months from the date of filing for publishing your patent application.

Generally the patent application is published within a month form request form early publication.

Step 7: Request for examination

The patent application is examined only after receiving request for examination that is RFE. Up on receiving this request the controller gives your patent application to a patent examiner who examinees the patent application with different patentability criteria like:

- ✓ Patentable subject matter
- ✓ Novelty
- ✓ Non-obviousness
- ✓ Inventive step
- ✓ Industrial application
- ✓ Enabling

The examiner creates a first examination report of the patent application upon reviewing it for above terms. This is called patent prosecution. Everything happening to patent application before grant of patent is generally called as patent prosecution.

The first examination report submitted to controller by examiner generally contains prior arts (existing documents before the date of filing) which are similar to the claimed invention, and same is reported to patent applicant.

Step 8: respond to objections

Majority of patent applicants will receive some type of objections based on examination report. The best thing to do it analyse the examination report with patent professional (patent agent) and creating a response to the objections raised in the examination report.

This is a chance for an inventor to communicate his novelty over prior arts found in the examination report. The inventor and patent agent create and send a response to the examination that tries to prove to controller that his invention is indeed patentable and satisfies all patentability criteria's.

Step 9: clearing all objections

This communication between controller and patent applicant is to ensure that all objections raised in the patent application are resolved. (if not the patent will not be granted) and the inventor has his fair chance to prove his point and establish novelty and inventive step over existing prior arts.

Up on finding the patent application in order of grant, it is grant to the patent applicant as early as possible.

Step 10: Grant of patent

The application would be placed in order for grant once it is found to be meeting all patentability requirements. The grant of patent is notified in the patent journal which is published time to time.

Indian Patent Office:

- The **Indian Patent Office** is administered by the Office of the Controller General of Patents, Designs & Trade Marks (CGPDTM).
- This is a subordinate office of the Government of India and administers the Indian law of Patents, Designs and Trade Marks.

Patent administration

The CGPDTM reports to the Department of Industrial Policy and Promotion (DIPP) under the Ministry of Commerce and Industry and has five main administrative sections:

- Patent Office
- Designs Registry
- Trademarks Registry
- Geographical indications Registry

- Rajiv Gandhi National Institute of Intellectual Property Management (NIIPM)
- Patent Information System

The patent office is headquartered at Kolkata with branches in Chennai, New Delhi and Mumbai, but the office of the CGPDTM is in Mumbai.

The Indian Patent Office has 526 Patent Examiners, 97 Assistant Controllers, 42 Deputy Controllers, 1 Joint Controller, and 1 Senior Joint Controller, all of whom operate from four branches. Although the designations of the Controllers differ, all of them (with the exception of the Controller General) have equal authority in administering the Patents Act.

International Patent Office:

- The **World Intellectual Property Organization** (**WIPO**) is one of the 17 specialized agencies of the United Nations (UN)
- WIPO was created in 1967 "to encourage creative activity, to promote the protection of intellectual property throughout the world"
- WIPO currently has 191 member states, administers 26 international treaties, and is headquartered in Geneva, Switzerland
- The current Director-General of WIPO is Francis Gurry, who took office on 1 October 2008.

1. Food and Agriculture Organization (FAO)
2 International Civil Aviation Organization (ICAO)
3 International Fund for Agricultural Development (IFAD)
4 International Labour Organization (ILO)
5 International Maritime Organization (IMO)
6 International Monetary Fund (IMF)
7 International Telecommunication Union (ITU)
8 United Nations Educational, Scientific and Cultural Organization (UNESCO)
9 United Nations Anti-Terrorism Coalition (UNATCO)
10 United Nations Industrial Development Organizations (UNIDO)
11 Universal Postal Union (UPU)
12 World Bank Group (WBG)
    12.1 International Bank for Reconstruction and Development (IBRD)
    12.2 International Finance Corporation (IFC)

12.3 International Development Association (IDA)

13 World Health Organizations (WHO)

14 World Intellectual Property Organizations (WIPO)

15 Worlds Meteorological Organization (WMO)

16 World Tourism Organizations (UNWTO)

17 Former specialized agencies

Present Indian Scenario

PATENTS IN THE INDIAN SCENARIO:

The laws pertaining to Patent in India is governed by the Patents Act, 1970 which has been amended twice by The Patents (Amendment) Act, 1999 and The Patents (Amendment) Act, 2002. The new Patent Act, 2002 has although been notified on June 25th 2002, however, currently only limited sections of it have been made applicable vide Gazette Notification from the Government of India, dated May 20, 2003. Although, it is being implemented in phased manner, however, it is a matter of time before the new Act shall be applicable in its entirety.

In the current scenario, the old Acts i.e. The Patent Act, 1970 and The Patent Rules, 2003 are applicable except for the sections made applicable through the Gazette Notification, as stated above.

WHAT IS PATENTABLE:

Patents are granted in respect of any invention in goods. An invention means any new and useful art, process, method or manner of manufacture, machine, apparatus or other article, or substance produced by manufacture, and includes any new and useful improvement in any of them.

No patent is granted in respect of claims for the substances themselves; however, claims for the methods or processes of manufacture are patentable. However, in compliance with its commitment under the TRIPS agreement, India has been given time to introduce product patent by the year 2005.

WHO CAN APPLY:

Both the Indian nationals and foreigners can make an application for patent in India. But, in case of foreigners applying for patent in India, it is necessary that the country of such applicant should also be providing such reciprocal rights to the Indian nationals.

Application for patents can be made by any person claiming to be the true and first inventor of the invention or by his assignee or legal representative. An application for patent can be made by any of these persons either alone or jointly with any other person. Two or more companies as assignees may also make an application jointly.

STEPS INVOLVED IN GRANT OF PATENT:

- Filing of an application for grant of a patent accompanied by either a provisional specification or a complete specification before any public disclosure of the invention.
- In case provisional specification accompanies the original application, then filing of the complete specification within 12 months from the date of filing of the provisional specification. The said period may be extended by a further period of 3 months by paying appropriate fee for extension.
- Overcoming objections, if any laid by the examiner after the technical examination of the application by the patent office.
- Acceptance of the application and advertisement of such acceptance in the official gazette.
- Overcoming opposition, if any, to the grant of a patent.
- Grant and sealing of the patent.
- Maintenance of patent by payment of renewal fee.
- Enforcement/revocation.

PATENT COOPERATION TREATY:

Patent Cooperation Treaty is the sister treaty of the Paris Convention, which is administered by the World Intellectual Property (WIPO). The PCT facilitates filing of patent applications under a single umbrella and provides for simplified procedure for the search and examination of such applications.

Under the Paris convention an inventor gets a grace period of 12-months to file a patent application in other member countries after filing in the home country. This period of grace is extended to 30-months under the PCT, whereby an inventor can file an "international patent application" in each of the PCT member countries within this prescribed period. In India a grace period of 31 months is granted for such "international patent application".

FILING PROCEDURE UNDER THE PCT:

1) International Phase

2) National Phase

1. International Phase

India being one of the contracting state in the "PCT", any Indian applicant may file an international

application in the standard format [Form "PCT" /RO/101] through any of the Indian Patent Offices as the

Receiving Office i.e. The Patent Office, Kolkata, and its branch Offices at New Delhi, Mumbai, Chennai

(RO/IN) along with the copy of Specification and Statutory Fees. Language of filing may be either in English or Hindi.

At the time of filing the said application the applicant is also required to mention the number of countries wherein eventually registration of patent is desired to be sought and also has to specify the name
of the International Search Authority and the International Preliminary Examination Authority.

The following are the documents that must accompany a PCT Application filed through an Indian
Patent office as the receiving office:

1. PCT Request (Form PCT/RO/101).
2. The complete specification in triplicate.
3. Power of Attorney
4. Certified priority Document.

On the receipt of the application, the patent office shall prepare a certified copy of the priority documents and transmit the same to the International Bureau and the International Search Authority with
intimation to the applicant. Thereafter the international search is conducted and the copy of the search report is also forwarded to the applicant.

2. National Phase

Once the formalities under step one are duly complied and the applicant receives the International
Search Report or once the Final International Preliminary Examination Report is complete and issued, the
application enters the National Phase.

Filing of National Phase Application in India requires the request for the grant of patent to be made
to the competent Receiving Office i.e. The Patent Office, Kolkata, and its branch Offices at New Delhi,
Mumbai, Chennai in the prescribed form i.e. Form 1A. Language of filing may be either in English or Hindi.

Further the following information/ documents are also required to be submitted along with the necessary fees with the Patent office:

1. Request (PCT/RO/101)
2. Drawings (where applicable)
3. P.A./G.P.A. (where applicable)
4. The Specification including drawing figures as published in the "PCT" Gazette;

5. Verified English translation of international application, if not in English;

6. International Search Report;

7. International Preliminary Examination Report (if India is elected for using the IPE results);

8. Certified Copies of the Priority Documents;

9. Particulars of amendments made to the specification/claims during the "PCT" International Phase;

10. Verified English translation of amendments filed during the international phase.

11. Such other information and documents that the patent office may require to be submitted.

----------BEST OF LUCK----------