BIOTECHNOLOGY AND GENETIC ENGINEERING

UNIT 1 BIOTECHNOLOGY

BY

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INTRODUCTION

This unit deals with an overview and introduction of biotechnology, societal perception and fundamental of biochemical engineering. Biotechnology developed in two phase viz., classical biotechnology (pre-vedic period to 1973) and modern biotechnology (1973 onward). The classical biotechnology was a kitchen technology. The modern biotechnology embraces several inter-disciplinary areas such as biology, physics, chemistry, mathematics, statistics, computer science, engineering, economics, management, laws, etc. It is hoped that biotechnology would be a boon to solve several problems to come in future when human population will reach from 6 to 10 billions by 2050. Secondly, people's participation in necessary for popularisation of biotechnology and providing guidelines for future research. The following single chapter covers the objectives of this unit:

SCOPE AND HISTORY OF BIOTECHNOLOGY

The term biotechnology was coined in 1917 by a Hungarian Engineer, **Karl Ereky**, to describe a process for large scale production of pigs. According to him all types of work are biotechnology by which products are produced from raw materials using living organisms. During the end of 20th century biotechnology emerged as a new discipline of biology integrating with technology; but the route of biotechnology lies in biology. There was no sudden sprout of this discipline, but some of the methods for production of products were developed centuries back. Therefore, biotechnology is concerned with exploitation of biological components for production of useful products. Biotechnology is defined by different organisations in different ways. It has been broadly defined as, "the development and utilization of biological processes, forms and systems for obtaining maximum benefits to man and other forms of life". Biotechnology is "the science of applied biological process" (*Biotechnology* : A Dutch Perspective, 1981). Following are some of the definitions given by other organisations :

These scientists are dev
against the HIV viruses

- *Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and service* [The Organisation for Economic Cooperation and Development (OECD), 1981].
- *The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological application of the capabilities of microorganisms, cultured tissue, cells, and parts their of* [The European Federation of Biotechnology (EFB), 1981; O'Sullivan, 1981].
- *The application of biochemistry, biology, microbiology and chemical engineering to industrial process and products and on environment* [International Union of Pure and Applied Chemistry (IUPAC), 1981.]
- Biotechnology is the "*controlled use of biological agents such as microorganisms or cellular components for beneficial use*" (U.S. National Science Federation).

In the definition given by OECD, "scientific and engineering principles" refer to microbiology, genetics, biochemistry, etc. and "biological agents" means microorganisms, enzymes, plant and animal cells. The meaning of these three definitions and others given by many organisations are more or less similar.

A unified definition of genetic engineering has been given by Smith (1996) as "*the formation of new combinations of heritable material by the insertion of nucleic acid molecules produced by whatever means outside the cell, into any virus, bacterial plasmid or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation*".

HISTORY OF BIOTECHNOLOGY

If we trace the origin of biotechnology, it is as old as human civilization. Development of biotechnology can be studied considering its growth that occurred in two phases: (*i*) the traditional (old) biotechnology, and (*ii*) the new (modern) biotechnology.

1. Traditional Biotechnology

The traditional biotechnology is really the kitchen technology developed by our ancestors using the fermenting bacteria. Kitchen technology is as old as human civilisation. During *vedic* period (5000–7000 BC), *Aryans* had been performing daily *Agnihotra* or *Yajna*. One of the materials used in Yajna is animal fat (i.e. *ghee*) which is a fermented product of milk. Similarly, the divine '*soma*' (a fermented microbial product used as beverage) had been offered to God. Summarians and Babylonians (6000 BC) were drinking the beer. Egyptians were baking leavened bread by 4000 BC. Preparation of curd, *ghee*, wine beer, vinegar, etc. was the kitchen technology. In spite of all development, preparation of curd, *ghee*, vinegar, alcoholic beverages, jalebi, idli, dosa, have become an art of the kitchen of all Indians (Table 1.1).

Brewing is one of the ancient methods of fermentation.

> Table 1.1 **Traditional fermented foods prepared in different parts of India**

Types of foods	*Substrate*	*Regions*	*Quality and uses*
Ambali	Millet and rice	South	Steamed cake used in snack
Bhatura	Wheat flour (*maida*)	North	Flat fried, lavened bread used with chhola
Chhurpi	Milk	Himalaya	Cheese like, mild sour, soft mass, used as curry
Dosa	Rice and black gram	South	Spongy, shallow fried, used as staple food
Dahi	Milk	North	Sour, thick gel with whey
Dhokla	Bengal-gram	West	Spongy cake used as snack
Gundrum	Leafy vegetable	Himalaya	Sun-dried, sour taste, used as-soup or pickles
Idli	Rice and black gram	South	Steamed spongy cake used in breakfast
Jalebi	Wheat flour (*maida*)	North	Crispy, deep-fried used as sweet confectionery
Khaman	Bengal gram	West	Spongy cake, used in breakfast
Khalpi	Cucumber	Himalaya	Sour pickles
Mesu	Bamboo shot	Himalaya	Sour pickles
Mishti dahi	Milk	East	Thick sweet gel
Nan	Wheat flour (*maida*)	North	Leavened flat baked bread used as staple food
Papad	Black gram flour (*besan*)	North	Circular wafers used as snack
Paneer	Milk	North	Soft cheese used as fried curry
Rabadi	Mixture of butter-milk-wheat/pearlmillet/barley	West	Cooked paste used as staple food
Srikhand	Milk	West	Concentrated sweetened preparation
Sinki	Radish tap root	Himalaya	Sun-dried sour soup pickles

Tari	Date palm	East	Sweet alcoholic beverage
Tharra	Mahua	North	Sweet alcoholic beverage obtained through distillation
Vadai (wada)	Black gram	North	Deep fired cake used as snack
Wari	Black gram	North	Spongy cake used as snack

The traditional biotechnology refers to the conventional technology which have been used for many centuries. Beer, wine, cheese and many foods have been produced using traditional biotechnology. Thus, the traditional biotechnology includes the process that are based on the natural capabilities of microorganisms. The traditional biotechnology has established a huge and expanding world market. In monetary term, it represents a major part of all biotechnology financial profits.

Who can forget the story of '*Makhan Chori*' (butter stealing) by Lord Sri Krishna during Mahabharat period ? Butter would have been produced following the same kitchen art. Besides breeding of strong productive animals, selection of desirable seeds for enhanced crop production has been the part of human activity since the time immemorial.

Role of Microorganisms in Fermentation: The causes of fermentation could be discovered after observing microorganisms using a microscope by Antony van Leeuwenhoek (1673–1723) at Delft (Holland). During 18th century a significant contribution was done by the chemists on the process and products of fermentation. In 1757, it was demonstrated that a milky precipitate could be obtained when the gas evolved from fermentation was passed through the lime water. It was called *lime water test*. A similar gas comes out from burning of charcoal. Henry Cavendish demonstrated that the gas evolved from brown sugar in water treated with yeast was absorbed by sodium hydroxide solution. Schele and J Priestley (1772–1774) confirmed the identity of oxygen gas present in air. Using analytical technique for carbon estimation, Antoine Lavoisier gave the chemical basis of alcoholic fermentation.

Antony von microscope.

A French man, Nichola Appert (1810) described the method of food preservation. In the same year Peter Durand also gave the use of tin container for food preservation. It was done by putting an air-tight vessel containing food material in the boiling water. It increased the importance of canning industry. Lack of oxygen in such a closed and heated vessel was reported by Gay Lussac. He concluded that oxygen was required for initiation of alcoholic fermentation, but not for further progress of fermentation. After 1830, Charles B. Astier gave the concept that *air is the carrier of all kinds of germs*.

In 1837, Theodore Schwann after a series of experimentation demonstrated that 'the development of the fungus (sugar fungus) on fruit juice causes fermentation'. He was the first to observe and describe the yeast in growing process. Charles Cagniard-Latour (1838) observed yeast budding using a microscope allowing 300–400 power magnification.

Justus von Liebig (1839), a well known chemist, proclaimed that all the activity of yeast cells was the result of chemical and physical reactions going on in the medium.

The study of microbiology was started since the first report of Louis Pasteur (1857) on lactic acid fermentation from sugar. He isolated the

Louis Pasteur

microorganisms (lactic yeast) that were associated with lactic acid and formed curd. The cells of lactic yeast were smaller than that of beer yeast. Lactic acid production got increased when he added chalk powder to fermentation medium. Pasteur showed the presence of lactic acid in curd by using a *polarimeter*.

In 1860, Pasteur provided a detailed report on the use of synthetic medium for microbiological studies. He concluded that:

(*i*) fermentation is carried out anaerobically by the living cells.

(*ii*) during alcoholic fermentation in synthetic medium, the yeast increased the weight with increase in C and N contents of overall batch. The increase in yeast protein in synthetic medium was accompanied by a related decrease in the ammonium nitrogen in the medium.

(*iii*) fermentation of sugar was required for multiplication of yeast cells.

(*iv*) similar phenomenon occurred in fermentation of lactic acid, tartaric acid, butyric acid, etc.

(*v*) because of not using pure culture, some of the fermentation processes were stopped.

(*vi*) growth and physiology of yeast differ when they are grown under aerobic and anaerobic conditions (it was later on called as '*Pasteur effect*'). Under anaerobic growth conditions a large amount of sugar was converted into alcohol, while under aerobic conditions large amount of sugar was converted into yeast cell mass.

MODERN BIOTECHNOLOGY

The two major features of technology differentiates the modern biotechnology from the classical biotechnology: (*i*) capability of science to change the genetic material for getting new products for specific requirement through recombinant DNA technology, and (*ii*) ownership of technology and its socio-political impact. Now the conventional industries, pharmaceutical industries, agro-industries, etc. are focusing their attention to produce biotechnology-based products.

The new or modern biotechnology embraces all methods of genetic modification by recombinant DNA and cell fusion technologies. It also includes the modern developments of traditional biotechnological processes. The new aspects of biotechnology founded in recent advancement of modern biology, genetic engineering and fermentation process technology are now increasingly find wide industrial application. But the rate of application will depend on: (*i*) adequate investment by the industries, (*ii*) improved system of biological patenting, (*iii*) marketing skill, (*iv*) economics of the new methods, and (*v*) public perception about the biotechnology products.

Canning process.

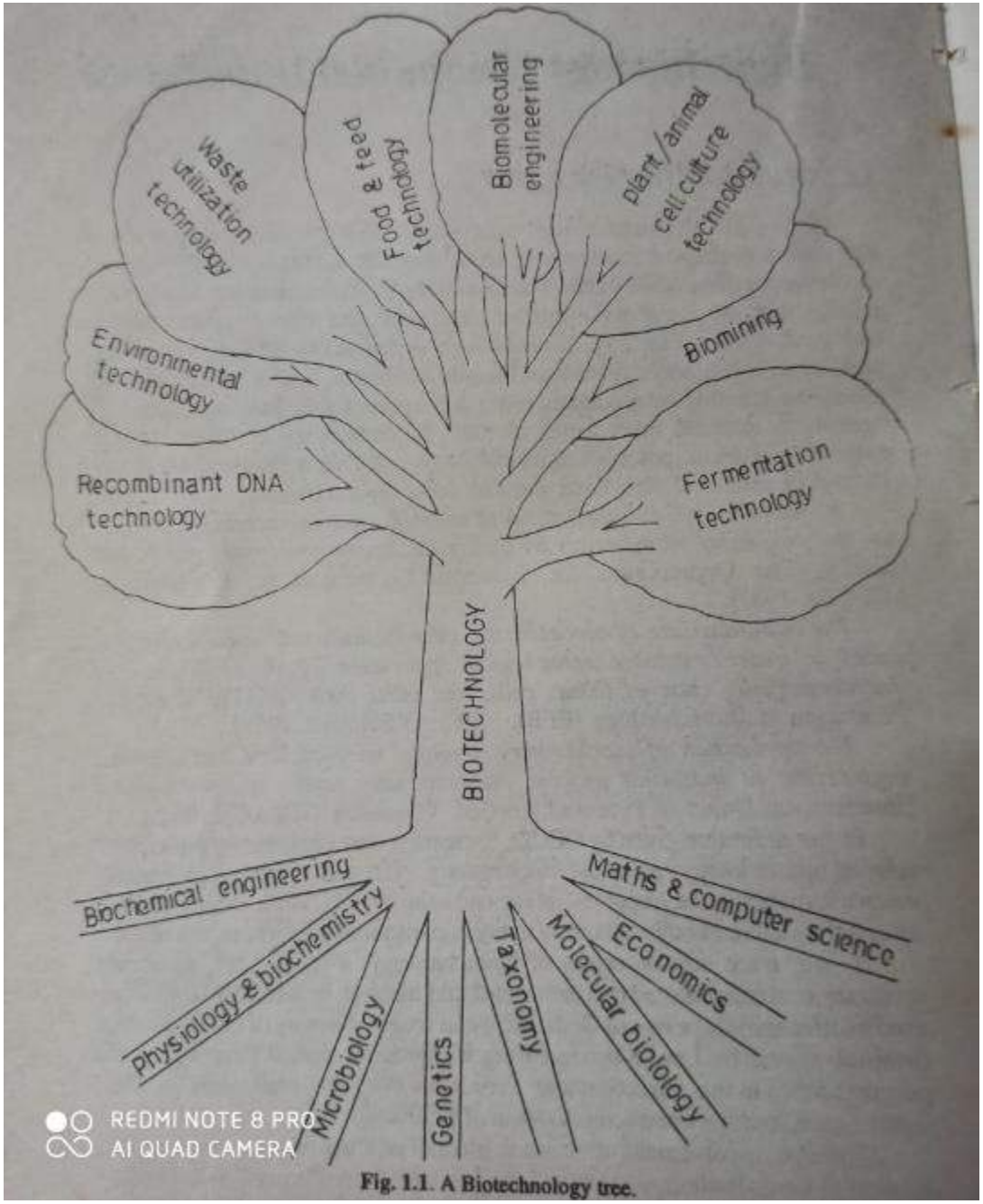


Fig. 1.1. A Biotechnology tree.

POTENTIALITIES AND CONSTRAINS OF BIOTECHNOLOGY

a) POTENTIALITIES

❖ **Increased crop productivity**

Biotechnology has helped to increase crop productivity by introducing such qualities as disease resistance and increased drought tolerance to the crops. , researchers can select genes for disease resistance from other species and transfer them to important crops.

❖ **Enhanced crop protection**

It is provide cost-effective solutions to pest problems crops such as corn, cotton, and potato have been successfully transformed through genetic engineering to make a protein that kills certain insects when they feed on the plants.

❖ **Improved nutritional value**

it has allowed new options for improving the nutritional value, flavor, and texture of foods.

Transgenic crops in development include soybeans with higher protein content, potatoes with more nutritionally available starch content and beans with more essential amino acids.

❖ **Better flavor**

Flavor can be altered by enhancing the activity of plant enzymes that transform aroma precursors into flavoring compounds. Transgenic peppers and melons with improved flavor are currently in field trials.

❖ **Fresher produce**

Genetic engineering can result in improved keeping properties to make transport of fresh produce easier, giving consumers access to nutritionally valuable whole foods and preventing decay, damage, and loss of nutrients

❖ **Environmental benefits**

When genetic engineering results in reduced pesticide dependence, we have less pesticide residues in foods, we reduce pesticide leaching into groundwater, and we minimize farm worker exposure to hazardous products.

b) CONSTRAINTS

The issues are catogorized as follows.

- Socio economic issues
- Cultural issues
- Legal issues
- Environmental issues and demerits
- Religious issues

Socio economic issues

- Scientific community assuring us that biotechnology is harmless, and promises marvelous advantages to humankind, even that it may be the key to our survival in an ever-changing world.
- On the other hand there exist a diverse array of arguments about the right of man to interfere in nature or God's process and the dangers to the environment, the food chain and ultimately

Cultural issues

- The ethics of biotechnology entails both a reflection on the immediate consequences of its use, and on the underlying social and cultural conditions of which it is a part. All technology modifies our relationship to our environment, to our work,and to ourselves, but biotechnology strikes much closer tohome, enabling us to modify life itself

Environmental issues

- Genetically engineered plants have also reduced the need of fertilizers thus minimized the pesticide pollution to rivers and costal waterresources.However the biotechnological tools and products caused many defects in environment. The benefits of a particular biotechnological intervention in the environment typically accrue directly to the sponsor, often a commercial interest However, the harms that may result from

Legal issues

- Legal issues are being arises in the use of biotechnological techniques. Particularly modern techniques such as stem cell technology, gene therapy, and human genome project have generated many issues in the societyand there is need to resolve them for the satisfaction of the person who is receiving treatment or getting benefit from these techniques

Religious issues

- Catholics criticize the Human stem cell research and embryo utilization and cnd consider this tool as immoral act.
- Human reproductive cloning is the production of a human fetus from a single cell by asexual reproduction.Human reproductive cloning has attracted more serious criticism from a number of religions, as it is seen as directly challenging the authority of God, and is tantamount to playing God.

FERMENTATION

- The word fermentation is derived from a latin verb

- This definition of fermentation had little meaning until the metabolic processes were known. In a micro-biological way, fermentation is defined as "any process for the production of useful products through mass culture of micro-organisms" whereas, in a biochemical sense, this word means the numerous oxidation - reduction reactions in which organic compounds, used as source of carbon and energy, act as acceptors or donors of hydrogen ions.

- The organic compounds used as substrate give rise to various products of fermentation, which accumulate in the growth medium Almost in all organisms metabolic pathways generating energy are fundamentally similar.

- In autophototrophs, (e.g. some bacteria, cyanobacteria and higher plants) ATP is generated as a result of photosynthetic electron transport mechanisms, whereas in chemotrophs the source of ATP is oxidation of organic compounds in the growth substrates.
- The oxidation reaction may be accomplished in the presence of oxygen (in aerobes) or in absence of oxygen (in anaerobes).Thus, in aerobic micro-organsim the process of ATP generation is referred to as cellular respiration, whereas in anaerobes or aerobes functioning under anaeorobic condition, it is known as anaerobic respiration or fermentation

- Although, fermentation (e.g. brewing and wine production) was done for many hundred years, yet during the end of 15th century, brewing became partially industrialized in Britain.

- Antony van Lecuwenhoek (1632-1723) developed method to observe yeasts and other micro-organsim under the microscope but this study could not be further strengthened. By early 19th century Cagniard - Latour and Schwann reported that the fermentation of wine and beer is accomplished by yeast cells.

- It was L. Pasteur who observed microorgansims associated with fermentation and causing many diseases in human beings. Detailed studies on

GENERAL REQUIREMENTS OF FERMENTATION PROCESS

- Most fermentations require liquid media, often referred to as broth; although some solid substrate fermentations (SSF) are operated.
- Fermentation media must satisfy all the nutritional requirements of the microorganism and fulfil the technical objectives of the process.
- All microorganisms require water, sources of energy, carbon, nitrogen, mineral elements and possibly vitamins plus oxygen if aerobic.
- The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific metabolite.
- In most industrial fermentation processes there are several stages where media are required. They may include several inoculum (starter culture) propagation steps, pilot scale fermentations and the main production fermentation. The technical objectives of inoculum propagation and the main fermentation are often very different, which may be reflected in differences in their media formulations.

Elemental composition

Element	Bacteria	Yeast	Fungi
Carbon	50-53	45-50	40-63
Hydrogen	7	7	7
Nitrogen	12-15	7.5-11	7-10
Phosphorus	2-3	0.8-2.6	0.4-4.5
Sulphur	0.2-1.0	0.01-0.24	0.1-0.5
Potassium	1.0-4.5	1-4	0.2-2.5
Sodium	0.5-1.0	0.01-0.1	0.02-0.5
Calcium	0.01-1.1	0.1-0.3	0.1-1.4
Magnesium	0.1-0.5	0.1-0.5	0.1-0.5
Chloride	0.5	--	--
Iron	0.02-0.2	0.01-0.5	0.1-0.2

CARBON SOURCE

- A carbon source is required for all biosynthesis leading to reproduction, product formation and cell maintenance. In most fermentations it also serves as the energy source.
- Molasses
- malted barley
- Starch and Dextrins
- Sulphite Waste Liquor
- Alkanes and Alcohols n-Alkanes
- Oils and fats

Factors influencing the carbon source

- Cost of the product
- rate at which it is metabolized
- geographical locations
- government regulations
- cellular yield coefficient

Nitrogen Sources

- Most industrial microbes can utilize both inorganic and organic nitrogen sources.
- Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and diammonium hydrogen phosphate, or ammonia. Ammonia can also be used to adjust pH of the fermentation.
- Organic nitrogen sources include amino acids, proteins and urea.
- Corn Steep Liquor
- Yeast Extracts
- Peptones
- Soya Bean Meal

Minerals

- All microorganisms require certain mineral elements for growth and metabolism. In many media, magnesium, phosphorous, potassium, sulphur, calcium and chlorine are essential components and must be added.
- Others such as cobalt, copper, iron, manganese, molybdenum and zinc are present in sufficient quantities in the water supplies and as impurities in other media ingredients.

Component	Range
*KH_2PO_4	1.0–4.0 (part may be as buffer)
$MgSO_4 \cdot 7H_2O$	0.25–3.0
KCl	0.5–12.0
$CaCO_3$	5.0–17.0
$FeSO_4 \cdot 4H_2O$	0.01–0.1
$ZnSO_4 \cdot 8H_2O$	0.1–1.0
$MnSO_4 \cdot H_2O$	0.01–0.1
$CuSO_4 \cdot 5H_2O$	0.003–0.01
$Na_2MoO_4 \cdot 2H_2O$	0.01–0.1

Chelators

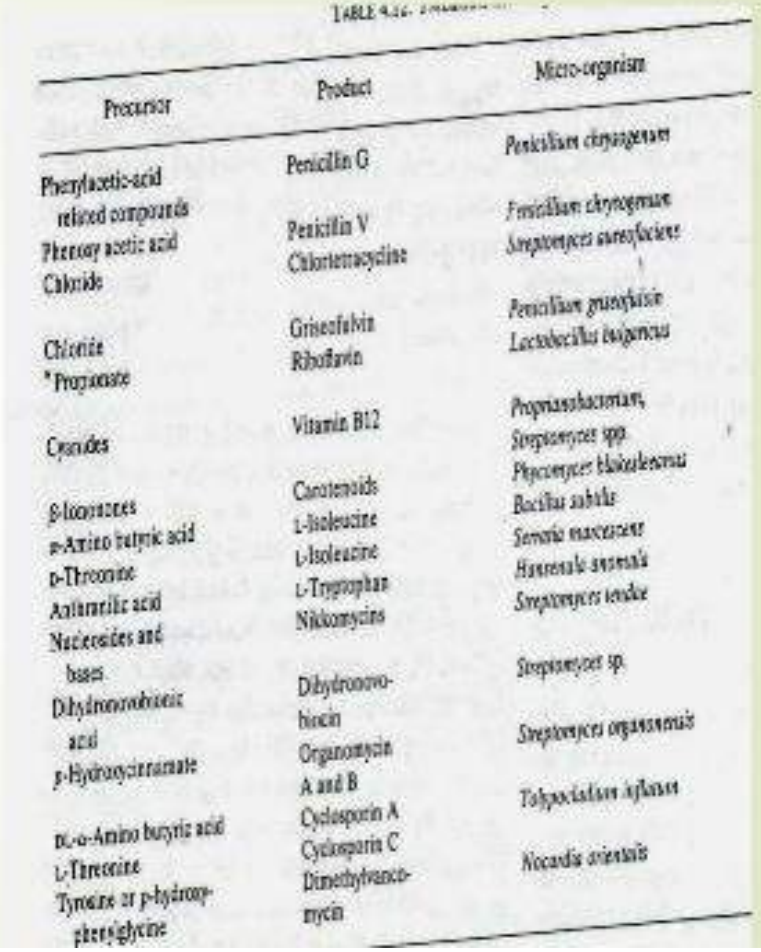
- Many media cannot be prepared without precipitation during autoclaving. Hence some chelating agents are added to form complexes with metal ions which are gradually utilised by microorganism
- Examples of chelators: EDTA, citric acid, polyphosphates etc.,
- It is important to check the concentration of chelators otherwise it may inhibit the growth.
- In many media these are added separately after autoclaving Or yeast extract, peptone complex with these metal ions

Vitamins and Growth Factors

- Many bacteria can synthesize all necessary vitamins from basic elements. For other bacteria, filamentous fungi and yeasts, they must be added as supplements to the fermentation medium.
- Most natural carbon and nitrogen sources also contain at least some of the required vitamins as minor contaminants

Precursors

- Precursors are defined as "substances added prior to or simultaneously with the fermentation which are incorporated without any major change into the molecule of the fermentation product and which generally serve to increase the yield or improve the quality of the product".
- They are required in certain industrial fermentations and are provided through crude nutritive constituents, e.g., corn steep liquor or by direct addition of more pure compounds.



Precursor	Product	Micro-organism
Phenylacetic-acid related compounds	Penicillin G	*Penicillium chrysogenum*
Phenoxy acetic acid	Penicillin V	*Penicillium chrysogenum*
Chloride	Chlortetracycline	*Streptomyces aureofaciens*
Chloride	Griseofulvin	*Penicillium griseofulvin*
* Propionate	Riboflavin	*Lactobacillus casperus*
Cyanides	Vitamin B12	*Propionibacterium*, *Streptomyces* spp.
β-Ionones	Carotenoids	*Phycomyces blakesleeanus*
α-Amino butyric acid	L-Isoleucine	*Bacillus subtilis*
D-Threonine	L-Isoleucine	*Serratia marcescens*
Anthranilic acid	L-Tryptophan	*Hansenula anomala*
Nucleosides and bases	Nikkomycins	*Streptomyces tendae*
Dihydroxynaphthoic acid	Dihydroxovo-bicin	*Streptomyces* sp.
p-Hydroxycinnamate	Organomycin	*Streptomyces organomycin*
DL-α-Amino butyric acid	A and B	*Tolypocladium inflatum*
L-Threonine	Cyclosporin A	*Nocardia orientalis*
Tyrosine or p-hydroxy-phenylglycine	Cyclosporin C	
	Dimethylvanco-mycin	

Inducers and Elicitors

- If product formation is dependent upon the presence of a specific inducer compound or a structural analogue, it must be incorporated into the culture medium or added at a specific point during the fermentation.
- The majority of enzymes of industrial interest are inducible. Inducers are often substrates such as starches or dextrins for amylase.
- In plant cell culture the production of secondary metabolites, such as flavanoids and terpenoids can be triggered by adding elicitors.

Enzyme	Inducer	Micro-organism
α-Amylase	Starch	*Aspergillus* spp.
	Maltose	*Bacillus subtilis*
Pullulanase	Maltose	*Aerobacter aerogenes*
α-Mannosidase	Yeast mannans	*Streptomyces griseus*
Penicillin acylase	Phenylacetic acid	*Escherichia coli*
Proteases	Various proteins	*Bacillus* spp.
		Streptococcus spp.
		Streptomyces spp.
		Aspergillus spp.
		Mucor spp.
Cellulase	Cellulose	*Trichoderma viride*
Pectinases	Pectin (beet pulp, apple pomace, citrus peel)	*Aspergillus* spp.
Nitrilase	Isovaleronitrile	*Rhodococcus rhodochrous*

Inhibitors

- Inhibitors are used to redirect metabolism towards the target product and reduce formation of other metabolic intermediates
- others halt a pathway at a certain point to prevent further metabolism of the target product.
- An example of an inhibitor specifically employed to redirect metabolism is sodium bisulphite

Product	Inhibitor	Main effect	Micro-organism
Glycerol	Sodium bisulphite	Acetaldehyde production repressed	*Saccharomyces cerivisiae*
Tetracycline	Bromide	Chlotetracycline formation repressed	*Streptomyces aureofaciens*
Glutamic acid	Penicillin	Cell wall permeability	*Micrococcus glutamicus*
Citric acid	Alkali metal/phosphate, pH below 2.0	Oxalic acid repressed	*Aspergillus niger*
Valine	Various inhibitors	Various effects with different inhibitors	*Brevibacterium roseum*
Rifamycin B	Di-ethyl barbiturate	Other rifamycins inhibited	*Nocardia mediterranei*
7-Chloro-6 demethyltetracycline	Ethionine	Affects one-carbon transfer reactions	*Streptomyces aureofaciens*

WATER

- All fermentation processes, except SSF, require vast quantities of water.
- Not only is water a major component of all media, but it is important for ancillary services like heating, cooling, cleaning and rinsing.
- A reliable source of large quantities of clean water, of consistent composition, is therefore essential.
- Assessing suitability of water
  - pH
  - dissolved salts
  - effluent contamination

Reuse of water is important

- It reduces water cost by 50%
- Effluent treatment cost by 10 fold

Oxygen

- Depending on the amount of oxygen required by the organism, it may be supplied in the form of air containing about 21% (v/v) oxygen or occasionally as pure oxygen when requirements are particularly high.
- The organism's oxygen requirements may vary widely depending upon the carbon source. For most fermentations the air or oxygen supply is filter sterilized prior to being injected into the fermenter.

Antifoams

- Antifoams are necessary to reduce foam formation during fermentation.
- Foaming is largely due to media proteins that become attached to the air-broth interface where they denature to form a stable foam "skin" that is not easily disrupted
- An ideal antifoam should have the following properties
  - Disperse readily and have fast action
  - Active at low concentrations
  - Long acting in preventing new foam
  - Should not be metabolized
  - Should not be toxic to m.o, humans etc
  - Cheap, should not cause problem in fermentation

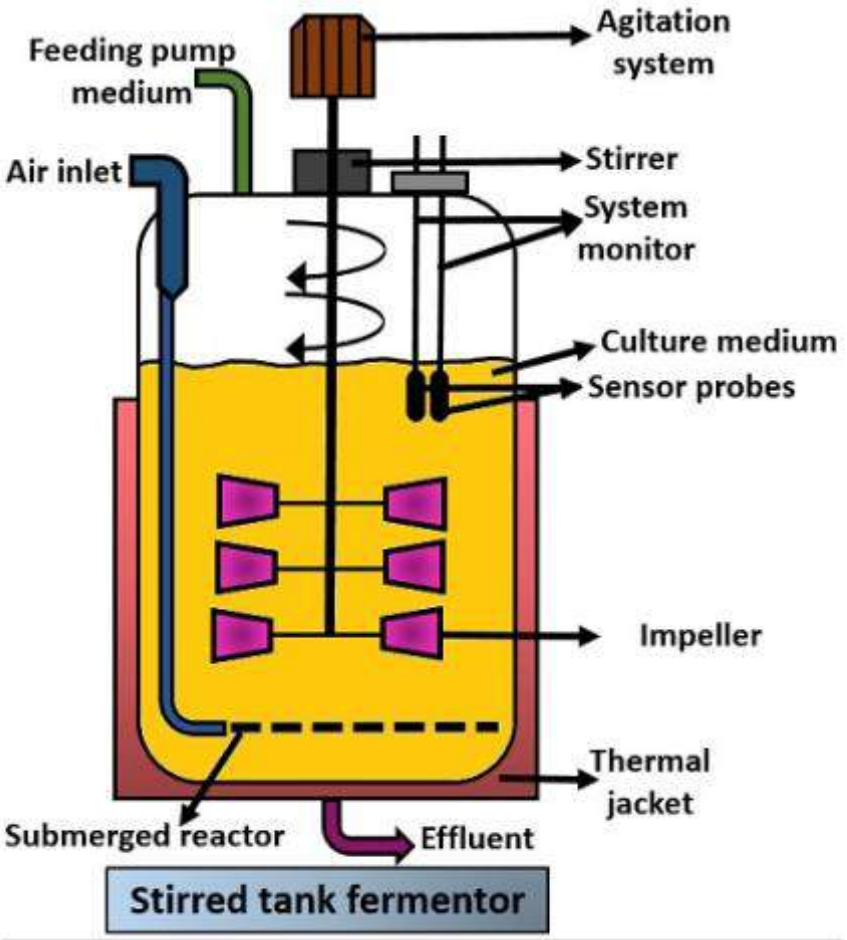
Types of Fermentor

A fermentor is mainly of 5 types, which includes:

- Stirred tank fermentor
- Airlift fermentor
- Fluidised bed fermentor
- Packed bed fermentor
- Photo fermentor

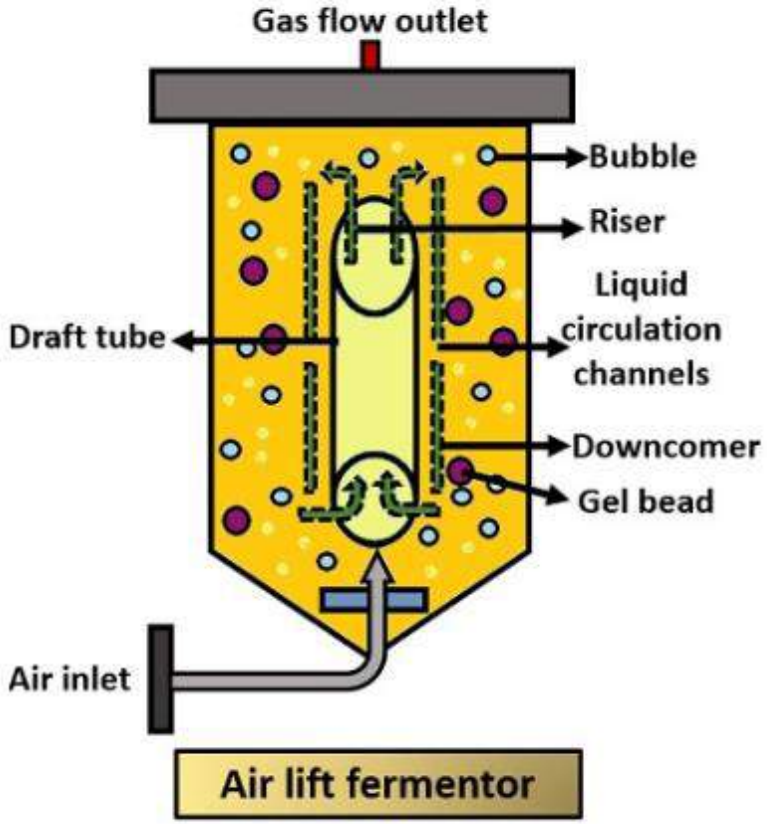
Stirred Tank Fermentor

- Motor driveshaft
- A variable number of impellers (more than one): The impellers have a 1/3rd diameter of the vessel.



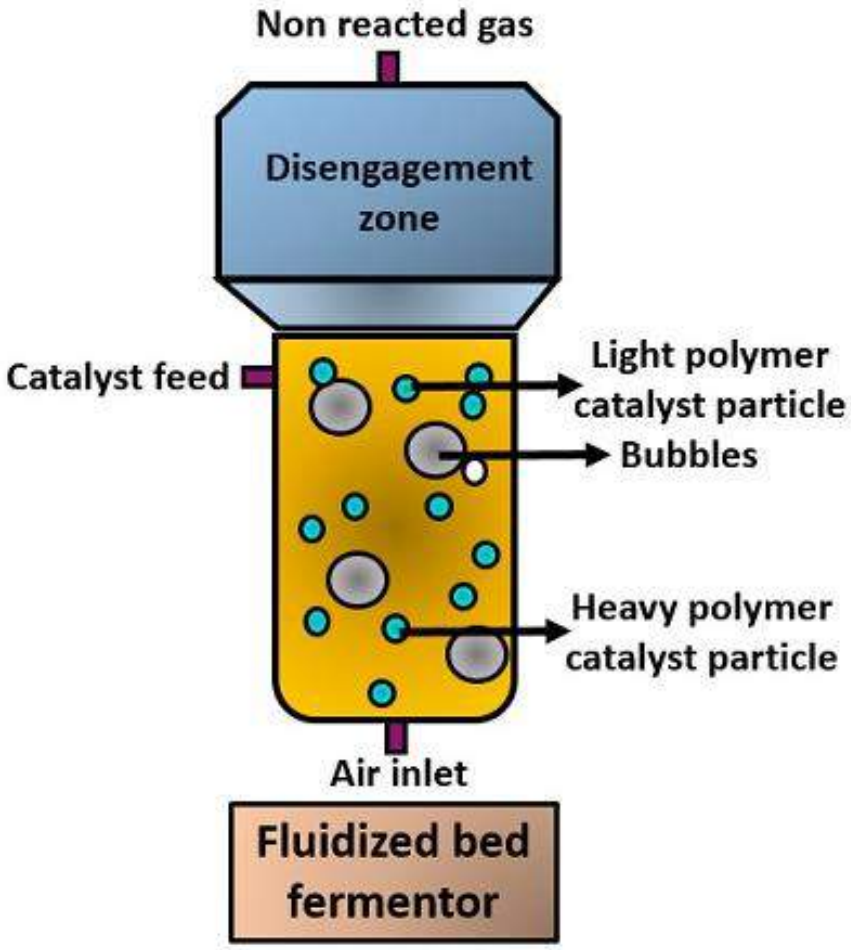
Airlift Fermentor

It consists of a single container inside which a hollow tube is present. This hollow tube refers to "**Draft tube**". There is a gas flow inlet present at the bottom of the fermentor allows the passage of oxygen. Gas flow inlet is attached with the perforated disc or tube that allows continuous distribution of air.



Fluidized Bed Fermentor

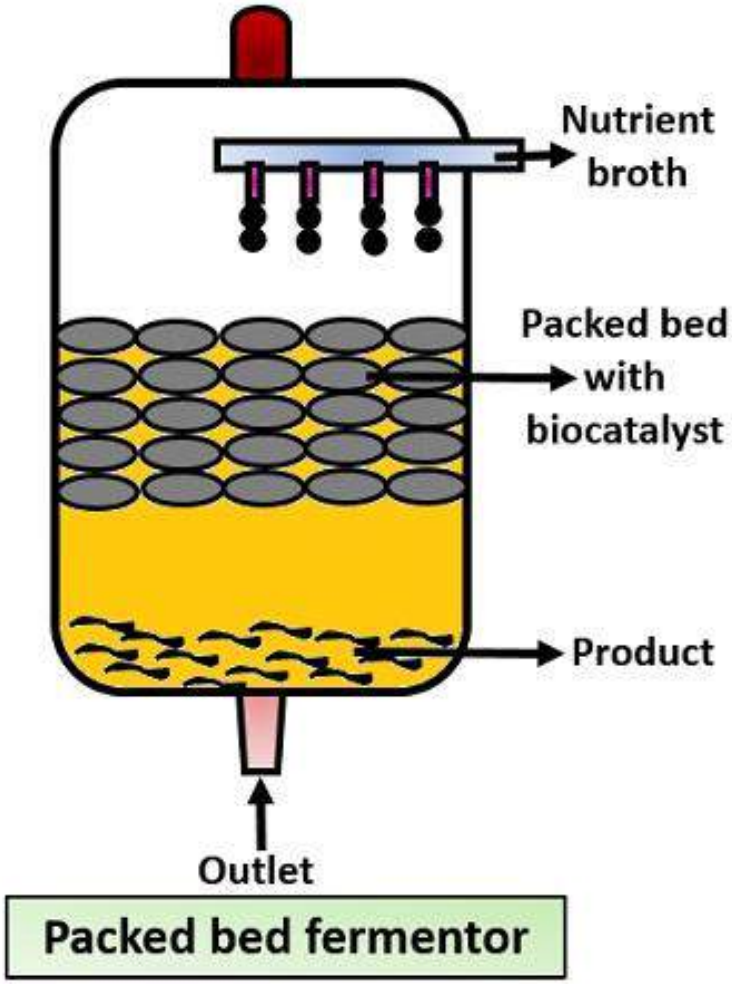
The top portion of this bioreactor is more expanded. This expansion reduces the velocity of the fluid. Its bottom part is slightly narrow.



Packed Bed Fermentor

It consists of:

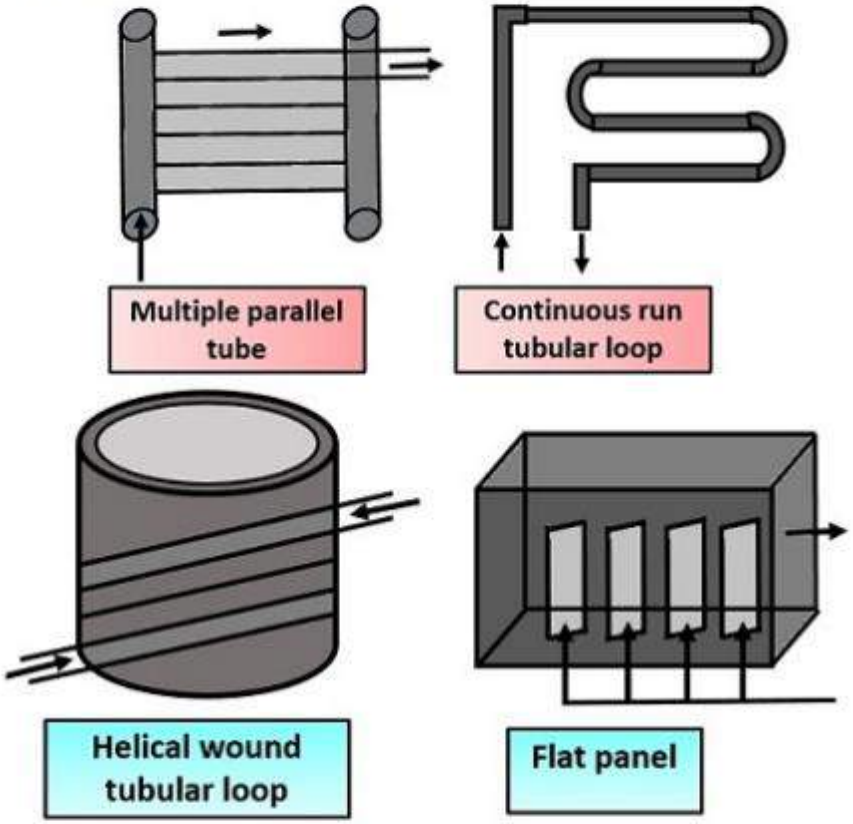
- A cylindrical vessel
- Bed of solid matrix packed with biocatalysts
- The solid matrix used for packed bed fermentor is generally:
- Porous or non-porous
- Highly compressible
- Rigid



In this kind of biofermentor, a nutrient broth continuously flows over the immobilized biocatalyst. After that, a product releases into the fluid at the bottom of the culture vessel and finally removed. In this bioreactor, a flow of fluid can be upward and downward.

Photo Fermentor

This fermentor works under the principle of light energy that involves direct exposure to the sunlight or through some artificial illumination. It uses widely for the production of p-Carotene, astaxanthin etc.



This type of bioreactor is basically made of glass or plastic. Photo fermentor consists of:

- A single container
- Number of tubes or panels

Algal Biotechnology

- Algal Biotechnology is "*the technological application of algae* (*both microalgae and macroalgae*) *or their derivatives to make or modify products or processes for specific use*". The phylogenetic diversity of the algae is also reflected in the diversity of habitats they can be found in, and their morphological, physiological and biochemical diversity. Algae already have wide application as sources of useful chemicals such as polysaccharides, carotenoids, phycobilin pigments, and long-chain polyunsaturated fatty acids. They have also found application in the food and feed industries, as fertilizers and growth promoters in agriculture, and in wastewater treatment
- Recently algae, especially the microalgae, are receiving renewed interest as potential sources of renewable fuels. The search for new products from new species as well new or improved applications continues. There are new developments in algae culture, harvesting and processing, and developments in molecular biology, metabolomics and the other 'omics' are creating opportunities for algal biotechnology.

Biotechnological importance of algae:

o Algae as renewable energy source; Chlorella, Dunaliella, Gracilaria and Sargassum produce fuels like diesel, gasoline, methane, butanol, ethanol and aviation fuel.

o They can grow on land or water (arid/saline/alkaline/marshy) unsuitable for crop cultivation.

o They scavenge green house gases and can be used for carbon dioxide mitigation.

o Algae are cheap source for waste water treatment and biogas production.

o Genetically engineered algae are used to enhance biofuel production and as source of protein and vitamin rich food and fodder.

o Algae are used as biofertilizer for crops as rich source of nitrogen, phosphorous, potassium, iodine, iron, calcium, silica and vitamins.

o Algae have been recommended for pesticide and heavy metal bioremediation.

o Algae are used in formation of biosolar cells.

o Algae as food; Alaria, Laminaria, Sargassum, Porphyra is popular as food in Japan and Europe.

o Algal storage materials like starch, gelatin and lipids are used as gelling agents in jellies, ice-creams, confectioneries and bacteriological media.

o Algae have therapeutic importance; Chlorellin from Chlorella is broad spectrum antibiotic.

o Algal pigments have antioxidant properties and therefore used in formulation of age proofing cosmetics.

WHY Spirulina ????

- **Protein**: Spirulina contains unusually high amounts of protein, between 55 and 70 percent by dry weight, depending upon the source

- It is a complete protein, containing all essential amino acids, though with reduced amounts of methionine, cystine, and lysine.

The Nutrition Facts of Spirulina 100g	
Moisture content	6-7g
Protein	60-70g
Fatty Acids	4-5g
Carbohydrate	15-18g
Chlorophyll	1-2g
Mixed Carotenoids	350-450mg
Beta Carotene	180-190mg
Phycocyanin	8-12g
GLA	1-2g
Calcium	400-600mg
Iron	50-100mg
Potassium	200-2000mg
Magnesium	200-300mg
Zinc	1-2.0mg
Vitamin A	100-200mg
Vitamin B1	1.5-4.0mg
Vitamin B2	3.0-5.0mg
Vitamin B6	0.5-0.7mg
Vitamin B12	0.05-0.2mg
Vitamin E	5.0-20mg

;ATIONS

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ls, crude fibers, etc.

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food-

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3. In therapeutic and natural medicines-

- Reduce body weight, cholesterol, stress.
- Lowers blood sugar level in diabetic(due to presence of B - linolenic acid)
- Prevents accumulation of cholesterol in body.
- Healthy eyes and skin (beta carotene)
- Beta carotene (anti cancer substance- UN National Cancer Research Institute)
- Increase lactation.

4. In cosmetics-

- Important role in maintaining healthy hair (vitamin A and B).
- Many herbal beauty products.
- Biolipstics and herbal face cream(Phycocyanin).
- Capable of replacing coal tar dye based cosmetics.

5. Poultry and cattle feed-

- Excellent, convenient source of protein and other nutrients.
- Used to feed cattle, fishes etc.

BIO DIESEL

- The potential use of microalgae as feedstock for biodiesel production has been receiving increased interest in recent years.
- It is advantageous to use microalgae for biodiesel production compared to other crop plants because it will not compromise production of food, fodder and other feedstocks derived from those crops.

Comparing Potential Biofuel Crops

Oil Producer	Fuel Production [kg/(ha year)]	Energetic Equivalent [kWh/(ha year)]
Oil palm	3,600–4,000	33,900–37,700
Jatropha	2,100–2,800	19,800–26,400
Tung oil tree (China)	1,800–2,700	17,000–25,500
Sugarcane	2,450	16,000
Castor oil plant	1,200–2,000	11,300–18,900
Cassava	1,020	6,600
Microalgae	91,000	956,000

BIOTECHNOLOGICAL APPLICATIONS OF MACRO ALGAE

•ALGAE AS FOOD & FODDER

- Algae species are used as food in several countries and in several forms.
- Algae species have proteins, vitamins(A, B, C and E), lipids and minerals.
- Laminaria species is the important edible seaweed in japan and the food item (kombu) is prepared from it.

- Aonori from Monostroma; Asakusa Nori from Porphyra are prepared in different countries. Porphyra has 35% protein, 45% carbohydrates,vitamins B & C.

- Nostoc is used as food material in south America.

- Many seaweeds such as Fucus, Laminaria, Ascophyllum and Sargassum are usedd as fodder.

- Rhodymenia palmata is used as food for sheep in narvey.

- Laminaria saccharina, Pelvitia, Ascophyllum,etc. species are used as food for cattle.

ALGAE IN INDUSTRY

- Many products of commercial and pharmaceutical importance have been derived from algae.
- ***Agar-Agar:-***
- Agar is obtained commercially from species of Gelidium, Gracilaria and condrus.
- Japan and South East Asia are the main production centers of Agar.
- The greatest use of agar is in food, Pharmaceutical and cosmetic industry.
- It is used for almost a century as stiffening agent in culture media.

•Carrageenan:-

- Carrageenan is obtained from the cell walls of Chondrus crispus and Gigartina stellata.
- Carrageenan is used in stabilisation of emulsions in paints and cosmetics. In alcohol and sugar industry it is used as a clearing agent.
- It is also utilised in the textile, leather and brewing industries.

•ALGINATE

- These are salts of algainic acid which occur in the cell wall of the brown algae belonging to the order Laminariales.
- Alginate are non-toxic and viscous and readily form gel, useful as thickner, emulsifier and gelling agent.
- Flame proof fabrics are also prepared from alginates.

•ALGAE AS BIOFERTILIZERS

- Many algae increase the water holding capacity besides the addition of their chemical constituent in the soil.

- In India, Turbinaria is used around palm tree while as sea weeds are used as compost.

- The species of Nostoc, Syctonema, Aulosira, Lyngobya, Microcoleus, Aphanothece, Anabaena, etc. Most of these can fix atmospheric nitrogen and increase the soil fertility.

- Due to their mucilaginous sheath, they are able to prevent soil erosion by binding the soil particles firmly.

- Blue-green algae are treated as bio-fertilizers from olden days.
- Nostoc, Oscillatoria, scytonema, Spirulina, etc. are used as fertilizers to rice fields.
- Cultivation of Spirulina is gaining importance as feed for fish, poultry and cattle.

Vesicular Arbuscular Mycorrhiza (VAM)

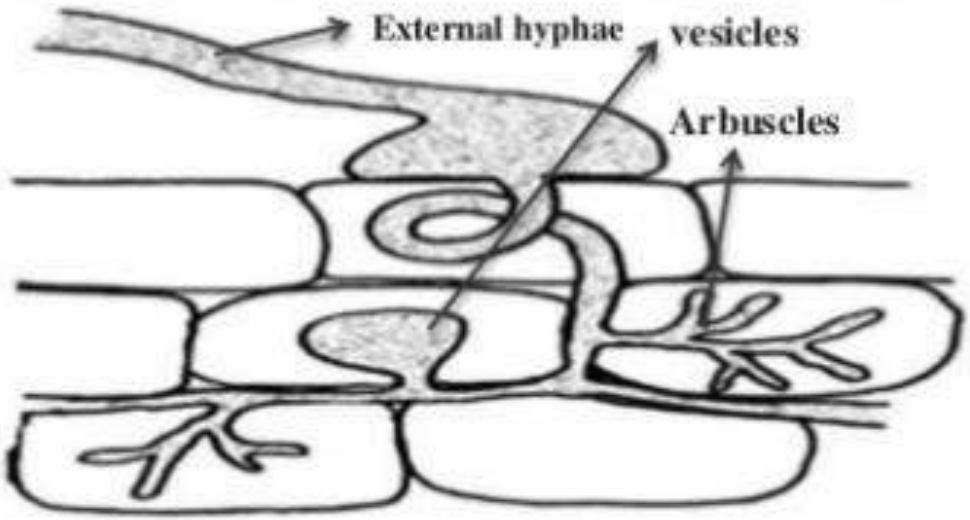
- The term mycorrhiza was taken from Greek language meaning **'fungus root'**. term was coined by Frank in 1885
- The mycorrhiza is a mutualistic association between fungal mycelia and plant roots.
- VAM is an endotrophic (live inside) mycorrhiza formed by aseptated phycomycetous fungi.
- VAM help in **nutrient transfer** mainly of phosphorus, zinc and sulfur.

- Mycorrhizae is the symbiotic association between plant roots and soil fungus of the 7 types of mycorrhizae,
- VAM plays a great role in inducing plant growth.
- VAM are symbiotic entophytic soil fungi, which colonize the roots of approximately 80% plants.
- The VAM hyphae also help is retaining moisture around the root zone of plants
- It increases the resistance to root borne or soil borne pathogens and Nematodes.

- They also **mobilize different nutrients** like Cu(copper), K(potassium), Al(aluminum), Mn(manganese), Fe (iron)and Mg (magnesium) from the soil to the plant roots.
- They posses vesicles (sac like structure) for storage of nutrients and arbuscular for funneling them into root system.

Morphology

- ✓ **External hyphae**
- ✓ **Arbuscles**
- ✓ **Vesicles**



Mechanism of Action

- The VAM forms an association with plant roots.
- It **penetrates in the root cortex** and spreads around the roots of the plant.
- As the name indicates, they posses sac like structure called **vesicules** which stores phosphorus as phospholipids.
- The other structure called arbuscule helps bringing the distant nutrients to the vesicules and root.

Mass production

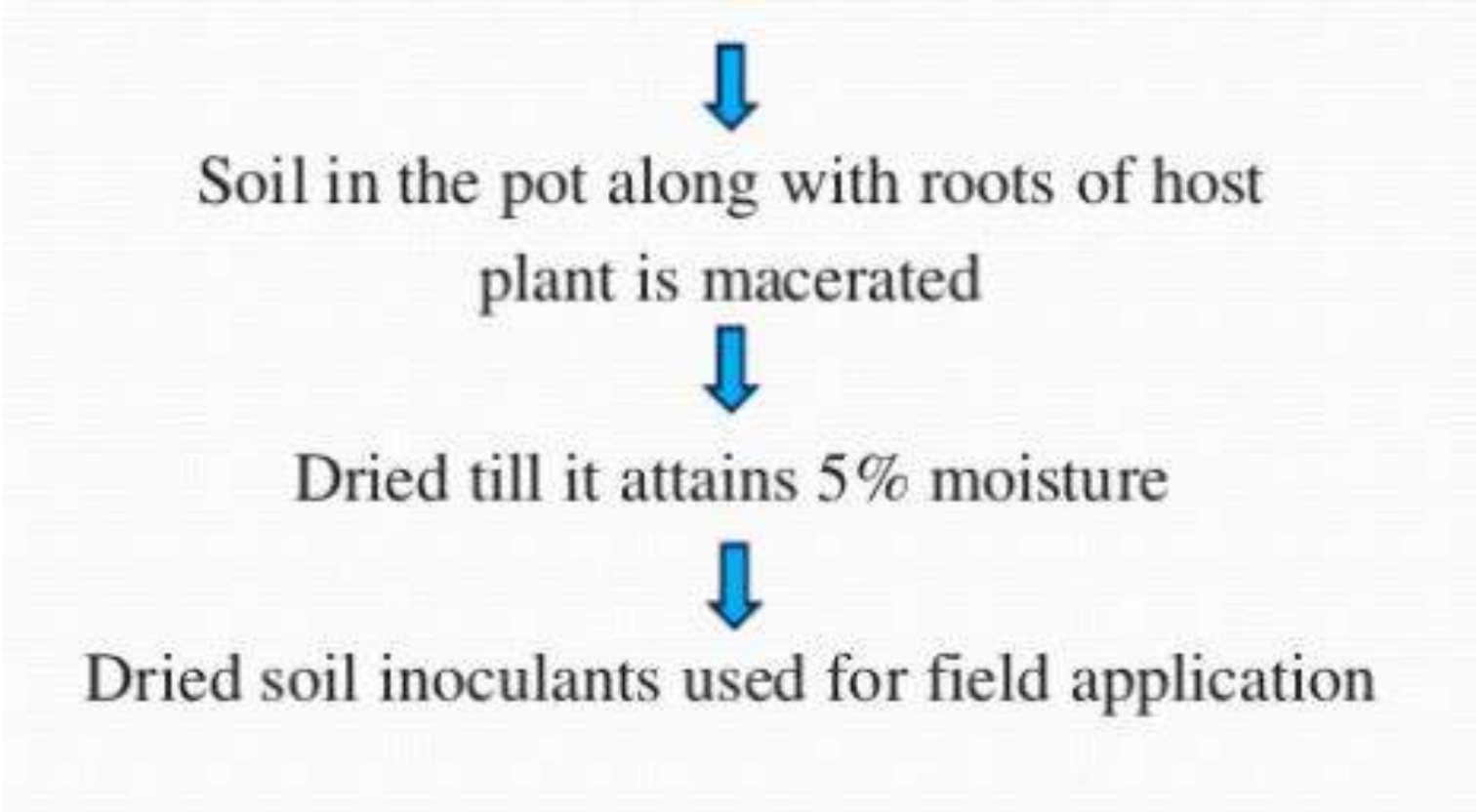
VAM spores isolated

Spores mixed with sterilized soil

Soil filled in pots

Host plant transplanted in pots

Kept 3-4 months in green house


Soil in the pot along with roots of host plant is macerated
Dried till it attains 5% moisture
Dried soil inoculants used for field application

Uses of VAM

- Enhances the feeding areas of the plant root is as the hyphae spreads around the roots.
- Mobilizes the nutrients from distantance to root.
- Stores the nutrients (sp. phosphorus).
- Removes the toxic chemicals (example : phenolics) which otherwise hinder nutrient availability.
- Provide protection against other fungi and nematodes
- It increase growth rate in plants (*citrus, maize, wheat, etc.*)
- It reduces sensitivity of crop towards high level of salts and heavy metals

Unit 2

Introduction

- Enzymes are macromolecular biological catalysts.
- Enzymes accelerate, or catalyze, chemical reactions.
- The molecules at the beginning of the process are called substrates and the enzyme converts these into different molecules, called products.
- Microbial enzymes are the biological catalysts for the biochemical reactions leading to microbial growth and respiration, as well as to the formation of fermentation products.

PROTEIN STRUCTURE

Scaffold to support and position active site

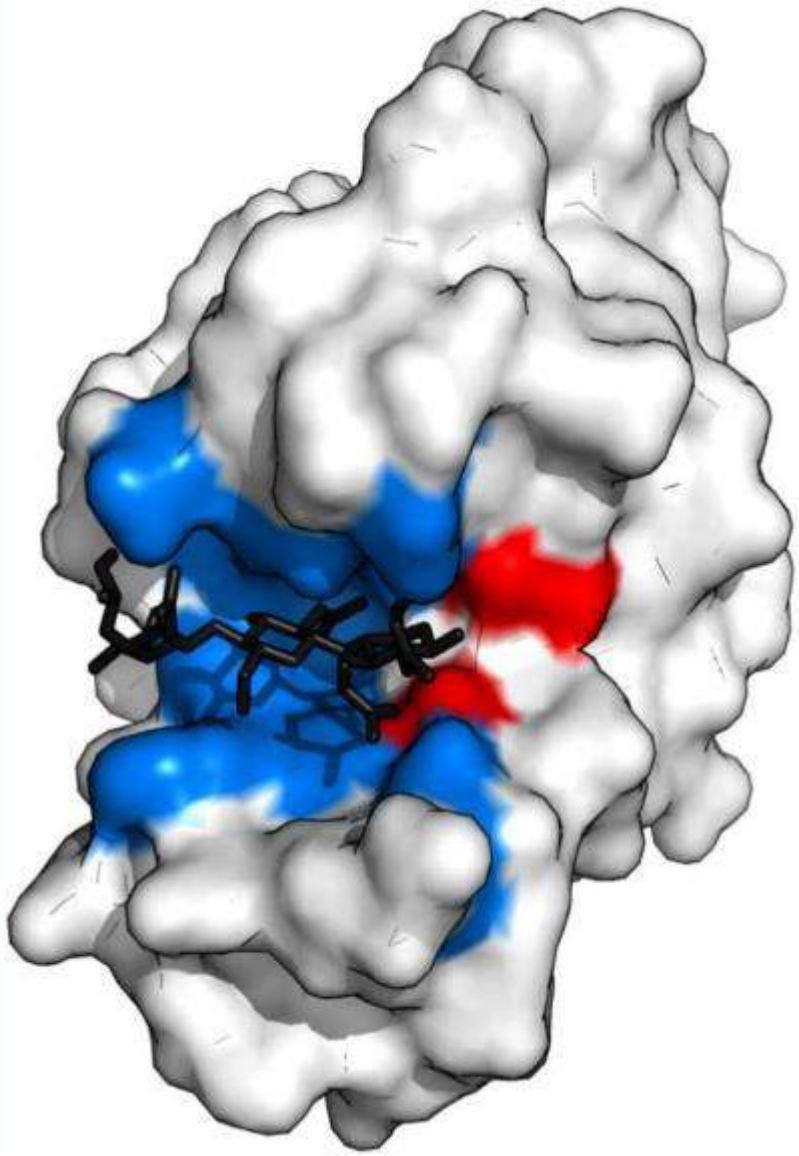
ACTIVE SITE

BINDING SITES

Bind and orient substrate(s)

CATALYTIC SITE

Reduce chemical activation energy



Types of Enzymes

- Produced only when the need arises
  Eg. When a cell is deficient of a particular nutrient.

- Produced always irrespective the amount of substrate.

History

- The first enzyme produced industrially was the fungal amylase Takadiastase which was employed as a pharmaceutical agent for digestive disorders.
- By 1969, 80% of all laundry detergents contained enzymes, chiefly Proteases.
- Due to the occurrence of allergies among the production workers and consumers, the sale of such enzyme utilizing detergents decreased drastically.

- Special techniques like micro-encapsulation of these enzymes were developed which could provide dustless protease preparation. It was thus made risk free for production workers and consumers.
- Microbial rennin is also one of the most significant enzymes. It has been used instead of Calf's rennin in cheese production.

Location of Enzymes

- Enzymes which are produced within the cell or at the cytoplasmic membrane are called as Endocellular enzymes.
- Enzymes which are liberated in the fermentation medium which can attack large polymeric substances are termed as Exocellular enzymes. Eg: Amylases & Proteases

Improved Prospects of Enzyme Application

- Microbial Genetics – High yields can be obtained by Genetic manipulation.
  Example – Hansenula polymorpha has been genetically modified so that 35% of it's total protein consists of the enzyme alcohol oxidase.
- Optimization of fermentation conditions (Use of low cost nutrients, optimal utilization of components in nutrient solution, temperature and pH)

- New cell breaking methods like Homogenizer, Bead mill, Sonication etc
- Modern purification processes like Counter current distribution, Ion-exchange chromatography, Molecular-sieve chromatography, Affinity chromatography and precipitation by using alcohol, acetone.
- Immobilization of enzymes
- Continuous enzyme production in special reactors.

Methods of Enzyme Production

Semisolid Culture

Submerged Culture

Semisolid Culture

The sterilized medium is spread on metal trays upto a depth of 1-10 centimeters.

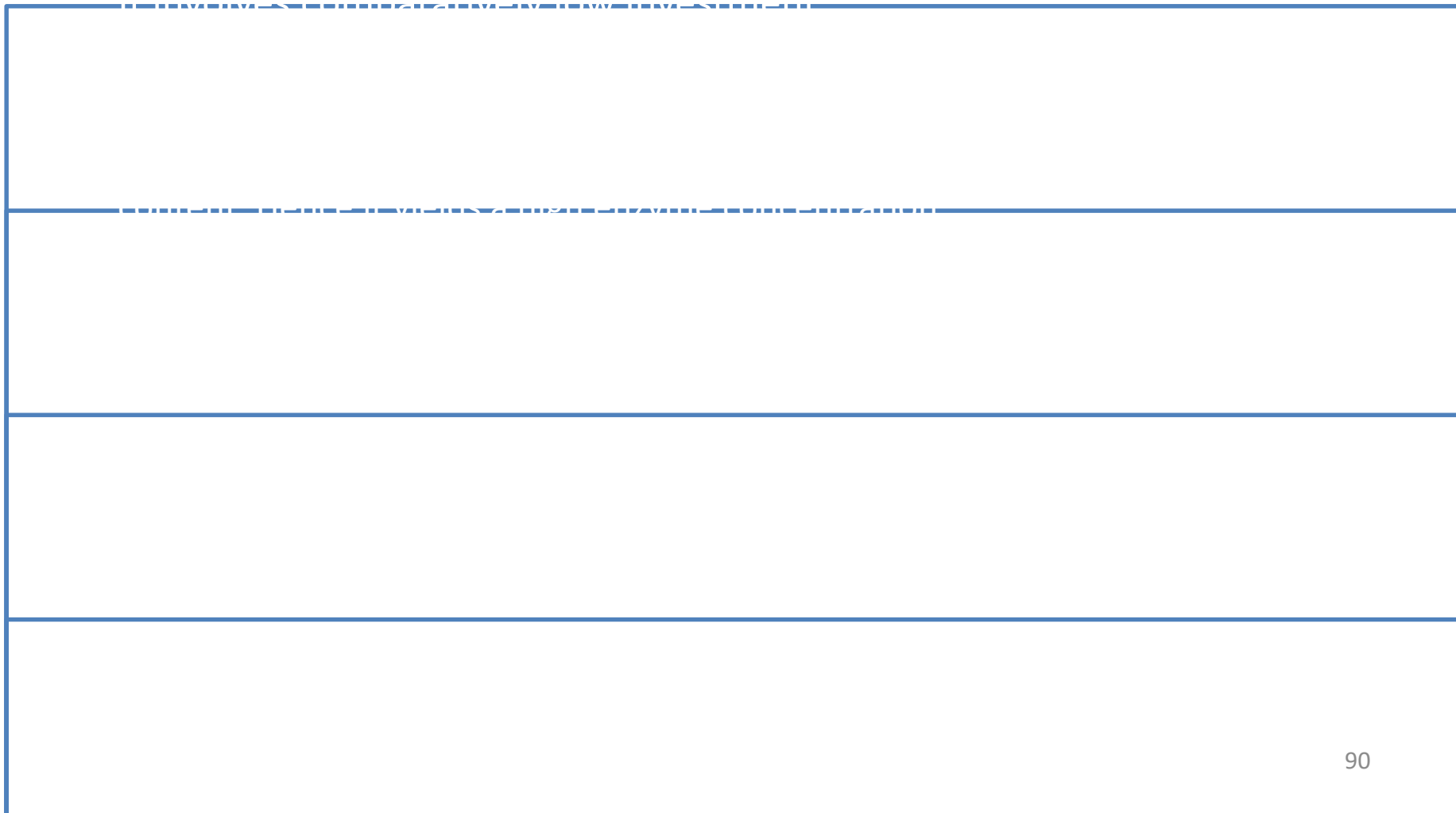
Culture is inoculated either in the autoclave a cooling or in trays.

High enzyme concentration in a crude fermen material.

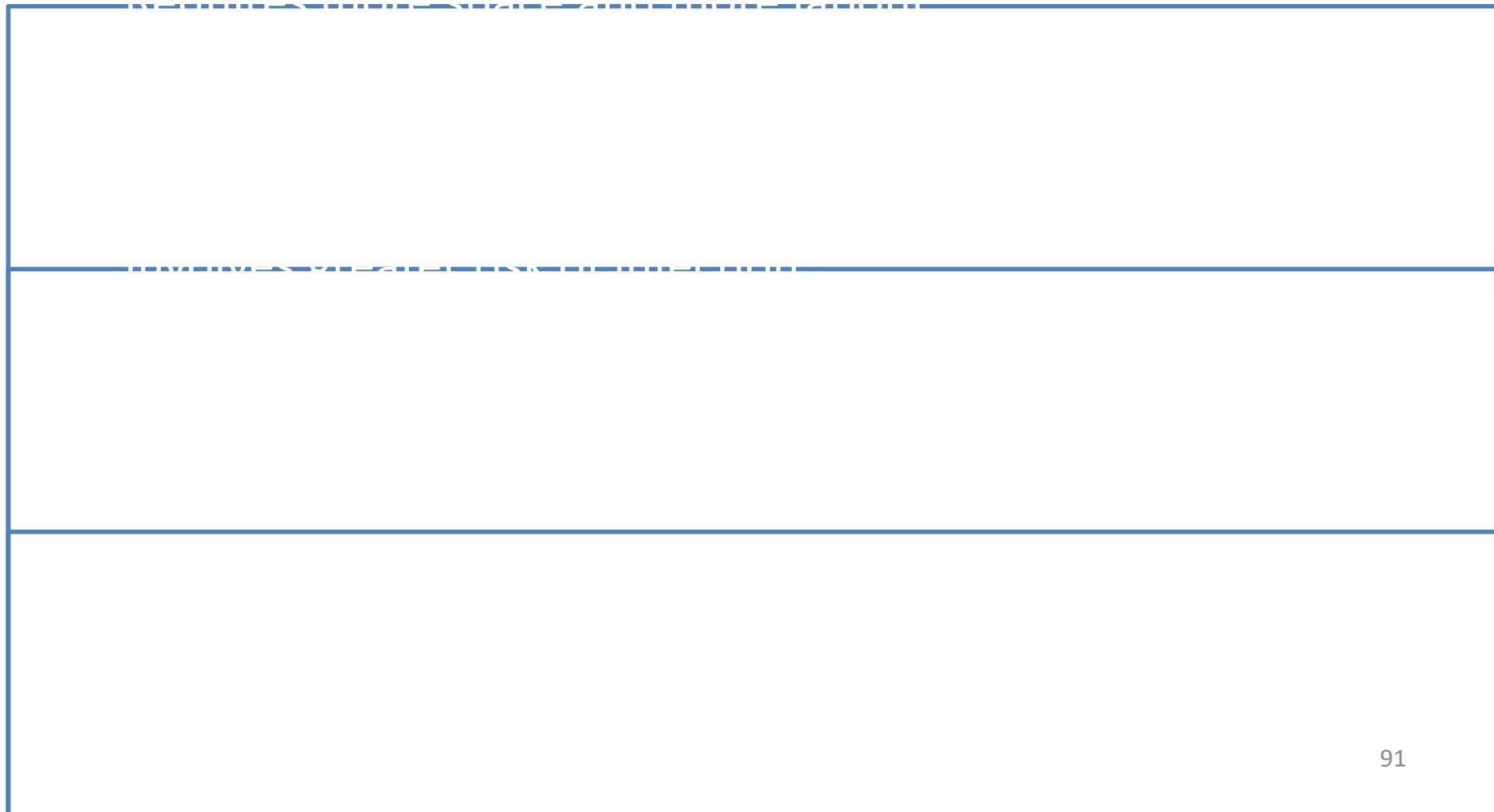
Enzymes produced by Semi-solid culture

Enzyme	Micro-organisms
α- Amylase	Aspergillus oryzae
Glucoamylase	Rhizopus spp.
Lactase	A. oryzae
Pectinase	A. niger
Protease	A. Niger & A. oryzae
Rennet	Mucor pusillus

Advantages of Semi-solid culture



Disadvantages of Semi-solid culture



Submerged Culture

- Fermentation equipment used is the same as in the manufacture of antibiotics.
- It's a cylindrical tank of stainless steel and it is equipped with an agitator, an aerating device, a cooling system and various ancillary equipment (Foam control, pH monitoring device, temperature, oxygen tension etc)
- Good growth is not enough to obtain a higher enzyme yield.

- Presence of inhibitors or inducers should also be checked in the medium.

Example – Presence of Lactose induces the production of β- galactosidase.

- As the inducers are expensive, constitutive mutants are used which do not require an inducer.
- Glucose represses the formation of some enzymes (α-amylases). Thus the glucose concentration is kept low.
- Either the glucose can be supplied in an incremental manner or a slow metabolizable sugar (Lactose or metabolized starch)

- Certain surfactants in the production medium increases the yield of certain enzymes.
- Non- ionic detergents (eg. Tween 80, Triton) are frequently used.

Advantages of Submerged culture

Requires less labor and space

Low risk of infection Automation is

easier

Disadvantage of Submerged Culture

- Initial investment cost is very high.

After fermentation

- Once fermentation is finished, the fermented liquor is subjected to rapid cooling to about 5^{o} C in order to reduce deterioration.
- Separation of micro-organisms is accomplished either by filtration or by centrifugation of the refrigerated broth with adjusted pH.
- To obtain a higher purity of the enzyme, it is precipitated with acetone, alcohols or inorganic salts (ammonium or sodium sulfate).
- In case of large scale operations, salts are preferred to solvents because of explosion hazards.

AMYLASE

Introduction

- Amylase is an enzyme that catalyses the hydrolysis of starch into sugars.
- Present in the saliva of humans
- Hydrolysis of Starch with amylase will first result in the formation of a short polymer Dextrin and then the disaccharide Maltose and finally glucose.
- Glucose is not as sweet as Fructose. Thus the next step would be the conversion of Glucose to Fructose by the enzyme Glucose isomerase.

Types of Amylases

α- Amylase

ß- Amylase

γ- Amylase

α- Amylase

- Also called as 1,4-α-D-glucan glucanohydrolase.
- Calcium metalloenzymes which cannot function in absence of calcium ions.
- Breaks down long carbohydrate chains of Amylose and Amylopectin.
- Amylose is broken down to yield maltotriose and Maltose molecules.
- Amylopectin is broken down to yield Limit dextrin and glucose molecules.

- Found in saliva and pancreas.
- Found in plants, fungi (ascomycetes and basidiomycetes) and bacteria (*Bacillus*)
- Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase.
- In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0

ß- Amylase

- Also called as 1,4-α-D-glucan maltohydrolase.
- Synthesized by bacteria, fungi, and plants.
- Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time.
- During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit.
- The optimum pH for β-amylase is 4.0–5.0

γ- Amylase

- Also termed as Glucan 1,4-α-glucosidase.
- Cleaves α(1–6) glycosidic linkages, as well as the last α(1–4) glycosidic linkages at the nonreducing end of amylose and amylopectin, yielding glucose.
- The γ-amylase has most acidic optimum pH of all amylases because it is most active around pH 3.

Effects of α-Amylases

- Break down the starch polymer but does not give free sugar

- Gives free sugars

Producing strains

- Bacteria – B. cereus, B.subtilis, B. amyloliquefaciens, B. polymyxa, B. licheniformis etc
- Fungi – Aspergillus oryzae, Aspergillus niger, Penicillum, Cephalosporin, Mucor, Candida eetc.

Applications

- Production of sweeteners for the food industry.
- Removal of starch sizing from woven cloth
- Liquefaction of starch pastes which are formed during the heating steps in the manufacture of corn and chocolate syrups.
- Production of bread and removal of food spots in the dry cleaning industry where amylase works in conjunction with protease enzymes

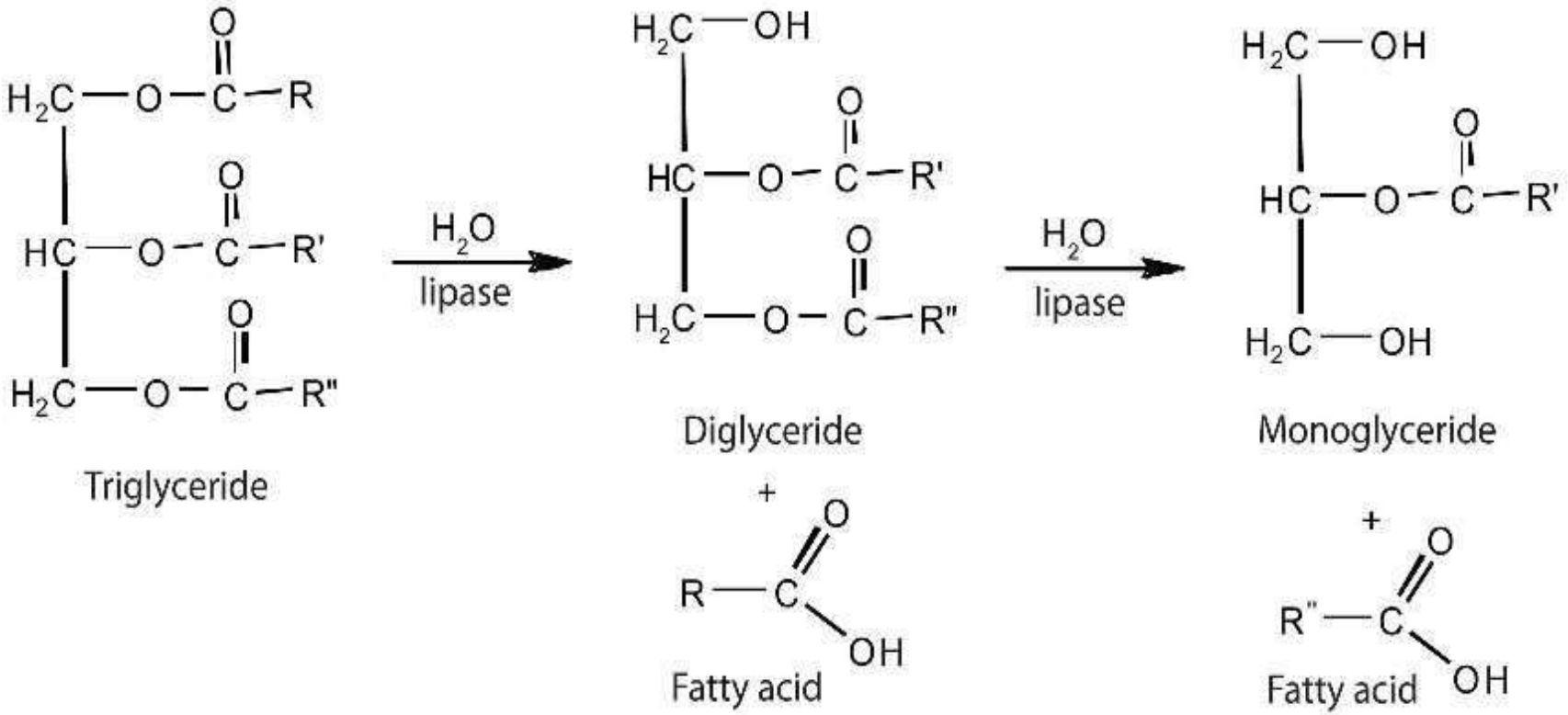
LIPASES

Introduction

- Lipases are also called as Glycerol ester hydrolases
- They are a subclass of esterases
- It splits fats into mono or di- glycerides and fatty acids.
- They are extracellular enzymes
- Mainly produced by Fungi
  Eg: Aspergillus, Mucor, Rhizopus, Peniciilum etc

- Bacteria producing lipases include species of Pseudomonas, Achromobacter and Staphylococcus.
- Yeasts like Torulopsis and Candida are also commercially used.

Mode of Action



- Enzyme production must be induced by adding oils and fats.
- But in some cases the fats have effect on the lipase production.
- Glycerol, a product of lipases action, inhibits lipase formation.
- Lipases are generally bound to the cells and hence inhibit an overproduction but addition of a cation such as magnesium ion liberates the lipase and leads to a higher enzyme titer in the production medium.

Applications

- Primarily marketed for therapeutic purposes as digestive enzymes to supplement pancreatic lipases.
- Since free fatty acids affect the odor and taste of cheese, and the cheese ripening process is affected by lipases, microbial affects during the aging process can be due to lipase action.
- In the soap industry, lipases from Candida cylindraceae is used to hydrolyze oils.

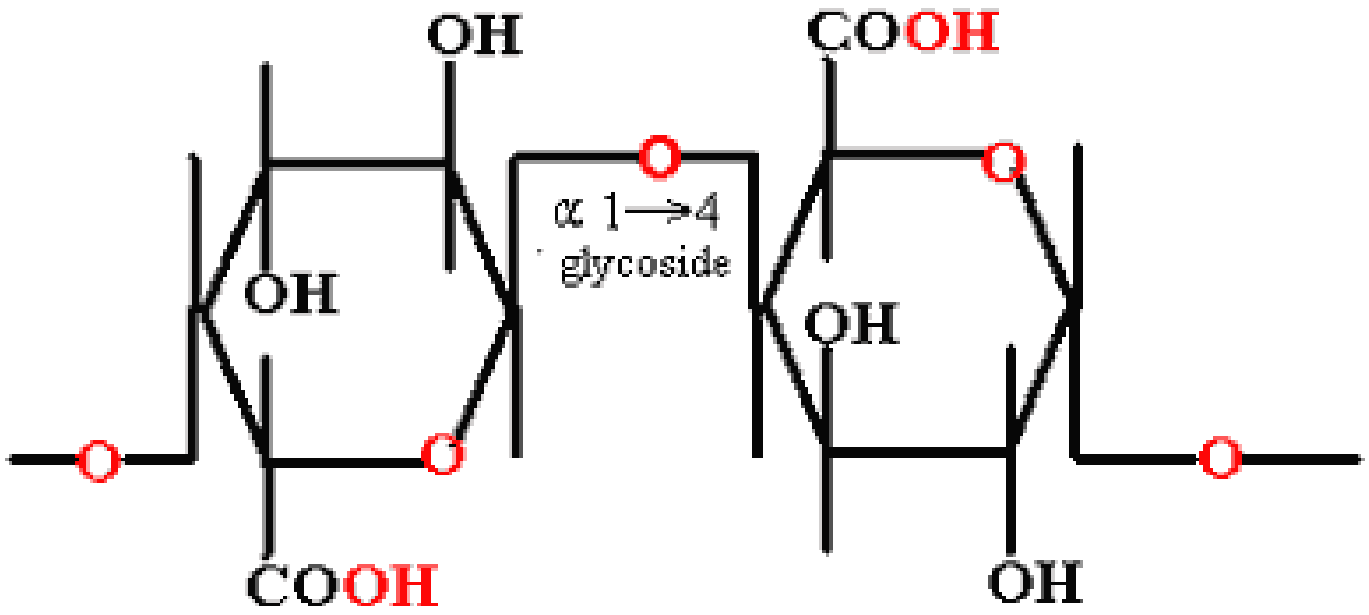
Pectinases

Introduction

- Pectinase is an enzyme that breaks down pectin, a polysaccharide found in plant cell walls.
- Pectic enzymes include Pectolyase, Pectozyme and Polygalacturonase.
- Pectin is the jelly-like matrix which helps cement plant cells together and in which other cell wall components, such as cellulose fibrils, are embedded.

- Basic structure of a pectin consists of α-1,4 linked Galactouronic acid with upto 95% of it's carboxyl groups esterified with methanol.
- Pectinase might typically be activated at 45 to 55 °C and work well at a pH of 3.0 to 6.5.

Mode of Action



Basic Unit of pectin: poly[α-(1>4)-D-galacturonic acid]. Blocks of this simple polymer alternate with "hairy," non-gelling regions containing side-chains with other unusual sugars

Production Strains

- Aspergillus niger, A. wentii, Rhizopus etc
- Fermentation with Aspergillus Niger runs for 60-80 hours in fed batch cultures at pH 3-4 and 37^{o} C using 2% sucrose and 2% pectin.

Applications

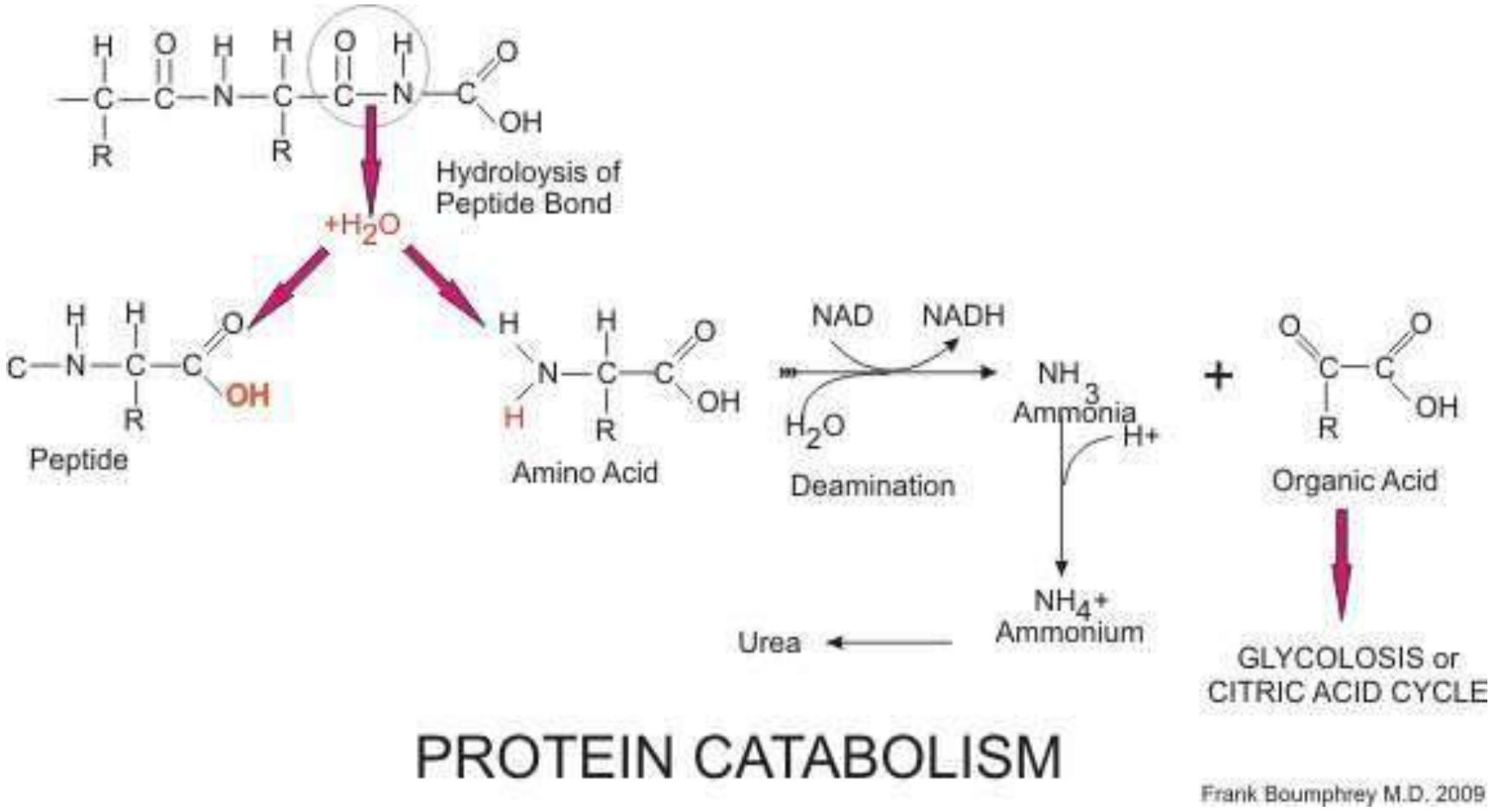
- Pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples.
- Pectinases have also been used in wine production since the 1960s
- Helps to clarify fruit juices and grape must, for the maceration of vegetables and fruits and for the extraction of olive oil.
- By treatment with pectinase, the yield of fruit juice during pressing is considerably increased.

Proteases

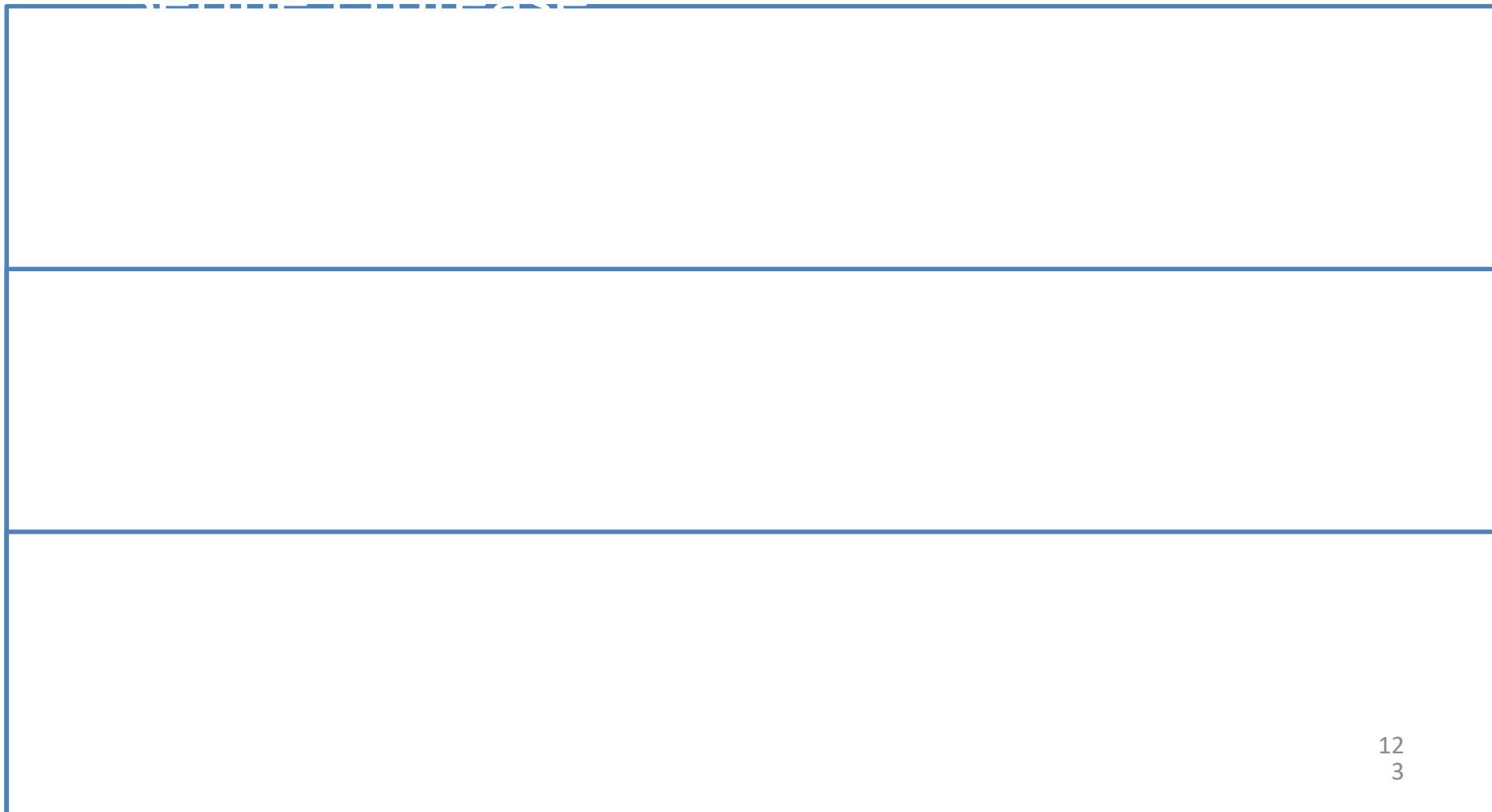
Introduction

- Protease (Mixture of Peptidases and Proteinases) are enzymes that perform the hydrolysis of Peptide bonds.
- Peptide bonds links the amino acids to give the final structure of a protein.
- Proteinases are extracellular and Peptidases are endocellular.
- Second most important enzyme produced on a large scale after Amylase

Mode of Action



Classification Based upon the residues in the Catalytic site



Classification Based upon the pH in which the Proteases are Active

Alkaline serine Proteases

Acid Proteases Neutral

Proteases

Alkaline Serine Proteases

- pH of the production medium is kept at 7.0 for satisfactory results.
- Have serine at the active site
- Optimum temperature maintained is 30^{o} to 40^{o} C.
- Important producers are B. licheniformis, B. amyloliquefaciens, B. firmus, B. megaterium, Streptomyces griseus, S. fradiae, S. rectus and fungi like A. niger, A. oryzae, A.flavus.

- Enzymes used in detergents are chiefly proteases from bacillus strains (Bacillopeptidases)
- Best known proteases are Subtilisin Carlsberg from B. licheniformis and Subtilisin BPN and Subtilisin Novo from B. amyloliquefaciens.
- These enzymes are not inhibited by EDTA (Ethylene diamine tetraacetic acid) but are inhibited by DFP (Di isopropyl fluorophosphate)

Proteases for the Use in Detergent industries

- Stability at high temperature
- Stability in alkaline range (pH- 9 to 11)
- Stability in association with chelating agents and perborates
- But shelf life is affected in presence of surface active agents.

Screening

- Because the enzymes should be stable in alkaline conditions, screening for better producers is done by using highly alkaline media.
- It was found that B. licheniformis and B. subtilis showed growth is the range of pH 6-7 by new strains were found to grow even in pH 10-11.
- Genetic Manipulation can also be carried out.

Fermentation Process

- To prepare a suitable encapsulated product, a wet paste of enzyme is melted at 50-70^{o} C with a hydrophobic substance such as polyethylene glycol and then converted into tiny particles.

Neutral Proteases

- They are relatively unstable and calcium, sodium and chloride must be added for maximal stability.
- Not stable at higher temperatures
- Producing organisms are B. subtilis, B. megaterium etc
- They are quickly inactivated by alkaline proteases.

Acid Proteases

- Similar to Mammalian pepsin
- It consists of Rennin like proteases from fungi which are chiefly used in cheese production
- They are used in medicine, in the digestion of soy protein for soya sauce production and to break down wheat gluten in the baking industry

Applications

- Textile industry to remove proteinaceous sizing.
- Silk industry to liberate silk fibers from naturally occurring proteinaceous material in which they are embedded.
- Tenderizing of Meat
- Used in detergent and food industries.

CONTENTS:

- Introduction
- Preparation of Immobilized Enzymes:
    - Methods of Irreversible Enzyme Immobilization
        a. Formation of Covalent Bonds
        b. Entrapment
    - Methods of Reversible Enzyme Immobilization
        a. Adsorption (Non Covalent Interaction)
        b. Chelation or Metal Binding
        c. Formation of Disulfide Bonds
- Applications of Immobilized Enzymes
    a. General Principles
    b. Enzyme Utilization in Industry

Introduction:

- Enzymes- biological catalysts- promote chemical reactions in living organisms
- Have the ability – catalyze reactions under very mild conditions with- high degree of substrate specificity- thus decreasing the formation of by-products
- Enzymes – can catalyze reactions in different states- individual molecules in solution, in aggregates with other entities and as attached to surfaces

- Attached- or “immobilized”- state has been of particular interest
- “immobilized enzyme” refers to “enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously”

Technological properties of Immobilized enzymes:

Advantages	Disadvantages
• Catalyst Reuse • Easier Reactor Operation • Easier Product Separation • Wider choice of Reactor	• Loss or reduction in activity • Diffusional limitations • Additional Cost

- The first industrial use of immobilized enzymes -1967 by Chibata and co-workers - developed the immobilization of *Aspergillus oryzae* aminoacylase for the resolution of synthetic racemic D - L amino acids
- In some industrial processes, whole microbial cells containing the desired enzyme are immobilized and used as catalysts

Major Products Obtained using Immobilized Enzymes:

Enzyme	Product
• Glucose isomerase	• High-fructose corn syrup
• Amino acid acylase	• Amino acid production
• Penicillin acylase	• Semi-synthetic penicillins
• Nitrile hydratase	• Acrylamide
• β-Galactosidase	• Hydrolyzed lactose (whey)

PREPARATION OF IMMOBILIZED ENZYMES

- Enzymes can be attached to a support via interactions ranging from reversible physical adsorption and ionic linkages to stable covalent bonds
- Ways of Immobilizing enzymes in two broad categories: irreversible and reversible methods
- The strength of the binding -inversely related to the ease with which it can be reversed
- Two conflicting objectives—stability and reversibility—are difficult to fulfill simultaneously
- The traditional approach has been to make the bond as strong as possible and sacrifice reversibility.

Methods of Irreversible Enzyme Immobilization:

- Irreversible immobilization- once the biocatalyst is attached to the support - cannot be detached without destroying either the biological activity of the enzyme or the support
- The most common procedures of irreversible enzyme immobilization are covalent coupling, entrapment or micro-encapsulation, and cross-linking

Formation of Covalent Bonds:

- An advantage of these methods -because of the stable nature of the bonds formed between enzyme and matrix- the enzyme - not released into the solution upon use
- A simple procedure -improves the activity - coupling reaction in the presence of substrate analogs
- Covalent methods for immobilization are employed when there is a strict requirement for the absence of the enzyme in the product.

- Wide variety of reactions -developed depending on the functional groups available on the matrix
- Coupling methods -divided in two main classes: (1) activation of the matrix by addition of a reactive function to a polymer and (2) modification of the polymer backbone to produce an activated group
- However, because of the covalent nature of the bond, the matrix has to be discarded together with the enzyme once the enzymatic activity decays

- The benefit of obtaining a leak-proof binding between enzyme and matrix resulting from these reactions is partially offset by the cost, in terms of generally low yield of immobilized activity and by the nonreversible character of this binding
- Enzymes attached covalently by disulfide bonds to solid supports represent one way to avoid this problem.

Covalent Coupling Methods of Enzymes: Activation of Matrix Hydroxyl Functions:

Activation Method	Group that reacts (with activated matrix)
• Tresyl chloride, sulfonyl chloride	• Thiol, amines
• Cyanogen bromide	• Amine
• Bisoxiranes (epoxides)	• Thiol, amine
• Epichlorohydrin	• Thiol, amine
• Glutaraaldehyde	• Amine
• Glycidol-Glyoxyl	• Amine
• N-Hydroxy-succinimidyl	• Amine

Entrapment

- The entrapment method -occlusion of an enzyme within a polymeric network that allows the substrate and products to pass through but retains the enzyme
- This method differs from the coupling methods described above-enzyme is not bound to the matrix or membrane
- There are different approaches to entrapping enzymes such as gel or fiber entrapping and micro-encapsulation.
- The practical use of these methods is limited by mass transfer limitations through membranes or gel

Methods of Reversible Immobilization:

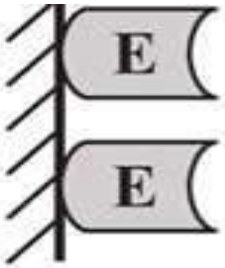
- Use of reversible methods for enzyme immobilization- **highly attractive** - mostly for economic reasons because - when the enzymatic activity decays the support can be regenerated and re-loaded with fresh enzyme
- Indeed, cost of the support - often **a primary factor** in the overall cost of immobilized catalyst
- Reversible immobilization of enzymes - important for immobilizing labile enzymes and for applications in bioanalytical systems

Adsorption (Non Covalent Interactions)

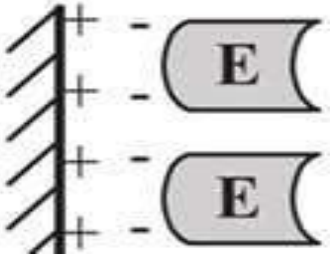
:

- Ionic Adsorption:

Adsorption



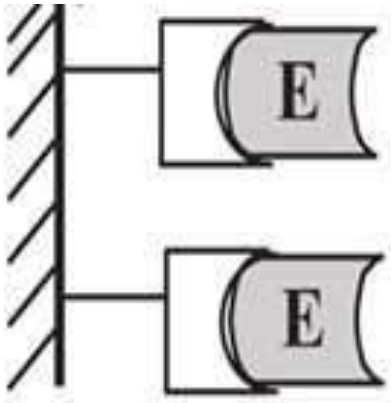
Ionic binding



- Problems - arise from the use of a highly charged support when the substrates or products themselves are charged; the kinetics are distorted as a result of partition or diffusion phenomena
- Therefore, enzyme properties, such as pH optimum or pH stability - may change
- Although this could pose a problem - it could also be useful to shift the optimal conditions of a certain enzyme towards more alkaline or acidic conditions - depending on the application

- Affinity Adsorption: principle of affinity between complementary biomolecules has been applied to enzyme immobilization
- Selectivity of the interaction is a major benefit of the method
- However - procedure often requires the covalent binding – of costly affinity ligand (e.g. antibody or lectin) to the matrix

Affinity binding



Chelation or Metal Binding:

- Transition metal salts or hydroxides deposited on the surface of organic carriers become bound by coordination with nucleophilic groups on the matrix
- Mainly titanium and zirconium salts have been used and the method is known as "**metal link immobilization**"
- Metal salt or hydroxide is precipitated onto support by heating or neutralization

- The bound proteins can be easily eluted by competition with soluble ligands or by decreasing pH
- Support is regenerated by washing with a strong chelator such as Ethylene Diamine Tetraacetic Acid (EDTA)
- These metal chelated supports – named as – Immobilized Metal-ion Affinity(IMA) adsorbents
- Different IMA-gels as supports for enzyme immobilization – has been studied using E.coli beta galactosidase as a model

Formation of Di-sulfide Bonds:

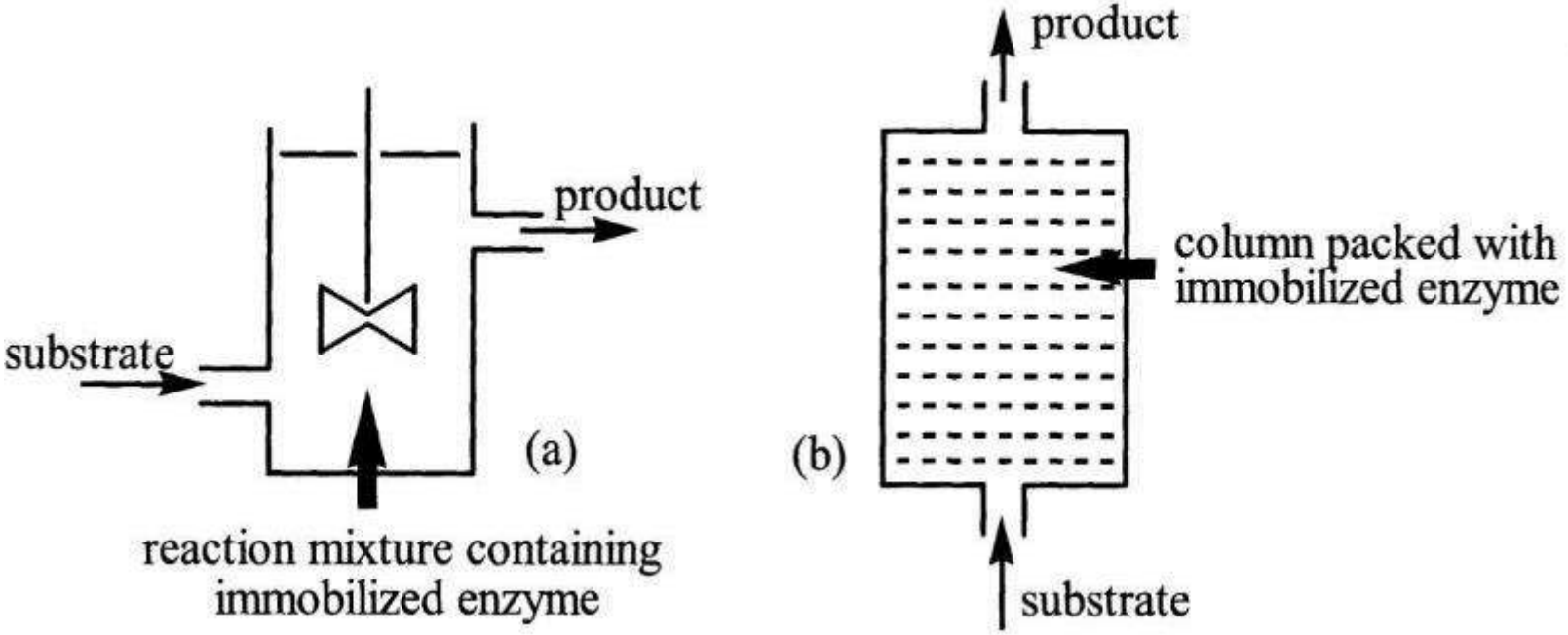
- Though a stable covalent bond is formed between matrix and enzyme, it can be broken by reaction with a suitable agent such as dithiothreitol (DTT) under mild conditions
- The reactivity of the thiol groups can be modulated via pH alteration, the activity yield of the methods involving disulfide bond formation is usually high—provided that an appropriate thiol-reactive adsorbent with high specificity is used

Applications of Immobilized Enzymes:

General Concepts:

- The immobilized enzyme system - should fit the requirements in terms of stability, activity, pH optimum and other characteristics should all be considered
- The property of immobilized enzymes - greatest industrial importance is the ease with which they can be separated from reaction mixtures
- Hence, in contrast to systems involving soluble enzymes - the reaction can be stopped by physical removal of the immobilized enzyme - without requiring such procedures as heat inactivation which might affect the products of the reaction
- Furthermore, the enzyme will still be active and largely uncontaminated, so can be used again

- For these reasons, immobilized enzymes are ideal for use in continuously operated processes
- Currently, continuous industrial processes involving immobilized enzymes – carried out in – (a)**Simple stirred tank reactors or** (b)**Packed bed reactors**



Enzyme utilization in Industry

Food and Drink Industry:

1. Use of yeasts(e.g. *Saccharomyces carlsbergensis*) in the **baking and brewing** industries - because they contain the enzymes for alcoholic fermentation; metabolize hexose sugars to produce pyruvate, but, whereas animals convert this to lactate under anaerobic conditions, the anaerobic end-product in yeasts is ethanol, with carbon dioxide being evolved

2. The **clarification of cider**, **wines and fruit juices** (e.g. apple) is usually achieved by treatment with fungal pectinases

Pectinases are a group of enzymes including polygalacturonases, which break the main chains of pectins, and pectinesterases, which hydrolyse methyl esters. Their action releases the trapped particles and allows them to flocculate

(pectins of fruit and vegetables play an important role in jam-making and other processes by bringing about gel formation)

3. **Cheese production** involves the conversion of the milk protein, K-casein, to para-casein by a defined, limited hydrolysis catalysed by chymosin (rennin)

Since chymosin - only be extracted from calves killed before they are weaned (pepsin is produced instead of chymosin after weaning) - the enzyme is in short supply - also ethical issues, there has been a large-scale search for an acceptable substitute

Proteases from animals (pepsin), plants (ficain and papain) and over a thousand micro-organisms have been tried, either on their own or mixed with calf chymosin

4. Papain is sometimes used as a **meat tenderizer**; some South American natives have traditionally wrapped their meat in leaves of papaya, the fruit from which papain is extracted

Papain (and other proteases) may also be used in the brewing industry to prevent chill hazes, caused by precipitation of complexes of protein and tannin at low temperatures

Other Industrial Applications:

1. **Washing powders** incorporating bacterial proteases

Commercial importance waned because of fears about the effect of enzyme dust on the respiratory system, but this problem was overcome by containment in granules which rupture only on contact with water

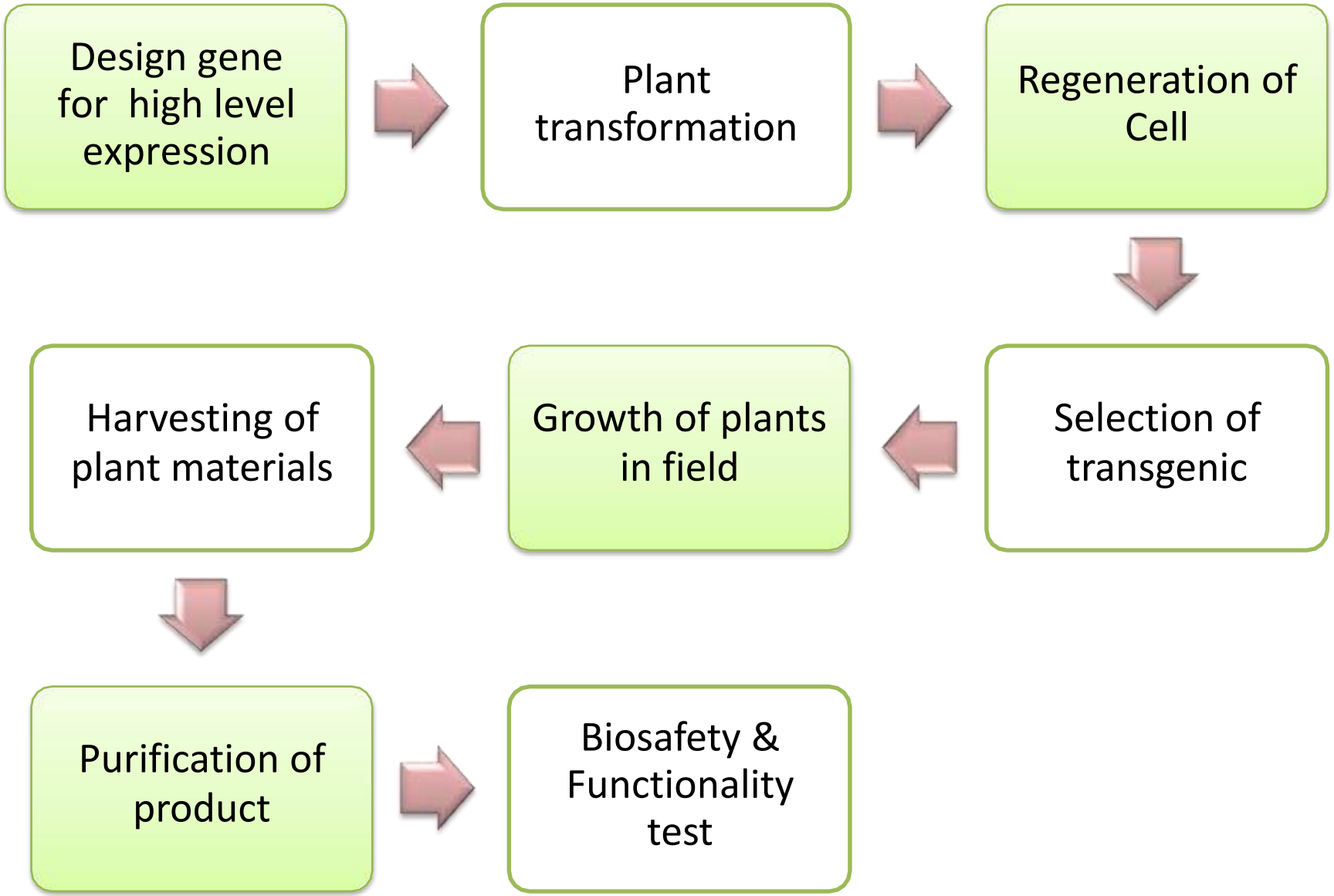
The enzymes in question, subtilisins from *Bacillus subtilis* mutants, are stable to alkali, high temperature (e.g. 65°C), detergents and bleaches. They will attack blood and other protein stains.

2. Bacterial proteases are also used in the **leather and textile industries** to loosen hair (or wool) and enable it to be separated from hide

Plants as Bioreactors

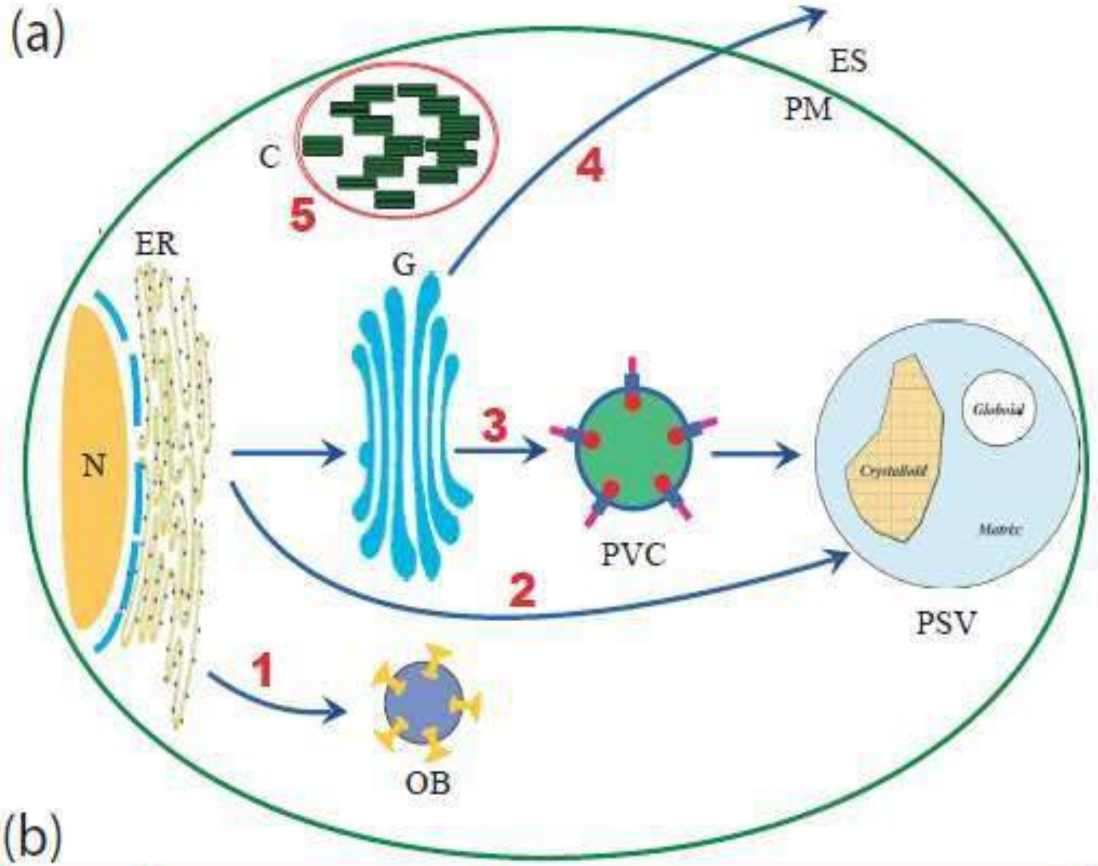
Introduction

- A Bioreactor is a device or vessels which are designed to obtain an effective environment for conversion of one material into some product by appropriate biochemical reactions
- Conversion is carried out by …… enzymes, microorganisms, cells of animals and plants, or sub cellular structures such as chloroplasts and mitochondria.
- Plants can be used as cheap chemical factories that require only water, minerals, sun light and carbon dioxide to produce thousands of chemical molecules with different structures.


Design gene for high level expression
Plant transformation
Regeneration of Cell
Selection of transgenic
Growth of plants in field
Harvesting of plant materials
Purification of product
Biosafety & Functionality test

G - Golgi;
PSV - Protein storage vacuole;
OB - Oil body;
C - Chloroplast; **ES** - Extracellular space;
PVC - Prevacuolar compartment.

(a)



(b)

Pathway	Storage Organelle	Targeting Mechanisms
1	Oil body	Oleosin
2	Protein storage vacuole	Golgi-bypassing via specific targeting sequences (e.g. alpha-TIP)
3	Protein storage vacuole	Vacuolar sorting receptor
4	Extracellular space	N-terminal signal peptide of secretory protein via default pathway
5	Chloroplast	Transit peptide or organelle transformation

Types of plant reactors

- Seed-based plant bioreactors
- Plant Suspension Cultures
- Hairy Root System Bioreactor
- Chloroplast bioreactor

Seed-based plant bioreactors

- An example is the successful expression of the human lysosomal enzyme alpha-L-iduronidase in *Arabidopsis thaliana* seeds.

- The advantage of these systems is that, proteins do not degrade at ambient temperature and are stable for long term storage.

Plant Suspension Cultures

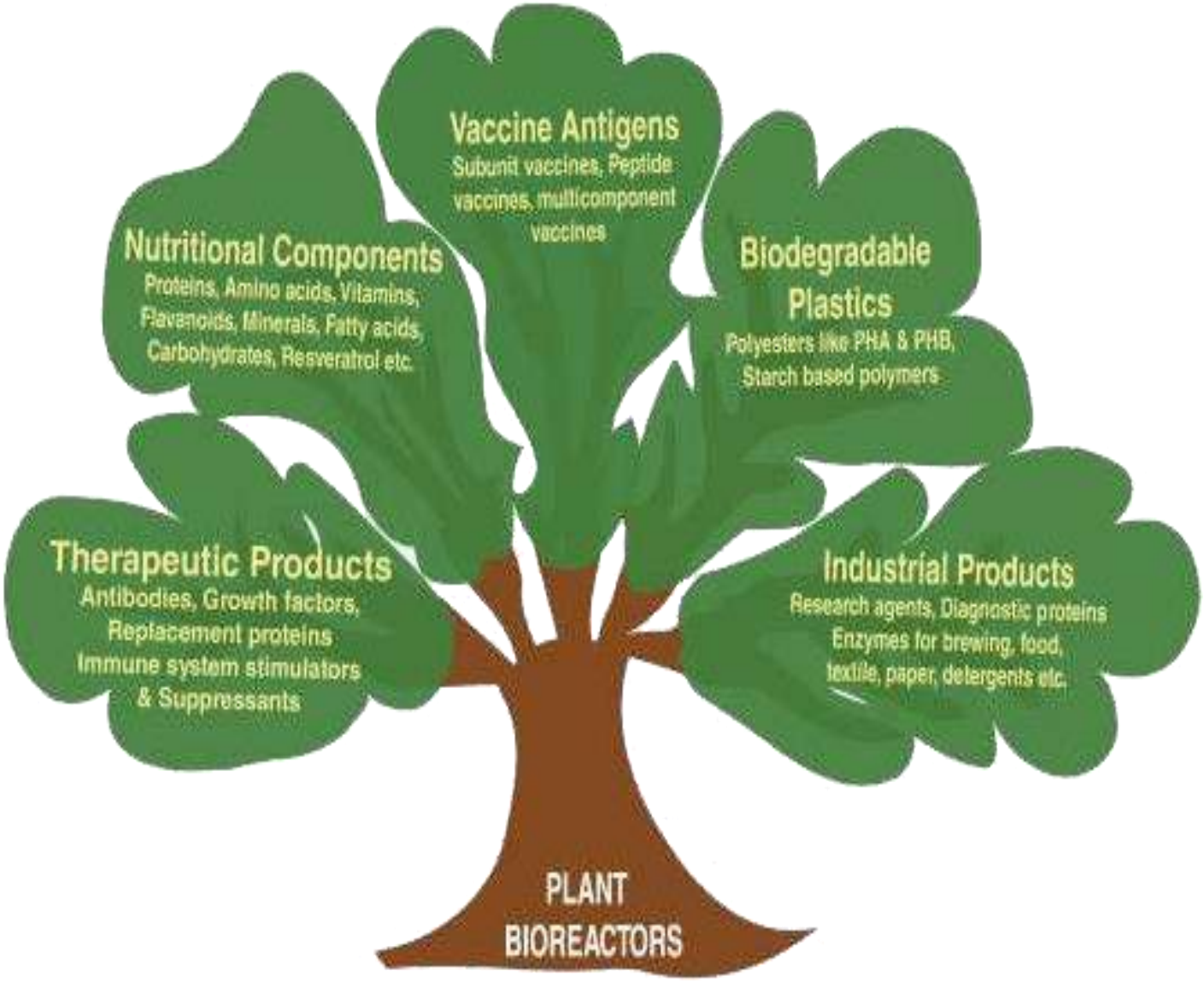
- Express recombinant proteins, secondary metabolites and antibodies transported to subcellular organelles.
- For example, is the expression of 80-kDa human lysosomal protein.

Hairy Root System Bioreactor

- It offers extreme biosynthetic stability and is suitable for making biopharmaceuticals as for example scopolamine in *Hyoscyamus muticus* L. hairy root culture.

Chloroplast bioreactor

- Insulin, interferon and other biopharmaceutical proteins can be made using Chloroplast bioreactor.
- Foreign genes are inserted into nuclear chromosomes and with peptides target expressed proteins into chloroplast.
- An example is the high yield in the expression of human serum albumin protein in chloroplast.



Source: www.plantbioreactor.co.in/images/00_112.jpg

- ***Vaccine antigens:***
- Antigens like Insulin, rotavirus enterotoxin, anthrax lethal factor, HIV antigen, foot and mouth disease virus antigen, heat stable toxin have been produced in plants.

- ***Therapeutic products:***
- The first successful production of a functional antibody, namely a mouse immunoglobulin IgGI in plants, was reported in 1989.

- In 1992, C.J. Amtzen and co-workers expressed hepatitis B surface antigen in tobacco to produce immunologically active ingredients via genetic engineering of plants

, Khandelwal *et al*., 2003; Sharma *et al*., 2004, Streatfield and Howard, 2003, Tiwari *et al*., 2009 and Youm et al., 2008.

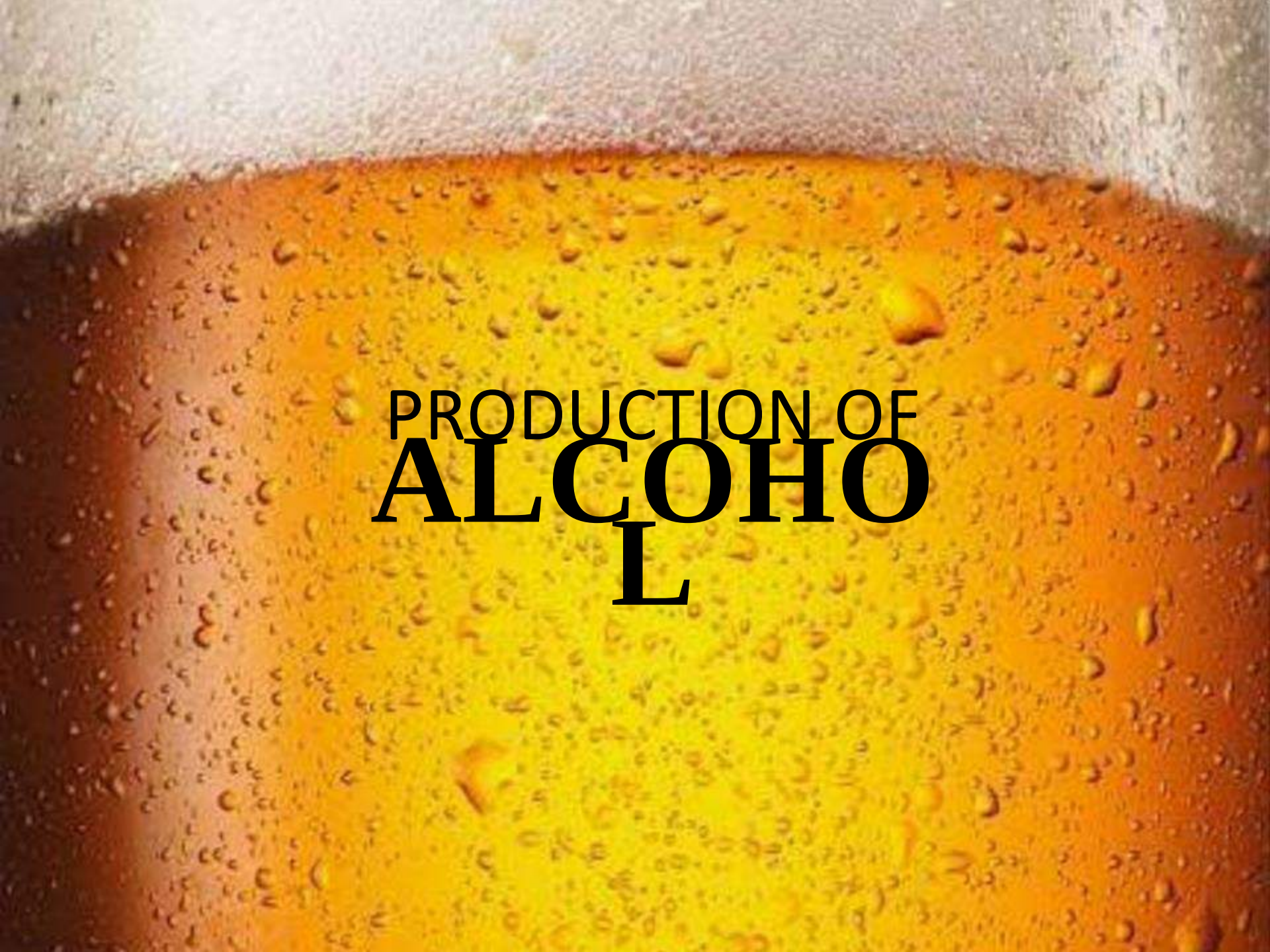
- ***Biodegradable plastics:***
  - Polyhydroxyalkanoates: biodegradable polymers which occur naturally in plants.
    - Plant was engineered to produce PHAs or PHBs in the various plant cell compartments.

- ***Industrial products:***
  - Most expensive Drug – **Hgc**
  - **hST** (Human somatotropin)
  - **rHLF** (Recombinant human lactoferrin)
  - **Synthetic fiber**: Produced from Potato and tobacco.

ADVANTAGES AND DISADVANTAGES

- Low cost source.
- Simple & Cost effective.
- Plant pathogens do not infect humans or animals.
- Produce large biomass.
- Easy storage for long time.

- Plant proteins have different sugar residues from human or animal proteins.


PRODUCTION OF
ALCOHOL

Introduction

Alcohol is any organic compound in which the hydroxyl functional group (-OH) is bound to a saturated carbon atom. The term alcohol originally referred to the primary alcohol ethanol (ethyl alcohol),which is used as a drug and is the main alcohol present in alcohol beverages.

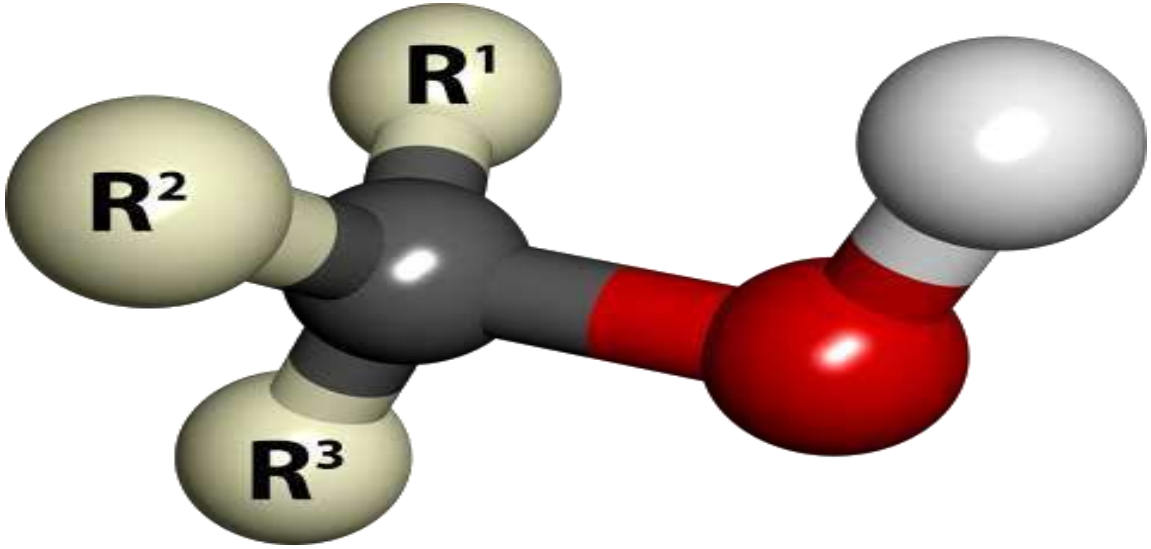


Fig – Ball and stick model of alcohol (-OH)

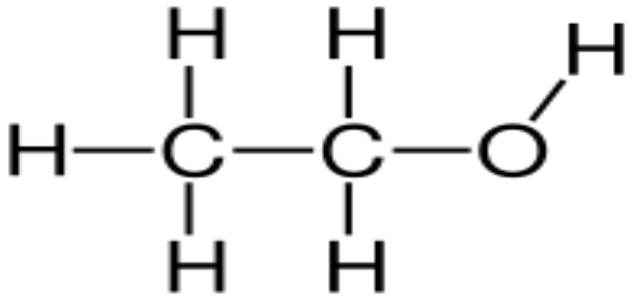
Rhazes (854CE -925CE),was a Persian polymath ,physician , alchemist and philosopher who discovered numerous compounds and chemicals including ***"alcohol"*** by developing several chemical instruments and methods of distillation.

Alcohol : structure and types

- An alcohol is often called with the name of the corresponding alkyl group followed by the word **"alcohol"**, methyl alcohol , ethyl alcohol , n-propyl alcohol .

- Alcohols are classified into primary (gen. formula : RCH_2OH) , secondary (sec-,s-) (gen. formula : RR'CHOH) and tertiary(tert-, t-)(gen. formula : RR'R"COH) based upon the numbers of carbon atoms connected to the carbon atom that bears the hydroxyl functional group.

- Ethanol , which is also called alcohol , ethyl alcohol and drinking alcohol is a simple volatile, flammable, colourless liquid alcohol having chemical formula C_2H_5OH.



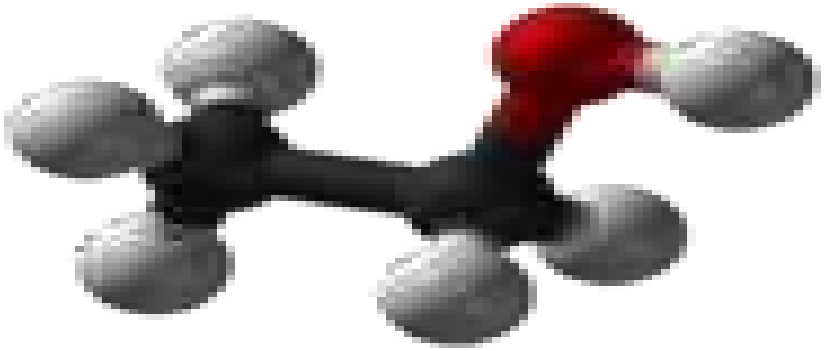


Fig:- structure of ethanol / drinking alcohol

Raw materials and micro-organisms

Micro-organisms:-- i)Yeast (*Saccharomyces cerevisae,Saccharomyces ellipsoideus, Kluyueromyces fragiles)*

ii) bacteria (*Zymononas mobilis , Candidas pseudotropicales , Candidas utilis)*

Raw materials:-- i)Sugary materials (e.g.:- molasses , sucrose , glucose etc.)

ii)Starchy materials (e.g.:- wheat , rice , maize , potato etc.)

ii) Cellulosic materials (e.g.:- agricultural waste , wood etc.)

PRE-TREATMENT of raw materials -

-

- Require some degree of pre-treatment ; actual process depends on the chemical component of the raw materials.

- Cellulosic substance have to be subjected to acidic or enzyme hydrolysis to release monosaccharide.

- Sugary raw materials require mild or no pre-treatment.

Biosynthetic pathway

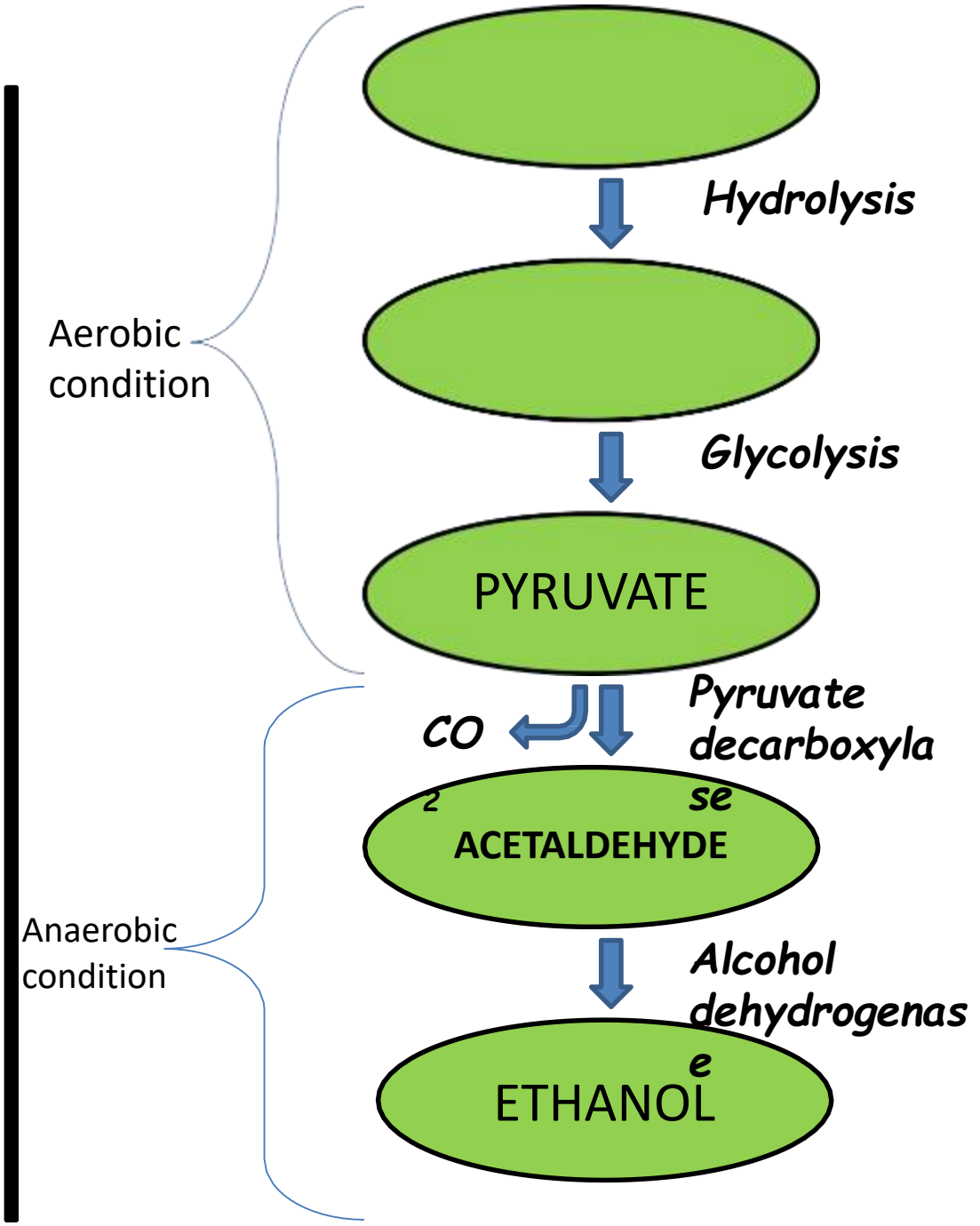
→The sequence of enzymatic steps in the synthesis of specific end-product in a living organism.

UNDER AEROBIC CONDITION :-

Excess glucose content in the medium, the micro-organism grow well without producing alcohol.

UNDER ANAEROBIC CONDITION :-

Excess glucose content in the medium ,the growth slows down and alcohol production occurs.



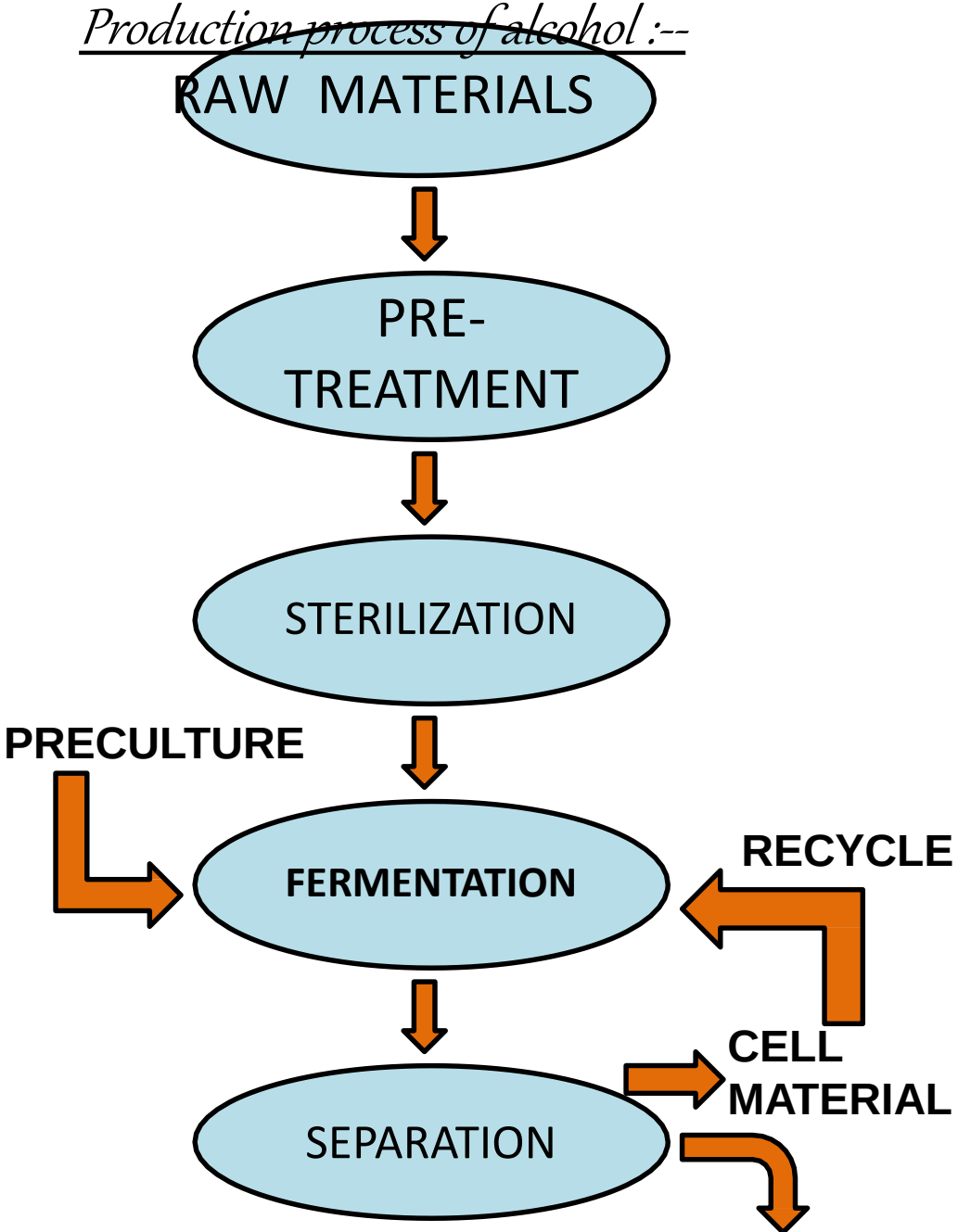
Regulation of synthesis

- Ethanol at high concentration in the medium inhibits it's own biosynthesis when yeast is used.

- Growth of yeast stops at 5% ethanol concentration (v/v in water).Yeast are sensitive to inhibition by endogenously synthesized ethanol and not to the ethanol added to the medium.

So , bacteria
Zymononas mobilis is used because of it's tolerance over a high concentration of alcohol (up to 13%)

Production process of alcohol :--



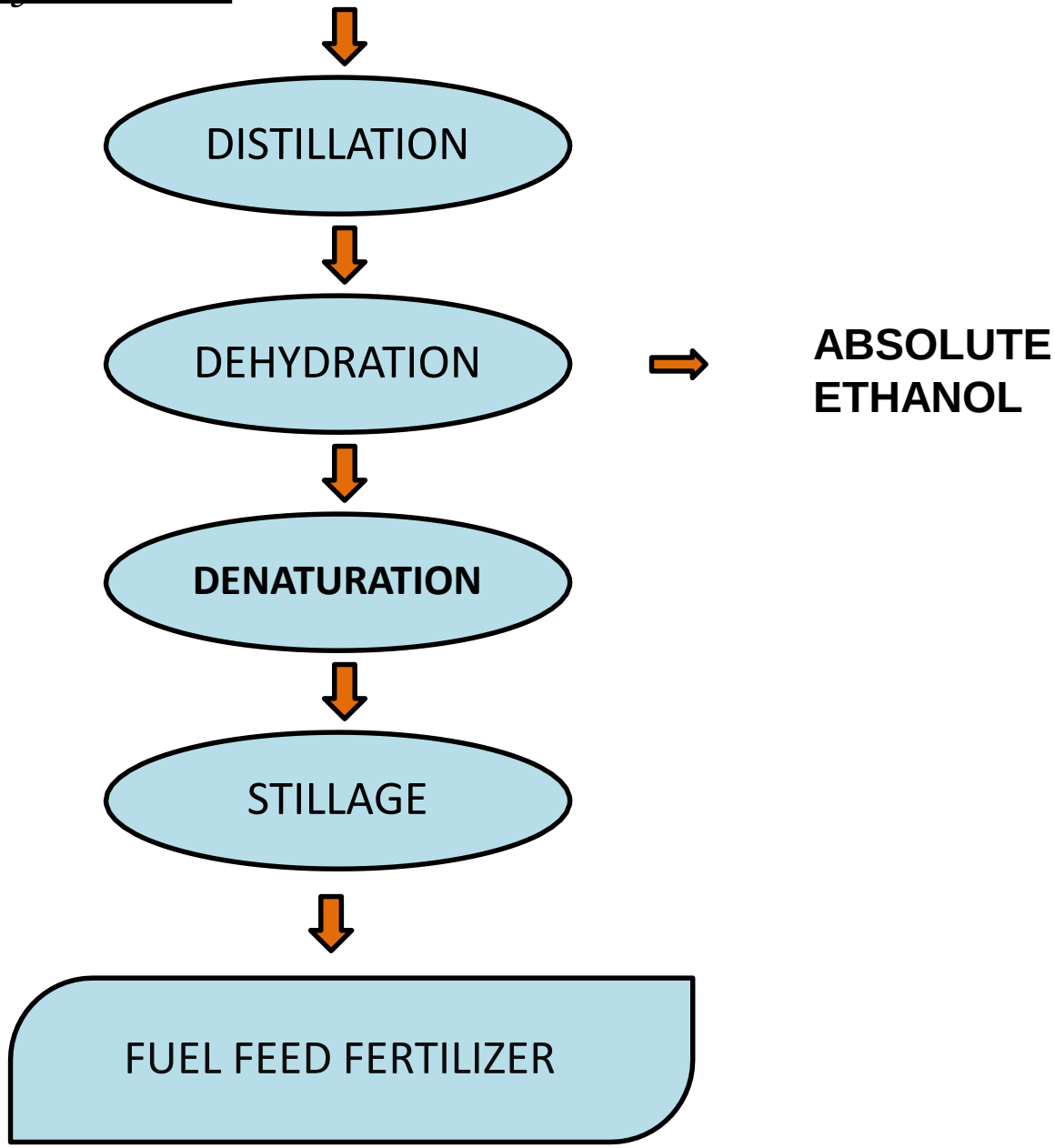
RAW MATERIALS :-

Starch , cellulose , molasses

PRE-TREATMENT :-

Hydrolysis, Clarification , filtration

Production process of alcohol :--



APPLICATION of alcohol :-

-

❑ ALCOHOLIC BEVERAGES

- Contains 3 – 40% alcohol by volume
- Produced and consumed by humans since pre-historic times.
- Natural fermentation produces trace amounts of alcohol such as 2-methyl-2-butanol and У- hydroxybutyric acid .

❑ ANTIFREEZE

- It commonly includes a 50% v/v (by volume) solution of ethylene glycol in water.

❑ MEDICAL

- Can be used as an antiseptic to disinfect the skin before injections are given , often along with iodine.
- Ethanol based soaps and gels (hand senitizers) are most common in restaurants as they don't require drying due to the volatility of the compound.

APPLICATION of alcohol :-

- **ALCOHOL FUEL**
  - Some alcohols , mainly ethanol and methanol , can be used as fuel .
  - Fuel performance can be increased in forced induction internal combustion engines by injecting alcohol into the air intake .
- **PRESERVATIVE**
  - Often used as a preservative for biological specimens in the fields of science and medicine.
- **SOLVENT**
  - They have applications in industry and science as reagents or solvents.
  - Because of it's relatively low toxicity , ethanol can be used as a solvent in medical drugs , perfumes , and vegetable essences such as vanilla.
  - in organic synthesis , alcohols serve as versatile intermediates.

Antibiotics

- Compound that kill or inhibit the growth of other organisms.
- Most Antibiotics are produced by filamentous fungi or Actinomycetes.
- They are derived from special microorganisms or other living systems, and are produced on an industrial scale using a fermentation process.
- Today, over 10,000 antibiotic substances have been reported.

- Antibiotics are produced by fermentation.

Any large-scale microbial process occurring with or without air is called Fermentation.

- The process may take a few days to obtain an extractable amount of product.
- Antibiotic production is done by the batch process.

Production of Antibiotics

- The mass production of antibiotics began during World War II with streptomycin and penicillin.

➓Now most antibiotics are produced by staged fermentations in which strains of microorganisms producing high yields are grown under optimum conditions .

- Production of antibiotics can be done by 3 methods.
- 1. **Natural microbial production** using Fermentation technology.

  Example: Penicillin
- 2. **Semi synthetic production** (post production modification of natural antibiotics).
- **Example:** Ampicillin
- 3. **Synthetic production of antibiotics** made synthetically in the lab.
- **Example:** Quinoline

Strains used for production

- Species are often genetically modified to yield maximum amounts of antibiotics.
- **Mutation** is often used -introducing mutagens such as ultraviolet radiation, x-rays
- Selection and further reproduction of the higher yielding strains can raise yields by 20-fold or more.
- Another technique used to increase yields is **gene amplification**, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids.

Raw Materials

- The compounds that make the fermentation broth are the primary raw materials required for antibiotic production.

➓The broth is an aqueous solution made up of all of the ingredients necessary for the proliferation of the microorganisms.

➓Typically, it contains;

- **Carbon source:** molasses, or soy meal,acetic acid, alcohols, or hydrocarbons

➓These materials are needed as a food source for the organisms.

- **Nitrogen Source :** Nitrogen is another necessary compound in the metabolic cycles of the organisms.

  ammonia salt is typically used.

Other Elements

- Trace elements needed for proper growth of antibiotic producing microorganisms such as:
- Phosphorus
- Sulfur
- Magnesium
- Zinc.
- Anti foaming agents to prevent foaming during fermentation such as:
- Lard oil
- Octadecanol

Steps in Production

- First the organism that makes the antibiotic must be identified.
- Desired microorganism must then be isolated.
- Then the organism must be grown on a scale large enough to allow the purification and chemical analysis of the antibiotic.
- The antibiotic tested against a wide variety of bacterial species.

It is important that sterile conditions be maintained throughout the manufacturing process, because contamination by foreign microbes will ruin the fermentation.

A) Starting a Culture

- Before the fermentation process the desired microbe must be isolated and its number must be increased by many times.

- A **starter culture** from a sample of previously isolated organisms is created in the lab.

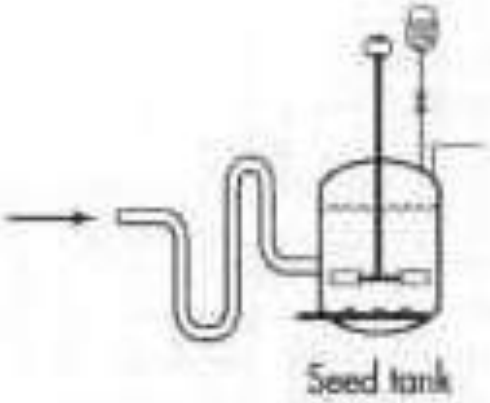
- A sample of the organism is transferred to an agar-containing plate.

- The initial culture is then transferred to shake flask containing nutrients necessary for growth.

- A suspension is formed which is then transferred to seed tanks for further growth.

Antibiotic-producing organism

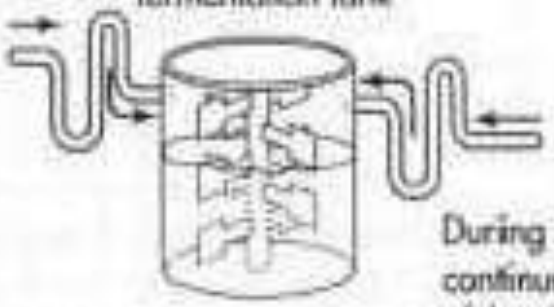
A culture is started by placing the sample of the organism into a shake flask with growth-promoting nutrients.



Seed tank

The seed tank is equipped with mixers to keep the growth medium active, and a pump to deliver sterilized air.

30,000 gallon fermentation tank



During fermentation, the microorganisms continue to grow and excrete large quantities of the desired antibiotic.

FERMENTATION

- The **seed tanks** are steel tanks designed to provide an ideal environment for growing microorganisms.
- The seed tanks are equipped with agitators which mix the growth medium with microbes and deliver sterilized, filtered air.

- After about 24-28 hours, the material in the seed tanks is transferred to the primary fermentation tank.

B) Fermentation

- The fermentation tank is a larger version of the seed tank, which is able to hold about 30,000 gallons.
- Microorganisms are allowed to grow and multiply.
- During this process, they excrete large quantities of the desired antibiotic.

- The tanks are cooled to keep the temperature between 73-81° F (23-27.2 ° C).
- It is constantly agitated, and a continuous stream of sterilized air is pumped into it.

- Anti- foaming agents are periodically added.

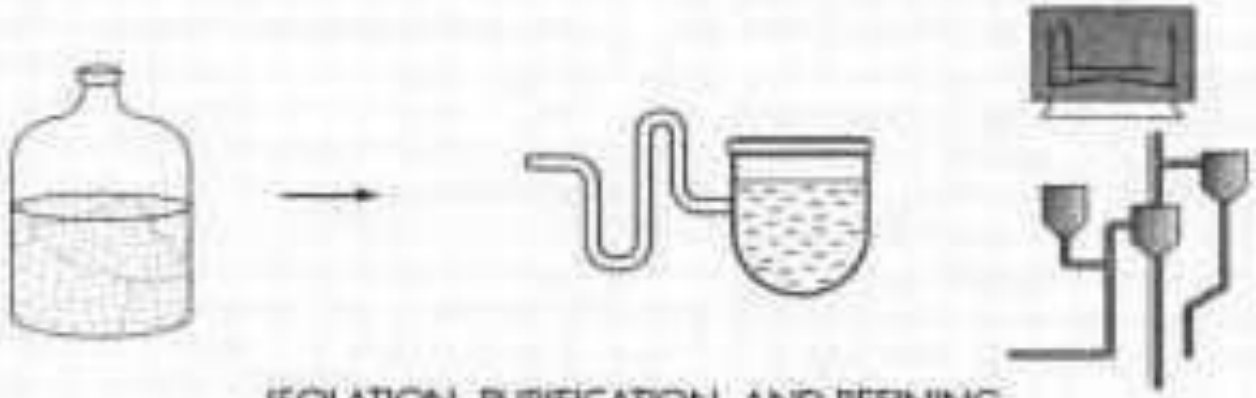
- Since pH control is vital for optimal growth, acids or bases are added to the tank as necessary.

C) Isolation & Purification

- After 3-5days, the maximum amount of antibiotic will have been produced.

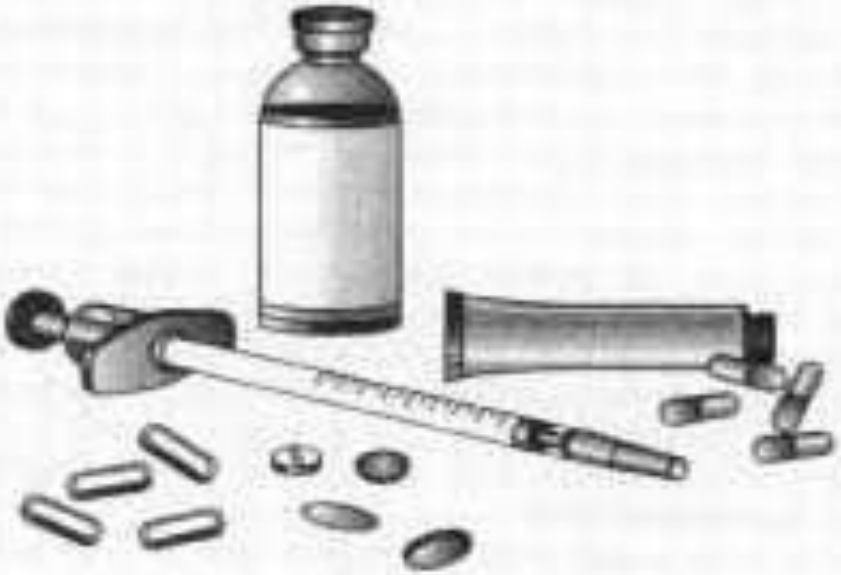
⑩The isolation process can begin.

- The isolation depend on the specific antibiotic produced, the fermentation broth is processed by various purification methods.



ISOLATION, PURIFICATION, AND REFINING

Once the antibiotic is isolated from the fermentation broth and purified using either the ion-exchange or solvent extraction method, a purified powder form of the antibiotic is produced.



PACKAGING AND SHIPPING

Water soluble Antibiotics

- Antibiotic compounds that are water soluble, an ion-exchange method is used for purification.

➓ The compound is first separated from the waste organic materials in the broth.

➓ Then sent through equipment, which separates the other water-soluble compounds from the desired one.

Oil soluble Antibiotics

- Solvent extraction method is used for the isolation of oil soluble or organic antibiotics.
- The broth is treated with organic solvents such as butyl acetate or methyl isobutyl ketone, which can dissolve the antibiotic.
- The dissolved antibiotic is then recovered using various organic chemical means.
- At the end of this step a purified powdered form of the antibiotic is obtained which can be further refined into different product types.

Refining/Packaging

- Antibiotic products can take on many different forms. They can be sold in solutions for intravenous bags or syringes, in pill or gel capsule form, or powders, which are incorporated into topical ointments.
- Various refining steps may be taken after the initial isolation.

➓For **intravenous bags**, the crystalline antibiotic can be dissolved in a solution, put in the bag, which is then hermetically sealed.

- For **gel capsules**, the powdered antibiotic is physically filled into the bottom half of a capsule then the top half is mechanically put in place.
- When used in **topical ointments**, the antibiotic is mixed into the ointment

Quality Control

- Quality control is of great importance in the production of antibiotics.

➉ Since it involves a fermentation process, steps must be taken to ensure that absolutely no contamination is introduced at any point during antibiotic production.

- During manufacturing, the quality of all the compounds is checked on a regular basis.

➉ Frequent checks of the condition of the microorganism culture during fermentation.

• Various physical and chemical properties of the finished product are checked such as pH, melting point, and moisture content.

Production of Biopestcides

Introduction

- **Biopesticide** is a formulation made from naturally occurring substances that controls pests by non toxic mechanisms and in **ecofriendly** manner.

- Biopesticides may be derived from animals (e.g. nematodes), plants (*Chrysanthemum*, *Neem*) and micro-organisms (e.g. *Bacillus thuringiensis*, *Trichoderma*, nucleopolyhedrosis virus).

- However, biopesticides are generally less toxic to the user and non-target organisms, making them desirable and sustainable tools for disease management.

MAJOR CLASSES OF PESTICIDES

1. *Microbial pesticides*

- Microbial pesticides are composed of microscopic living organisms (viruses, bacteria, fungi, protozoa) or toxin produced by these organisms
- Applied as conventional insecticidal sprays, dusts, or granules.
- Their greatest strength is their specificity as most are essentially nontoxic and non pathogenic to animals and humans.
- Microbial pesticides includes insecticides, fungicides, herbicides and growth regulators of microbial origin.

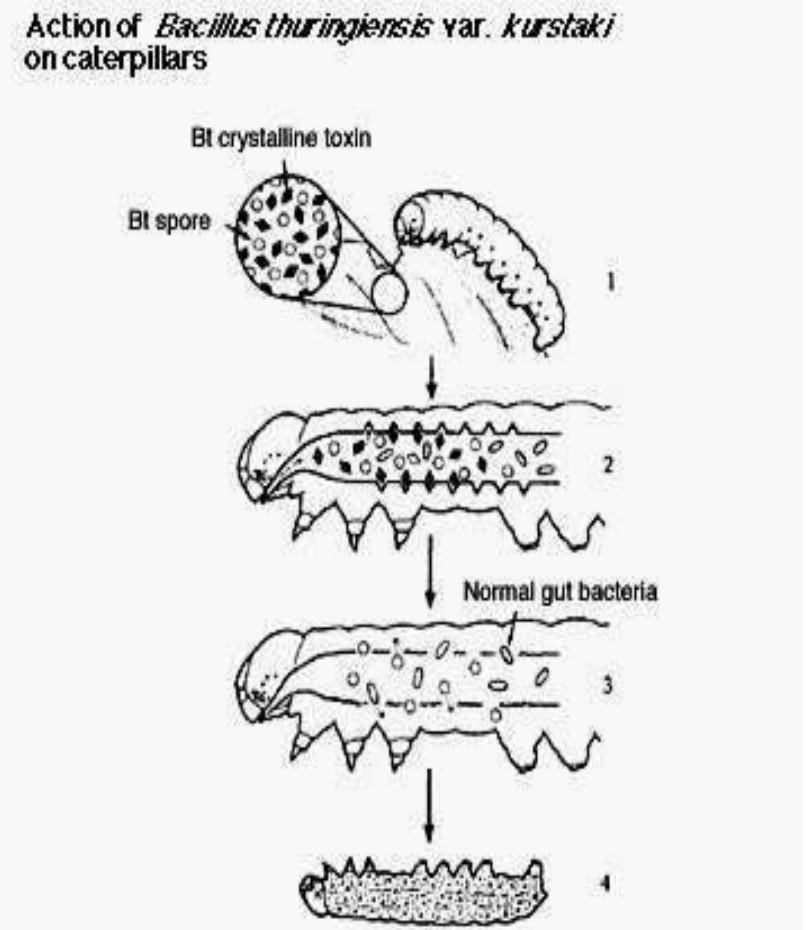
Table 41.18 The Use of Bacteria, Viruses, and Fungi As Bioinsecticides: An Older Technology with New Applications

Microbial Group	Major Organisms and Applications
Bacteria	*Bacillus thuringiensis* and *Bacillus popilliae* are the two major bacteria of interest. *Bacillus thuringiensis* is used on a wide variety of vegetable and field crops, fruits, shade trees, and ornamentals. *B. popilliae* is used primarily against Japanese beetle larvae. Both bacteria are considered harmless to humans. *Pseudomonas fluorescens,* which contains the toxin-producing gene from *B. thuringiensis,* is used on maize to suppress black cutworms.
Viruses	Three major virus groups that do not appear to replicate in warm-blooded animals are used: nuclear polyhedrosis virus (NPV), granulosis virus (GV), and cytoplasmic polyhedrosis virus (CPV). These viruses are more protected in the environment.
Fungi	Over 500 different fungi are associated with insects. Infection and disease occur primarily through the insect cuticle. Four major genera have been used. *Beauveria bassiana* and *Metarhizium anisopliae* are used for control of the Colorado potato beetle and the froghopper in sugarcane plantations, respectively. *Verticillium lecanii* and *Entomophthora* spp., have been associated with control of aphids in greenhouse and field environments. *Coelomyces,* a chytrid, also is used for control of mosquitoes.

Some of the important microbial pesticides

Bacillus thuringiensis .

- Spores and crystalline insecticidal proteins of *B. thuringiensis* used to control insect pests
- Applied as liquid sprays
- Trade names such as DiPel and Thuricide.
- Highly specific, environmentally friendly, with little or no effect on humans, wildlife, pollinators, and most other beneficial insects, and are used in organic farming;
- Control lepidopterous pests like american bollworm in cotton and stem borers in rice.
- When ingested by pest larvae, Bt releases toxins which damage the mid gut of the pest, eventually killing it.
- Main productional substrains *kurstaki, galeriae and dendrolimus*



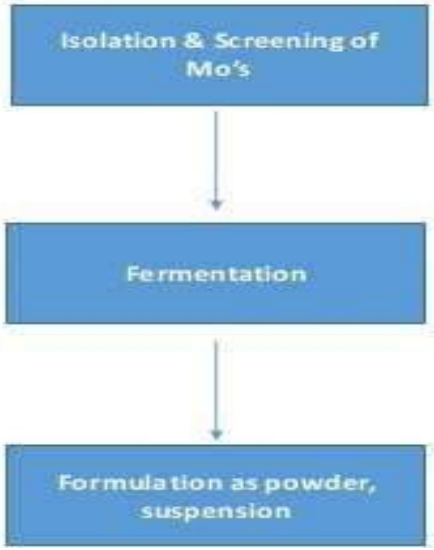
b. *Agrobacterium radiobacter* (Agrocin)

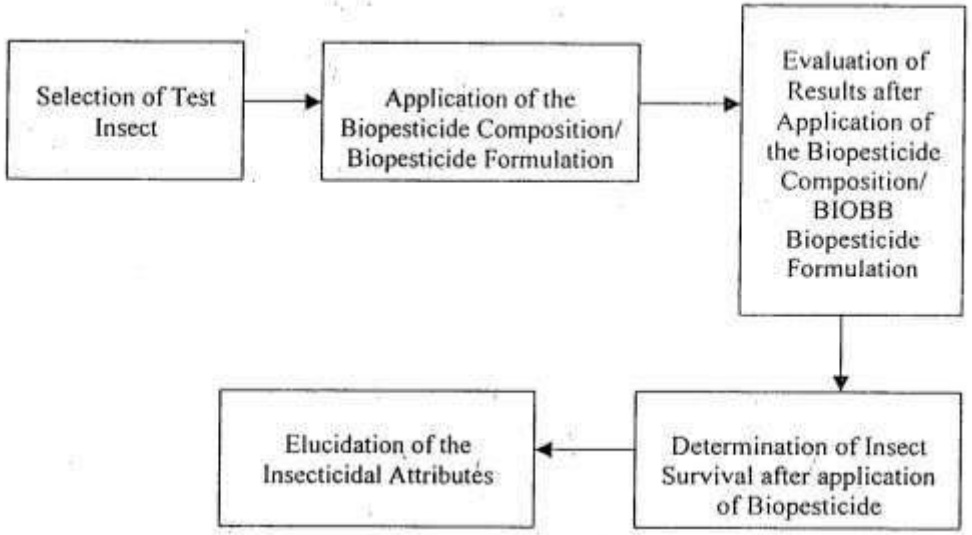
•*Agrobacterium radiobacter* is used to treat roots during transplanting, that checks crown gall.

•Crown gall is a disease in peaches, grapevine, roses and various plants caused by soil borne pathogen *Agrobacterium tumefaciens***.**

•The effective strains of *A. radiobacter* posses two important features:
✓They are able to colonize host roots to a higher population density.
✓They produce an antibiotic, agrocin, that is toxic to *A. tumefaciens*.

Large scale production of microbial pesticides





2. *Plant Pesticides*

- **Plants that produce substances or chemicals that have detrimental effect on the pest organism**
- **Pyrethrum (*Chrysanthemum)* flowers contain active pyrethrins extracted and sold in the form of an oleoresin. This is applied as a suspension in water or oil, or as a powder. Pyrethrins attack the nervous systems of all insects, and inhibit female mosquitoes from biting and insect repelling.**
- **Neem does not directly kill insects on the crop. It acts as an anti-feedant, repellent, and egg-laying deterrent, protecting the crop from damage. The insects starve and die within a few days. Neem also suppresses the hatching of pest insects from their eggs.**

Pyrethrum (*Chrysanthemum)*

Neem

3. Biochemical pesticides

- **They are naturally occurring substance to control pest by non-toxic mechanisms.**
- **Biochemical pesticides include substances as insect sex pheromones, that interfere with mating that attract insect pest to traps.**

- **The synthetic attractants are used in one of four ways:**

i. **As a lure in traps used to monitor pest populations;**

ii. **As a lure in traps designed to "trap out" a pest population;**

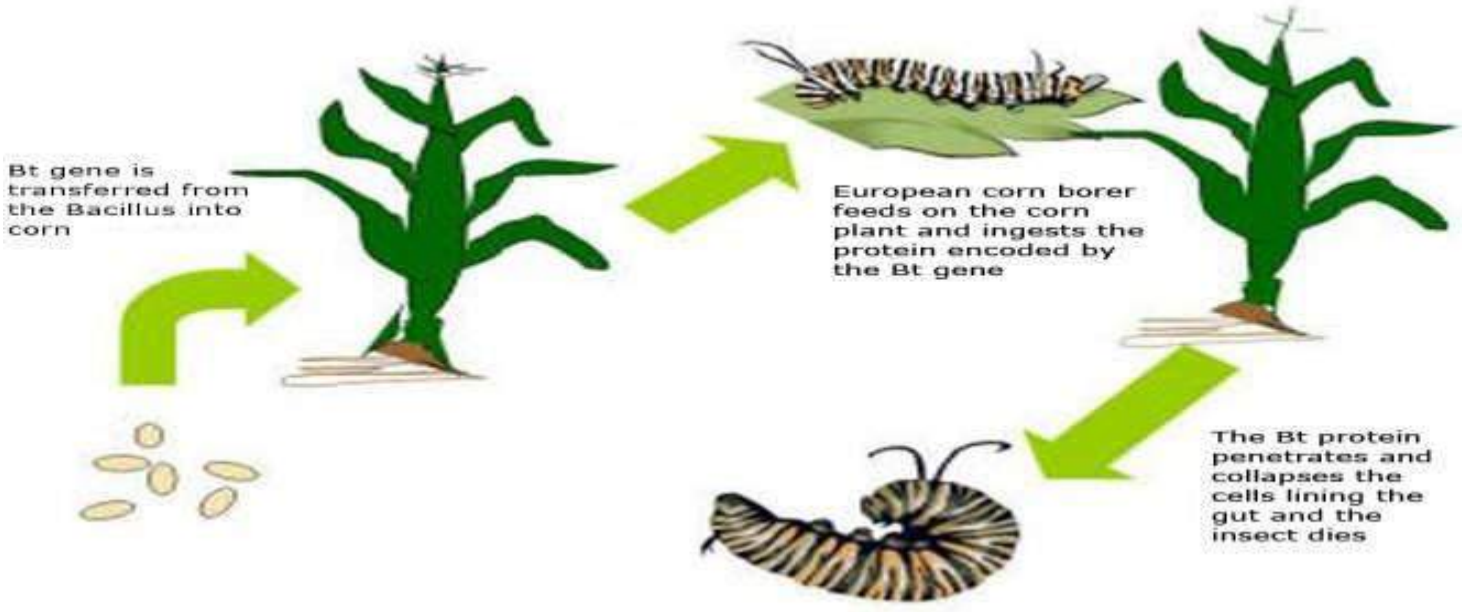
iii. **As a broadcast signal intended to disrupt insect mating**

iv. **As an attractant in a bait containing an insecticide**

Rice Weevil (*Sitophilus oryzae*) pheromone trap

4. Plant-incorporated-protectants (PIPs)

- Plant-incorporated protectants are pesticidal substances produced by plants and the genetic material necessary for the plant to produce the substance.
- For example, scientists can take the gene for a specific Bt pesticidal protein and introduce the gene into the plant's genetic material.
- The new Bt cotton product contains the dual genes Cry IA(c) and Cry IF, transformed with *Agrobacterium tumefaciens* and incorporated through back crossing



5. Predators

- They consume several to many prey over the course of their development, they are free living and they are usually as big as or bigger than their prey.

- Lady beetles, rove beetles, many ground beetles, lacewings, true bugs such as Podisus and Orius, syrphid fly larvae, mantids, spiders, and mites.

Lacewings

Lady bird beetle

6.Parasitoids

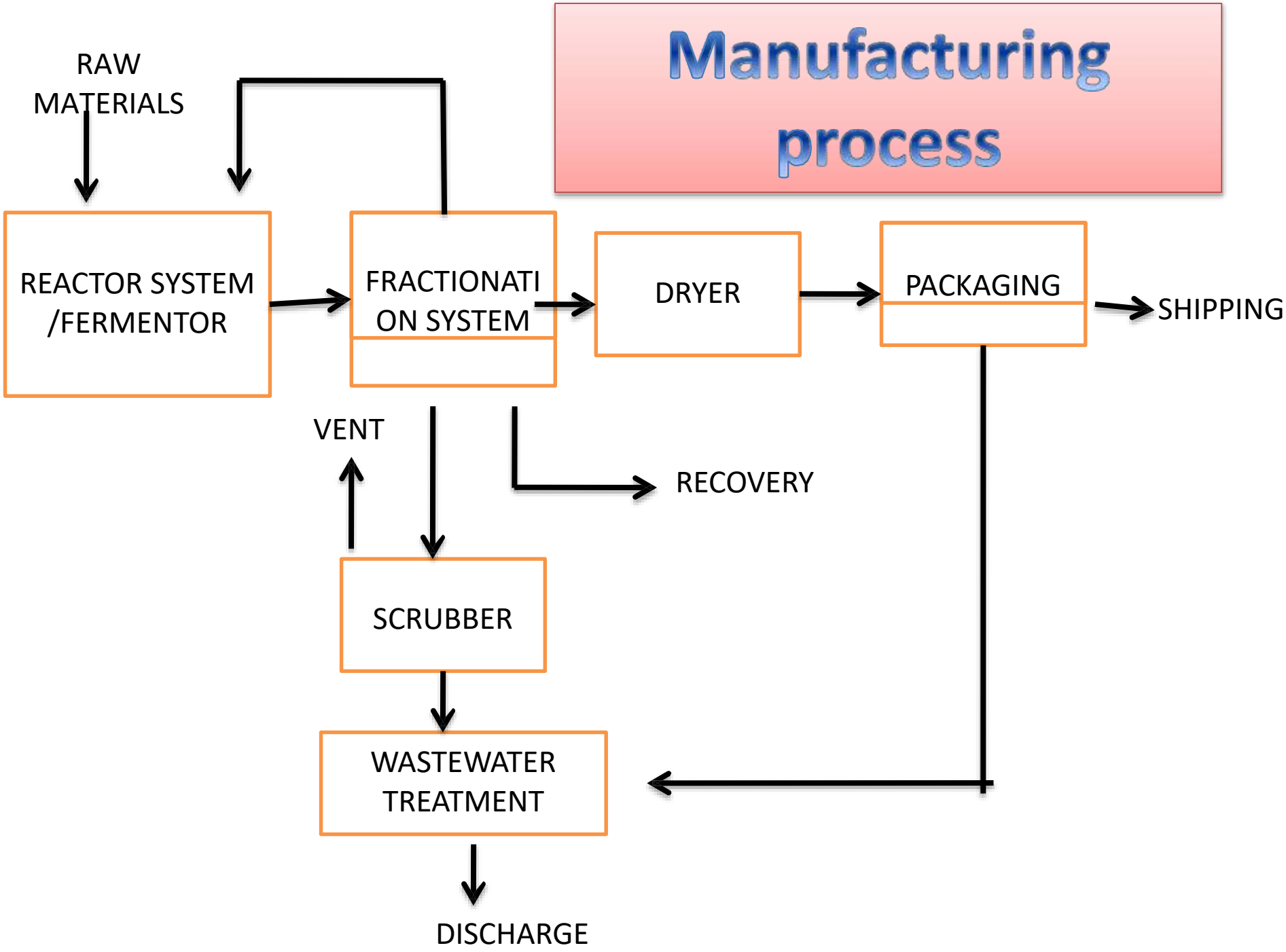
Fig: Trichogramma

- Parasitoids are almost the sam… hosts, and their development … host insect.
- An adult parasitoid deposits one or more eggs into or onto the body of a host insect or somewhere in the host's habitat.
- The larva that hatches from each egg feeds internally or

externally on the host's tissues and body fluids, consuming it slowly.

- Later in development, the host dies and the parasitoid pupates inside or outside of the host's body.
- E.g., *Bathyplectes, Trichogramma, Encarsia, muscidifurax* etc.

Manufacturing process



•RAW MATERIAL

•May be organic or inorganic compounds
•Different raw material for different pesticide

•REACTOR SYSTEM

•Chemical process takes place in the presence of chemicals such as oxidation, nitration, condensation, etc.

•FRACTIONATION SYSTEM

•Separation process in which certain quantity of a mixture(solid,liquid,solute,suspension or isotope) is divided up in a number of smaller fractions in which composition change
•Recovery

•DRYER

•Removal of water or other solvent by evaporation from solid, semi-solid or liquid
•Final production step before selling or packaging products.

•SCRUBBERS

•To remove priority pollutants from pesticide product using scrubbing liquor

•Wastewater go to treatment plant

•PACKAGING

•Packed in dry and clean containers e.g., drums type depend on type of pesticide

•Capacity 10,25,50,100,200 lits.

•Temper-proof, closer to avoid leakage,sturdy

•FORMULATION

•Processing a pesticide into granules, liquid, dust and powder to improve its properties of storage, handling, application, effectiveness, or safety.

•Dry mixing, grinding of solids, dissolving solids and blending

Advantages

- ✓ Inherently less harmful and less environmental load,
- ✓ Designed to affect only one specific pest or, in some cases, a few target organisms,
- ✓ Often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems .
- ✓ When used as a component of Integrated Pest Management (IPM) programs, biopesticides can contribute greatly.

Disadvantages

- Slow effect
- Lack persistence and wide spectrum activity
- Rapidly degraded by UV lights so residual action is slow.
- Seasonal availability of plants products indicates the needs for storage.
- They are not available easily
- Poor water solubility and generally not systemic in nature
- All products applied followed by growers have not been scientifically verified.


SINGLE-
CELL
PROTEIN

WHAT ARE SINGLE CELL PROTEINS ?

- SCP are dried cells of micro organisms which can be used as dietary protein supplement.
- They are used as animal feed & can be used for human feed as protein supplement.
- Also called 'Novel Food' & 'Minifood'.

HISTORY

- Part of our diet since ancient times.
- Earlier known as '**Microbial Protein**'.
- Name was introduced by **Prof. Scrimshow** of MIT in 1967
- In 1950's British Petroleum initiated production of SCP on commercial basis.
- **Pruteen** was the 1st commercial SCP used as animal feed additive
- Pruteen was produced from bacteria **Methylophilus methylotrophus** cultured on methanol & had 72 % protein content.

RAW MATERIALS

- Production of SCP requires micro-organisms that serve as the protein source and the substrate that is biomass on which they grow.
- There is a variety of both the sources that can be used for the production of SCP.
- The biomass used can be plant biomass or organic biomass.
- The micro-organisms used belong to the group of Algae, Fungi and Bacteria.

MICRO ORGANISMS

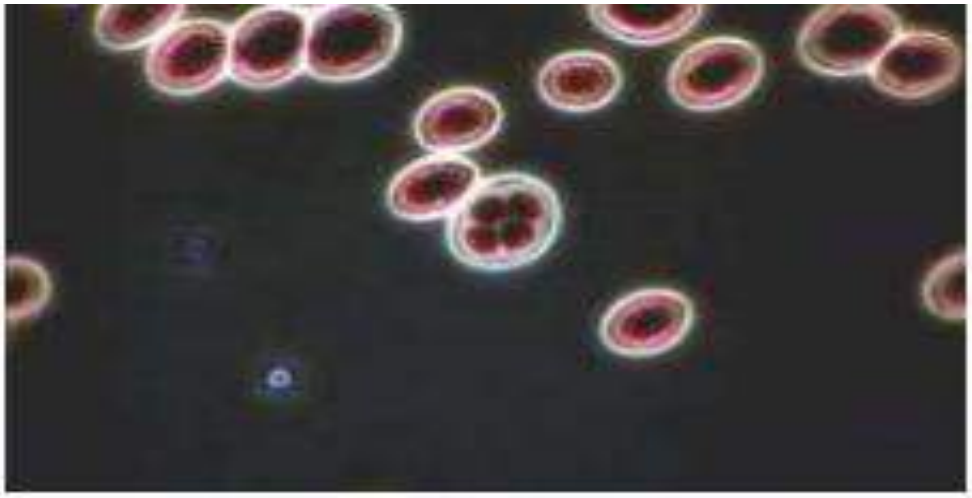
- Micro-organisms used are fungi , yeast, algae & bacteria.
- The following table shows average different compositions of main groups of micro– organisms (% dry wt.)

COMPOSITON	FUNGI	ALGAE	YEAST	BACTERIA
PROTEIN	30- 40 %	40- 60 %	45- 55 %	50- 65 %
FAT	9-14 %	8-10 %	5-10 %	3-7 %
NUCLEIC ACID	7-10 %	3-8 %	6-12 %	8-12 %

ntus

m

erevisae

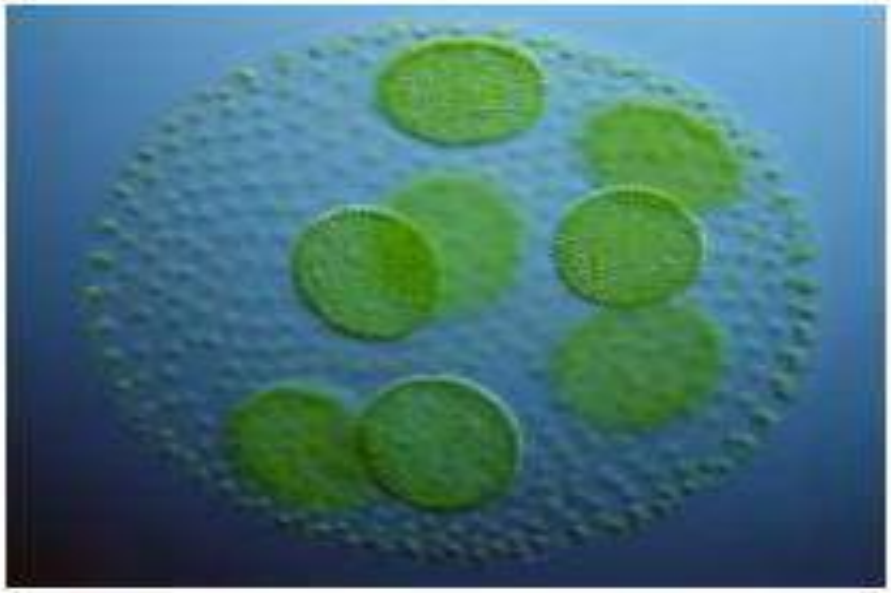


FUNGI

losa

oescens

im



COMPARISION OF MICRO-ORGANISMS

	ADVANTAGES	DIS ADVANTAGES
FUNGI	Easy to grow & harvest	Lower growth rates & lower protein content
ALGAE	Easy to grow & harvest & high quality protein	Non –digestible cellulosic cell wall, concentrate heavy metals
YEAST	Larger in size, lower NA content , familiarity & acceptability	Poor digestibility, low protein content, slow growth rate
BACTERIA	High protein content, digestible cell wall	High NA content, small in size, low density

SCP PRODUCTION

- Selection of suitable strain
- Fermentation
- Harvesting
- Post harvest treatment
- SCP processing for food

Selection of strain

- It a very critical step as the quality of protein depends totally on the microbe that is used for the production.
- Thus careful selection of the strain should be done.
- Care should be taken that the selected strain should not produce any toxic or undesirable effects in the consumer.

Fermentation

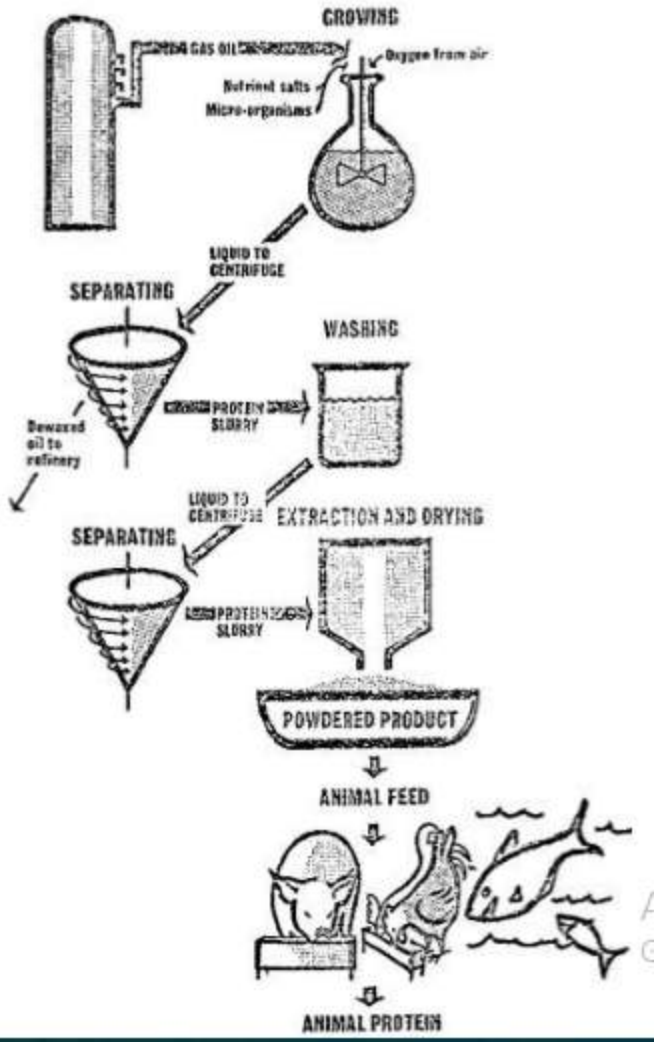
- It can be carried out in the fermentor which is equipped with aerator, thermostat, pH, etc. or in the trenches or ponds.
- Microbes are cultured in fed- batch culture.
- Engineers have developed deep lift fermentor & air lift fermentor .

Harvesting

- When the colonies of microbes are fully developed, they are then harvested.
- The bulk of cells are removed from the fermentor by decantation.

Post harvest treatment

- After harvesting, the cells are subjected to a variety of processes.
- Post harvesting treatments includes steps like separation by centrifugation, washing, drying, etc.



PROCESSING FOR FOOD

It includes

1. Liberation of cell proteins by destruction of indigestible cell wall.

A. MECHANICAL METHODS

- Crushing, crumbling, grinding, pressure homogenization, etc.

B. CHEMICAL METHODS

- Enzymes & salts are used to digest or disrupt the cell wall.
- Salts like NaCl, sodium dodecyl sulfate, etc. whereas nuclease enzymes are used.

C. PHYSICAL METHODS

- Freeze- thaw, osmotic shock, heating & drying.

2. Reduction of nucleic acid content

- Chemical & enzymatic treatments are preferred.
- Chemicals which are used includes acidified alcohol, salts, acids & alkalies.
- Use of such chemicals leads to formation of lygino-alanine which causes hypersensitivity skin reactions.
- Enzymes which are used include ribonuclease & nuclease enzymes .
- These enzymes can be used exogenously or can be induced endogenously.

ADVANTAGES

- Rapid successions of generations.
- Easily modifiable genetically.
- High protein content of 43-85% in dry mass.
- Broad spectrum of original raw material used for production, which also includes waste products.
- Production in continuous cultures
- consistent quality not dependant on climate in determinable amount
- low land requirements, economically beneficial.
- Utilization of solar energy
- Cellular, molecular and genetic alterations.

DISADVANTAGES

- High content of nucleic acids leading to elevated levels of uric acid.
- Development of kidney stone and gout if consumed in high quality.
- Possibility for the presence of secondary toxic metabolites.
- Poor digestibility
- Stimulation of gastro-intestinal
- Hypersensitivity skin reactions.

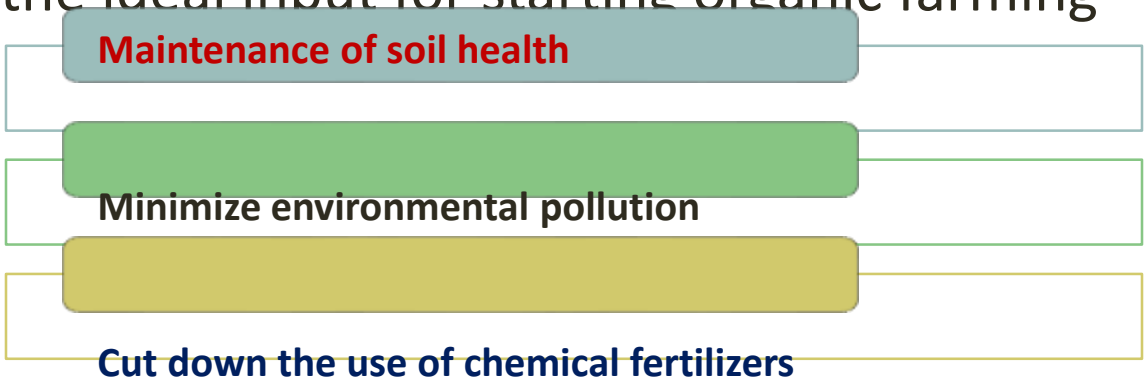
Definition

Selected strains of beneficial soil microorganisms

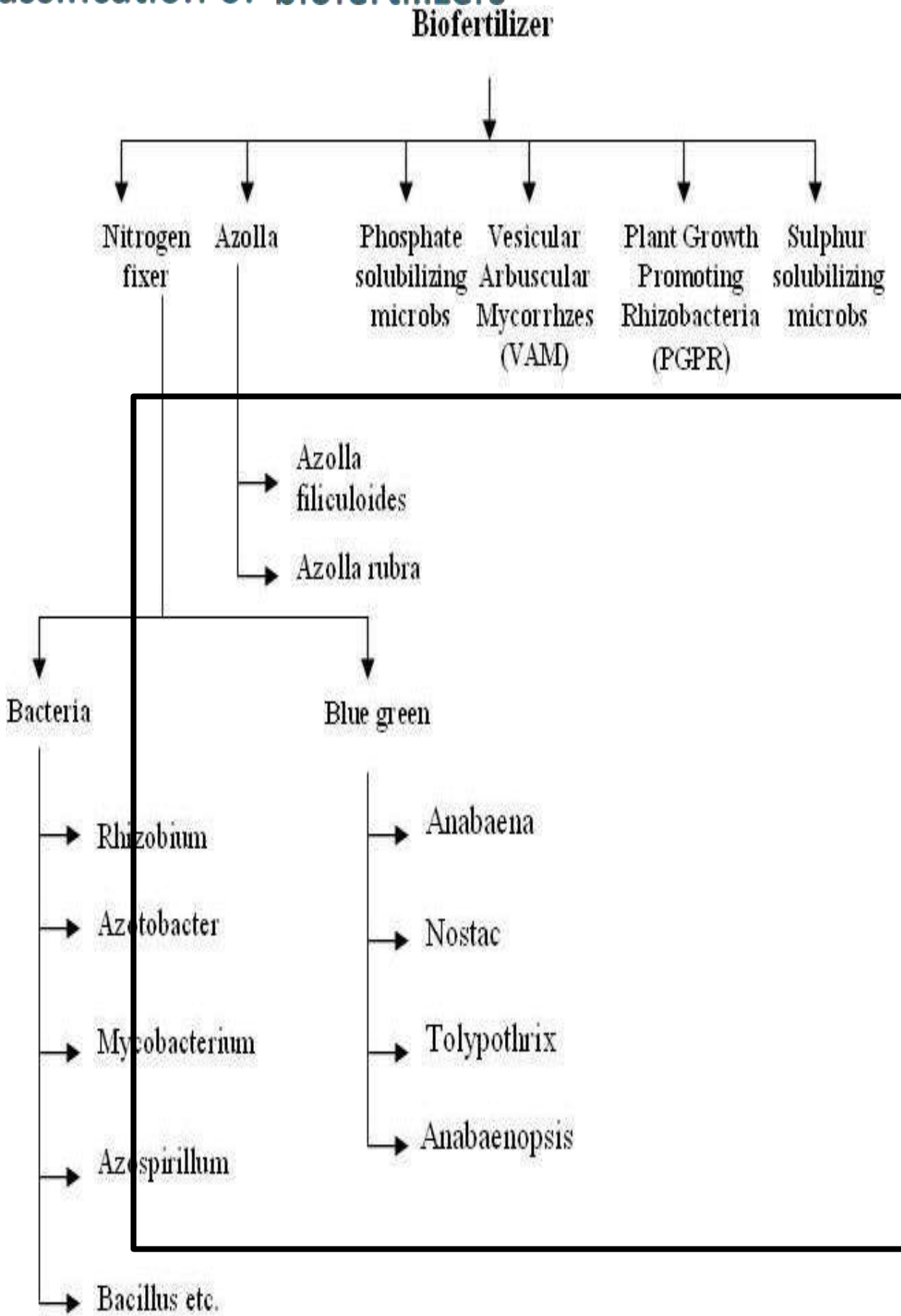
cultured in the laboratory and packed in a suitable carrier which increase the availability or uptake of nutrients for plants

Biofertilizer / microbial inoculants

- Biofertilizers are low cost renewable sources of plant nutrients
- Improve soil fertility and crop productivity
- They are the ideal input for starting organic farming



Classification of biofertilizers



Role of biofertilizers

*Makes availability of nutrients.

*Make the root rhizosphere more lively.

*Growth Promoting Substances are produced.

*More root proliferation.

*Better germination.

*Improve quality and quantity of produce.

*Improve fertilizer use efficiency.

*More biotic and abiotic stress tolerance.

*Improve soil health.

*Residual Effect.

*Make the system more sustainable.

Stages of development of biofertilizers

Mass production

- Isolated bacterial cultures were subculture in to nutrient broth
- The cultures were grown under shaking condition at 30±2°C
- The culture incubated until it reaches maximum cell population of 10^{10} to 10^{11}
- Under optimum condition this population level could be attained within 4-5 days for *Rhizobium* 5-7 days for *Azospirillum* and 6-7 days for *Azotobacter.*
- The culture obtained in the flask is called **Starter culture**
- For large scale production , inoculum from starter culture is transferred in to large flasks / fermentor and grown until required level of cell count is reached

Media used for mass culturing as follows:

- Rhizobium: YEMA(yeast extract mannitol Agar+ congored)
- Azospirillum:Dobereiners mallic acid broth with Sodium chloride
- Azatobacter: Waksmanna No.77broth
- Phosbacteria: Pikovaskys broth
- Pseudomonas: Kings B broth
- Trichoderma: PDB

Prepare appropriate media for specific to bacterial inoculant in required quantity

Inoculated with specific bacterial strain for aseptic condition

Incubated at 30±2°C for 5-7 days in rotary shaker. Observe growth of the culture and estimate the population (starter culture)

The above the media is prepared in large quantities in fermentor

Sterilized and cooled well

Media in a fermentor is inoculated with the log phase of culture grown in large flask (usually 1-2 % of inoculum is sufficient)

Cells are grown in fermentor by providing aeration & continuous stirring

Broth is checked for the population of inoculated organisms Cells

are harvested with the population load of 109 cells/ml

Carrier material

- The use of ideal carrier material is necessary for the production of good quality of biofertilizer

Ideal carrier material should be

- **Cheaper in cost**
- **Locally available**
- **High organic matter content**
- **No toxic chemical**
- **Water holding capacity of more than 50%**
- **Easy to process**

A : Press mud ; B : Lignite : C: Charcoal : D: Coconut Shell : E: Rice Husk : F: Cellulose Powder : G: Leaf Manure : H: Peat

Preparation of inoculants packet

- Neutralized and sterilized carrier material is spread in a clean, dry, sterile metallic or plastic
- Bacterial culture drawn from the fermentor is added to the sterilized carrier and mixed well by manual or mechanical mixer
- Inoculants are packed in a polythene bags sealed with electric sealer

Specification of the polythene bags

- Polythene bags should be of low density grade
- Thickness of bag should be around 50-75 micron
- Packet should be marked with the
- Name of the manufacture
- Name of the product
- Strain number
- The crops to which recommended
- Method of inoculation
- Date of manufacture
- Batch number
- Date of expiry
- Price
- Full address

Bio - fertilizers application methods

- Seed treatment/pelleting
- Root dipping
- Set treatment
- Soil applications

Seed treatment

- Seed treatment is a most common method adopted for all types of inoculants. The seed treatment is effective and economic
- The seeds are treated with biofertilizer are kept in shed for 30 mins and then seed bec sowing

Set treatment

- Sugarcane, cut pieces of potato and base of banana suckers
- Prepare the culture suspension by 1kg of biofertilizer with  (1:50) 

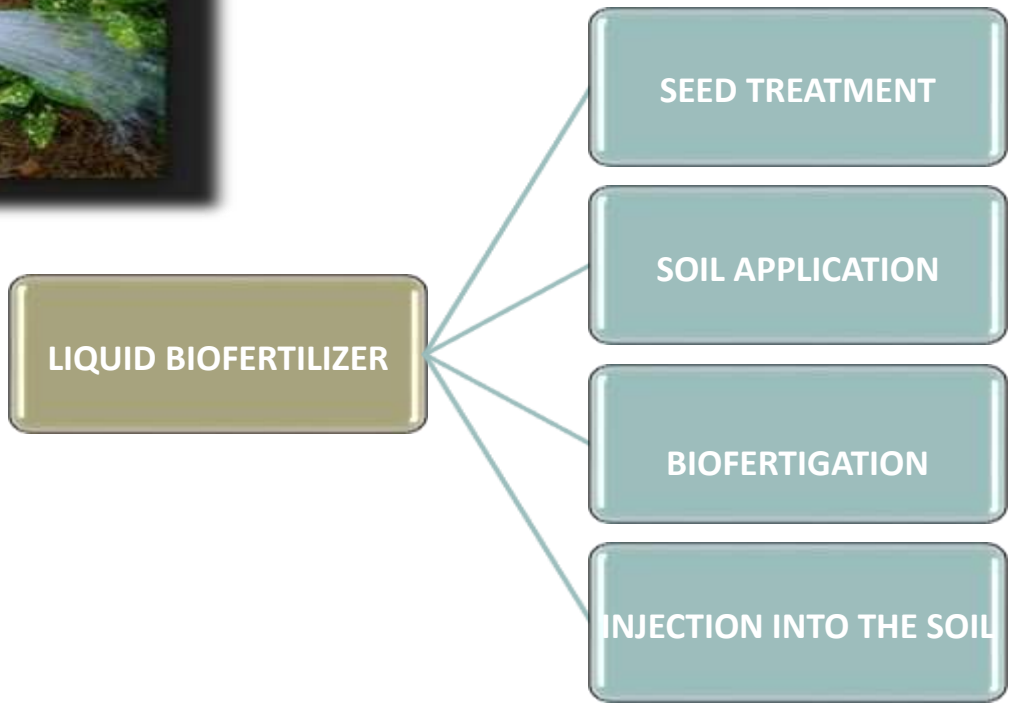
Seedling dipping

- The seedlings are uprooted from nursery and cleaned their roots in water dipped in solution of biofertilizer and kept in atleast 20 mins and transplant immediately
- Ratio about 1:10
- For root dipping : Dissolve the 1 pkt of biofertilizer with 20 litres of water (200-300 plants)
- One packet in 2 litres is sufficient to treat 200-300 sets under cutting method

Soil application

- The mixture of biofertilizer + compost + soil applied on land before sowing of seed or transplanting of the main field
- The mixture of biofertilizer and cattle manure/soil sprinkled with water is then broadcasted into the soil at the time of sowing or at the time irrigation in standing crop

Application of liquid biofertilizer



Disadvantages

- Biofertilizers require special care for long-term storage because they are alive.
- Must be used before their expiry date.
- If other microorganisms contaminate the carrier medium or if growers use the wrong strain, they are not as effective.
- Biofertilizers lose their effectiveness if the soil is too hot or dry.

BIO GAS PRODUCTION

Why Biogas?

- Dealing with wastes has become a nightmare for various people all over the world and no doubt has brought about sanitation problems.
- Power has also become a major concern especially in the less privileged rural areas.
- Integration of these two problems would be a plus point for various communities.
- From Biogas, various components can be powered by properly making use of the gas obtained. Less pollutant manure can be obtained ultimately.

Bioga
s

- Biogas is clean environment friendly fuel.
- Biogas is generated when bacteria degrade biological material in the absence of oxygen, in a process known as anaerobic digestion.
- Biogas generally comprise of 55-65 % methane, 35-45 % carbon dioxide, 0.5-1.0 % hydrogen sulfide and traces of water vapour.
- The heating value of biogas is about 60% of natural gas and about 25% of propane. [Average calorific value of biogas is 20 mj/m3].
- Biogas has corrosive nature and storage of biogas is not practical.

Process Of Bio-digestion

- Anaerobic digestion is basically a simple process carried out in a number of steps that can use almost any organic material as a substrate.
- Conventional anaerobic digestion is a "liquid" process, where waste is mixed with water to facilitate digestion. Since biogas is a mixture of methane and carbon dioxide it is a renewable fuel.

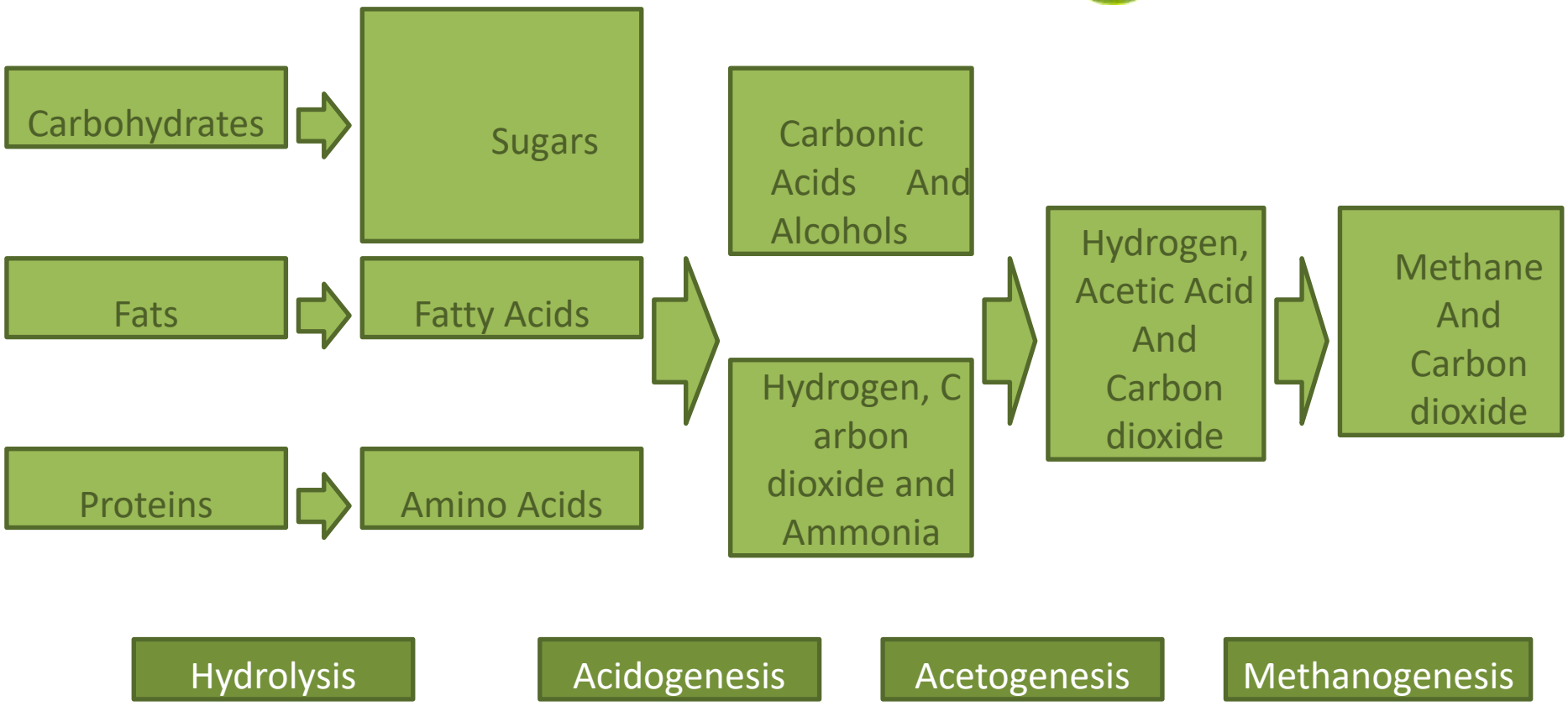
Process Of Bio-digestion

Biogas production process (Anaerobic digestion) is a multiple-stage process in which some main stages are:

Liquefaction Acid Production

Acetate Production Methane Production

Process Of Bio-digestion



Process Of Biodigestion

(1) LIQUEFICATION

- Complex organic matter is degraded to basic structure by hydraulic bacteria.
  - Protein -> Polypeptide and Amino Acid
  - Fat -> Glycerin and Fatty Acid
  - Amylase -> Monosacride and Polysacride

(2) ACID PRODUCTION (Acidogenesis)

- Simple organic matters are converted into H_2 and CO_2
- Acting bacteria in this process are called hydrogen-producing bacteria and acid-producing bacteria.

Process Of Biodigestion

(4)ACETATE PRODUCTION (Acetogenesis)

- The short-chain fatty acids are metabolized by synthrophic acetogenic and homoacetogenic bacteria into acetate, carbon dioxide, and hydrogen.

(5)METHANE PRODUCTION (Methanogenesis)

- In this process, acetic acid, H2, CO2, are converted into CH4.
- Methane-producing bacteria have strict PH requirement and low adaptability to temperature.

Biogas Production Potential From Different Wastes

	Raw Material	Biogas Production	Methane Content in Liters/Kg	biogas %
1	Cattle Dung	40		60.0
2	Green leaves and twigs	100		65.0
3	Food Waste	160		62.0
4	Bamboo Dust	53		71.5
5	Fruit waste	91		49.2
6	Bagasse	330		56.9
7	Dry Leaves	118		59.2
8	Non edible Oil Seed Cakes	242		67.5

Utilization Of Biogas

- **Cooking:** A biogas plant of 2 cubic meters is sufficient for providing cooking fuel needs of a family of about five persons.
- **Lighting:** Biogas is used in silk mantle lamps for lighting purposes. The requirement of gas for powering a 100 candle lamp (60 W) is 0.13 cubic meter per hour.
- **Power Generation:** Biogas can be used to operate a dual fuel engine to replace up to 80 % of diesel-oil. Diesel engines have been modified to run 100 per cent on biogas. Petrol and CNG engines can also be modified easily to use biogas.
- **Transport Fuel:** After removal of CO2, H2S and water vapor, biogas can be converted to natural gas quality for use in vehicles.

Benefits Of Biogas

- Availability of power at affordable rates
- Reduces pollution
- Reduces time wastage while collecting firewood
- Reduces reliance on fossil fuels
- Saves on the environment (Reduces deforestation)
- Improves living standards in rural areas
- Reduces global warming
- Produces good quality enriched manure to improve soil fertility.
- Effective and convenient way for sanitary disposal of organinc wastes, improving the hygienic conditions.
- As a smokeless domestic fuel it reduces the incidence of eye and lung diseases.

Countries Having Biogas Production Potential From Different Wastes

- India
- U.S.A
- Pakistan
- Bangladesh
- Germany
- China

Disadvantages Of Biogas

- The process is not very attractive economically on a large industrial scale.
- It is very difficult to enhance the efficiency of biogas systems.
- Biogas contains some gases as impurities, which are corrosive to the metal parts of internal combustion engines.
- Not feasible to set up at all the locations.

BIOPOLYMERS

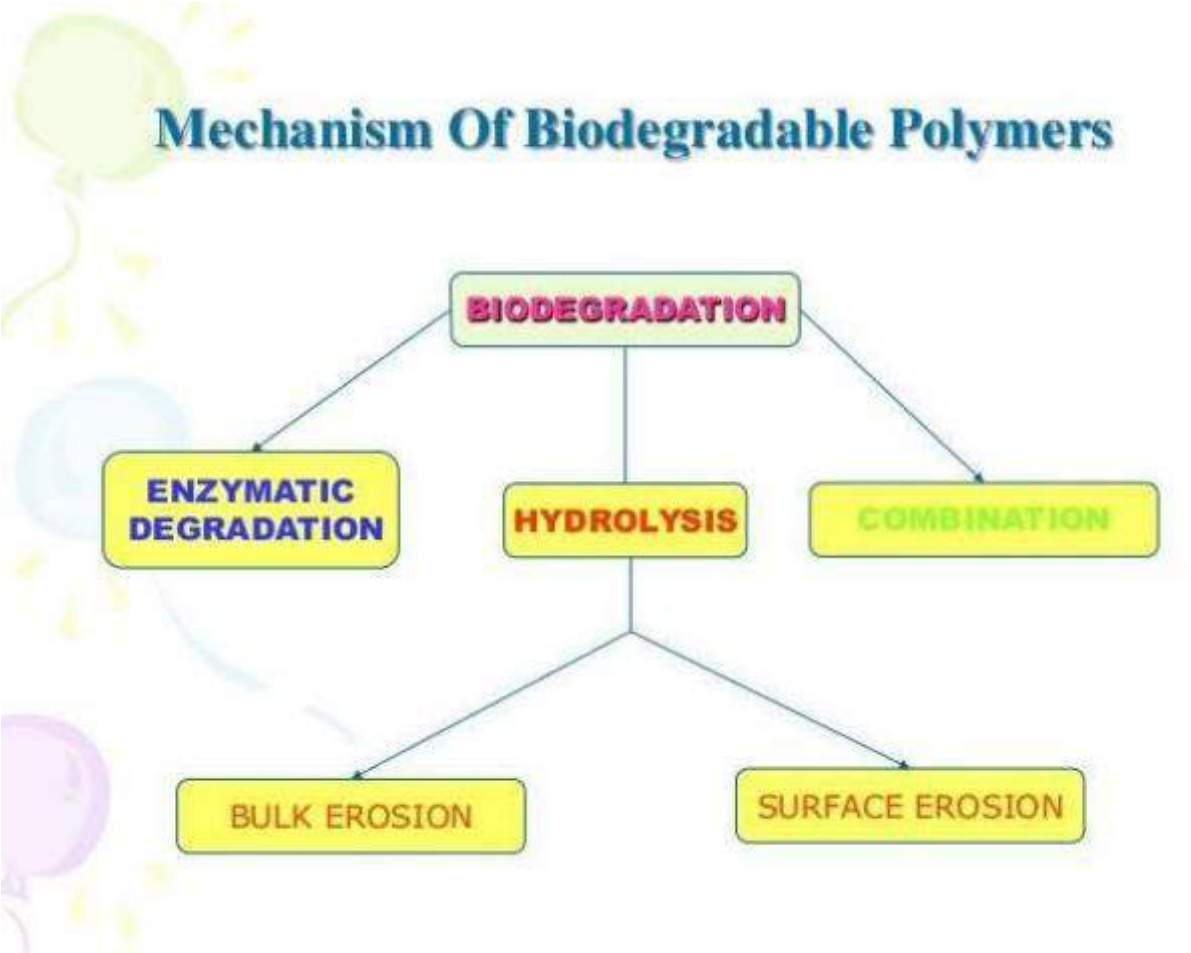
INTRODUCTION

- Bio polymer is a polymer that is developed from living beings.
- It is a biodegradable i.e., they are broken down into CO2 and water by micro organisms.
- In addition some of them are compostable i.e., they can be put into composting process.
- Ex: cellulose, starch, chitin, proteins, peptides, DNA and RNA.
- The most common biopolymer is Cellulose. It is also the most abundant organic compound on this planet. It comprises of 33% of all plant component on Earth.

WHY BIOPOLYMER?

- Polymers have become an essential part of our daily life. Having its numerous advantages it finds its use in every field. On the other side these polymer products account for approx. 150 million tons of non bio degradable waste every year. Such waste leads to various problems including pollution, soil erosion and other environmental problems.
- In order to over come this biopolymers have been found to replace the synthetioc polymers as they are degradable by microbes after its purpose, tthereby making the environment clean and safe.

PRODUCTION


Mechanism Of Biodegradable Polymers
BIODEGRADATION
ENZYMATIC DEGRADATION
HYDROLYSIS
COMBINATION
BULK EROSION
SURFACE EROSION

BIOPOLYMER CLASSIFICATION

- STARCH BASED POLYMER

  Starch acts as a natural polymer and can be obtained from wheat, tapioca, maize and potatoes. It is composed of glucose and can be obtained by melting starch. This polymer is not present in animal tissues.

  SOURCE

  Tapioca, corn, wheat and potatoes

- USES:

  It is used for molding process

- SUGAR BASED POLYMER:

  Sugar form the starting materials for these polymers. Polyactides are resistant to water and can be manufactured by methods like vacuum forming, blowing and injection molding.

- SOURCE:

  Potatoes, maize, wheat and sugar beet

- USES:

  It is used as surgical implants

- CELLULOSE BASED BIOPOLYMER:

  This polymer is composed of glucose and is the primary constituent of plant cellular walls.

- SOURCE:

  Natural resources like cotton, wood, wheat and corn

- USES:

  Packing cigarettes, CDS and confectionary.

- SYNTHETIC BASED BIOPOLYMERS

  These polymers are manufactured from synthetic components, they are completely compostable and bio-degradable.
- SOURCE:

  Petroleum
- Ex: Aliphatic Aromatic co polyester

- CHARACTERISTICS OF BIOPOLYMERS;

  Inert

  - Permeability
  - Non toxicity
  - Mechanical strength
  - Controlled rate of degradation
  - Tensile strength
  - Bio compatibility

APPLICATIONS

- PACKAGING SECTOR:

  BIOBAGS-made up of cornstarch, biodegradable and compostable biopolyester and vegetable oiland it is 100% biodegradable.

- AGRICULTURE SECTOR:

  Containers such as biodegradable plant pots and disposable containers and bags, fertilizers and chemical storage bags.

- AUTOMOBILE SECTOR:

Natural fibres aresubstitued for glass fibres as reinforcement materials in plastic parts of commercial vehicles and their waste products can be composted.

- MEDICAL SECTOR:
  - Biopolymer for occullar vascular orthopedic skin adhesive and surgical glues.
  - Many biomaterials like heart valve replacement and blood vessels are made up of teflon and poly urethane.

ENVIRONMENTAL BENEFITS

- They are carbon neutral and can always be renewed and are sustainable as they are composed of living materials.
- These polymers can reduce CO2 levels in the atmosphere and also decrease carbon emissions. It is because bio-degradation of these chemical compounds can release carbon dioxide that can be re-absorbed by crops grown as a substitute in their place.
- It is also compostable which means there is less chance of environmental pollution from this compound. This is one of the primary advantages of this chemical compound.
- They reduce dependency on non-renewable fossil fuels and are easily biodegradable and can decrease air pollution.
- It greatly reduces the harmful effect of plastic use on the environment. Long term use of biopolymer use will limit the use of fossil fuel.

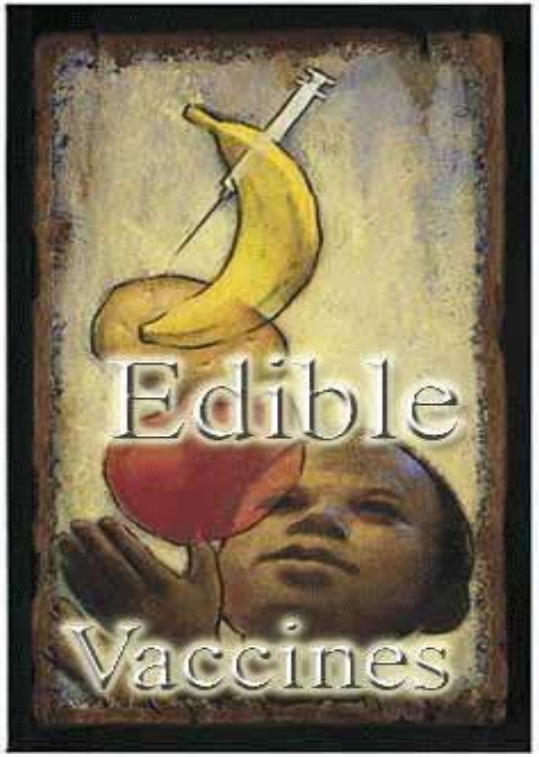
ENVIRONMENTAL IMPACTS

- Most of the biopolymers are not commercially viable due to their higher cost.
- Biopolymers are too unstable for long term industrial use and consumes more energy.
- They do not possess the strength and storage comparable to that of the conventional polymers.
- Trauma and death of marine species resulting from slow degradation of biodegradable plastic products in marine environments is caused.
- Soil and crop degradation resulting from the use of compost that may have unacceptably high organic and or metal contaminants.

EDIBLE VACCINE

EDIBLE VACCINE

- In the edible vaccine, Transgenic plants are used as vaccine production systems.
- The genes encoding antigens of bacterial and viral pathogens can be expressed in plants in a form in which they retain native immunogenic properties.
- Initially thought to be useful only for preventing infecti found application in prevention of autoimmune diseases therapy, etc.
- Edible vaccines are currently being developed for a r animal diseases.
- As Hippocrates said , Let "thy food be thy medicine"



Why edible vaccine?

Needle free

•Oral vaccines provide "mucosal immunity" at various sites by secreting antibodies.

•Don"t need to worry about re-use, misuse and lack of sterilization. Thus, low risk of infection.

Cheap

•Estimated cost of $0.005 to grow antigen for one dose of hepatitis B vaccine in an unprocessed form.

• Administering oral vaccines would require little or no
training at all.

storage

- Heat-stable; do not require cold-chain maintenance.
- If the local/native crop of a particular area is engineered to produce the vaccine, then the need for transportation and distribution can be eliminated.

safe

- Most importantly, they trigger the immunity at the mucosal surfaces such as mouth which is the body's first line of defense.
- Needs no purification.
- Edible vaccine activates both mucosal and systemic immunity

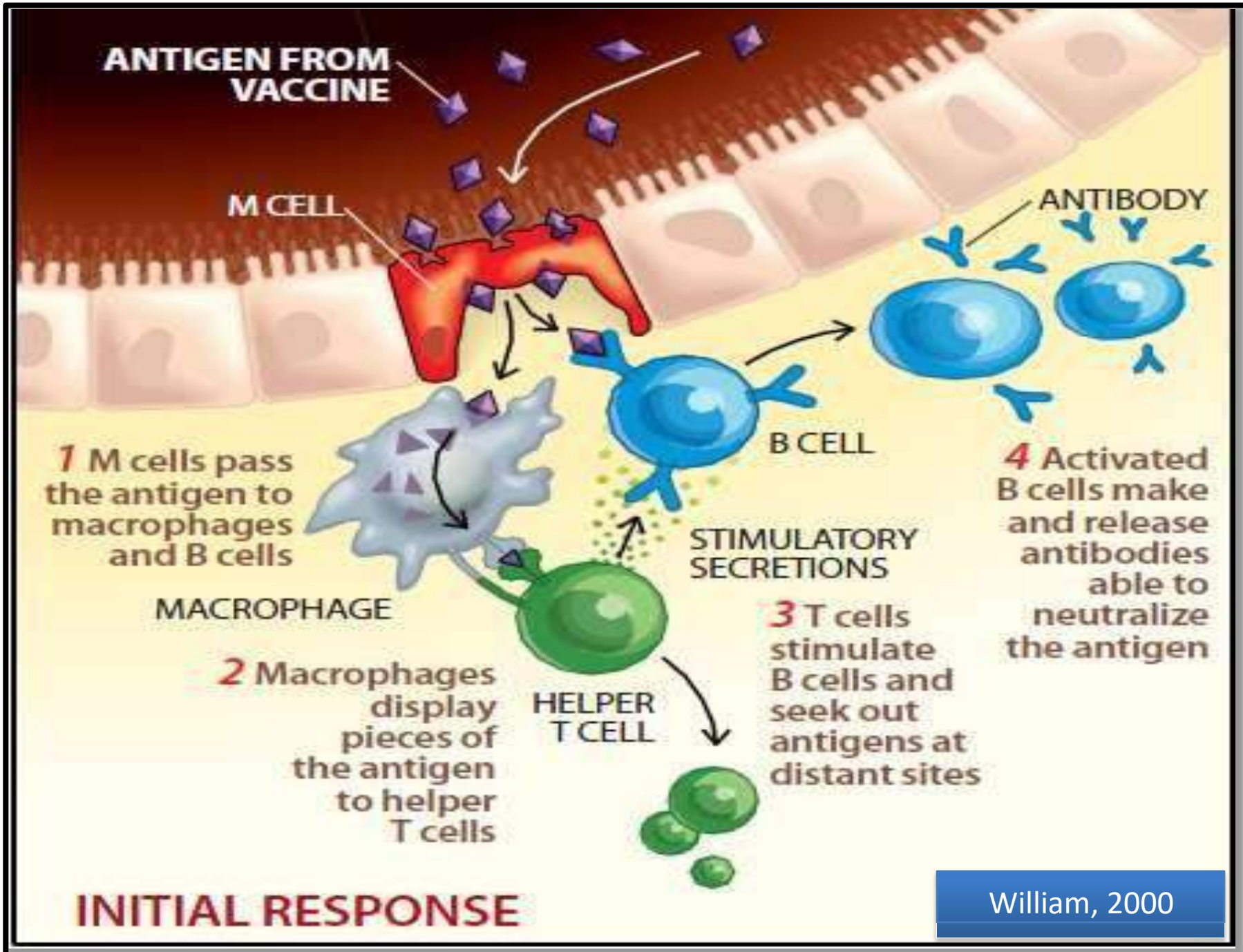
Mechanism of action

- The goal of oral vaccination is to stimulate the mucosal and systemic immunity against pathogen.

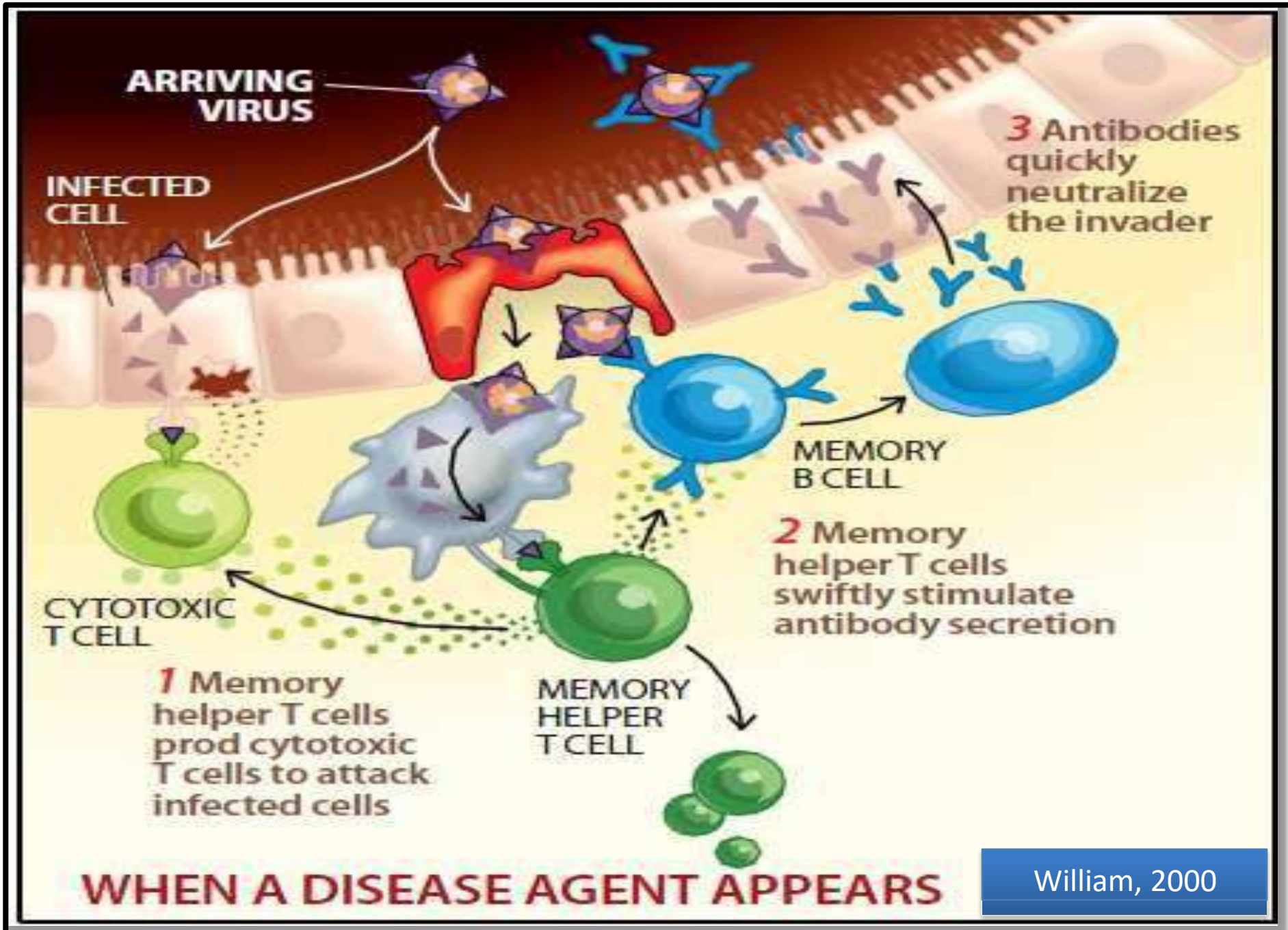
- Edible vaccine when taken orally undergoes the mastication process and the majority of plant cell degradation occur in the intestine as a result of action of digestive or bacterial enzyme on edible vaccine .

- Peyer‟s patches (PP) are an enriched source of Ig A producing plasma cells and have the potential to populate mucosal tissue and serves as mucosal immune effector site.

- The breakdown of edible vaccine near PP , consisting of the 30-40 lymphoid nodules on the outer surface of intestine and contain follicles.
- These follicles act as the site from which antigen penetrates the intestinal epithelium ,thereby accumulating antigen within organized lymphoid structure .
- The antigen then comes in contact with M-cell .
- M cell passes the antigen to macrophages and B cell.
- These B cell activates the T cell to provide immune response .
- In this way the immunity is activated by the edible vaccine.



William, 2000



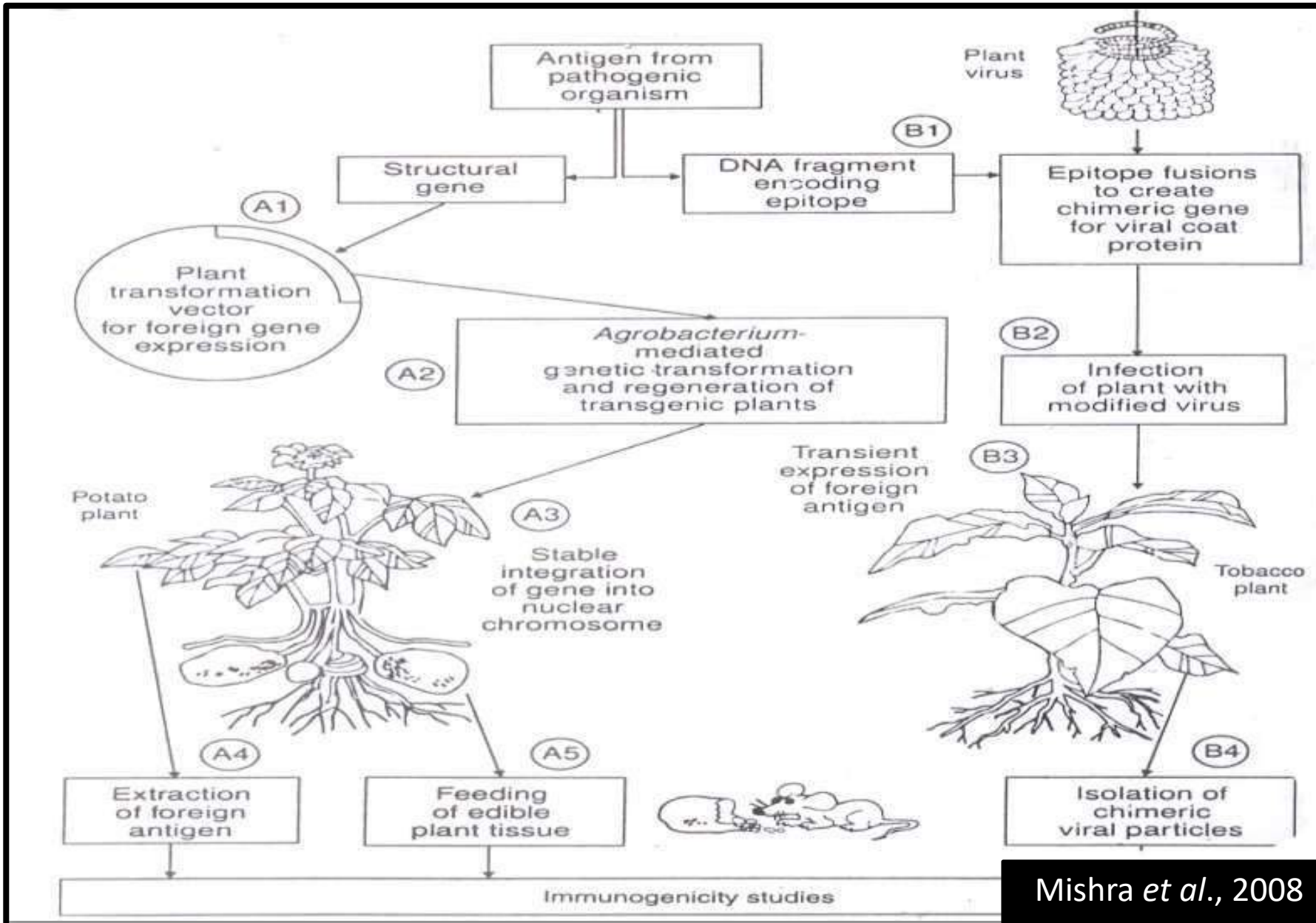
William, 2000

Developing an edible vaccine

- Two ways ……

- **In one case** , the entire structural gene is inserted into plant transformation vector between 5‟ and 3‟ regulatory element ; this will allow the transcription and accumulation of encoding sequence in the plant.

- **In the second case** , epitope within the antigen are identified ,DNA fragment encoding these can be used to construct gene by fusion with a coat protein gene from plant virus e.g. TMV or CMV .



Mishra *et al*., 2008

Production of edible vaccine antigen in plant tissu

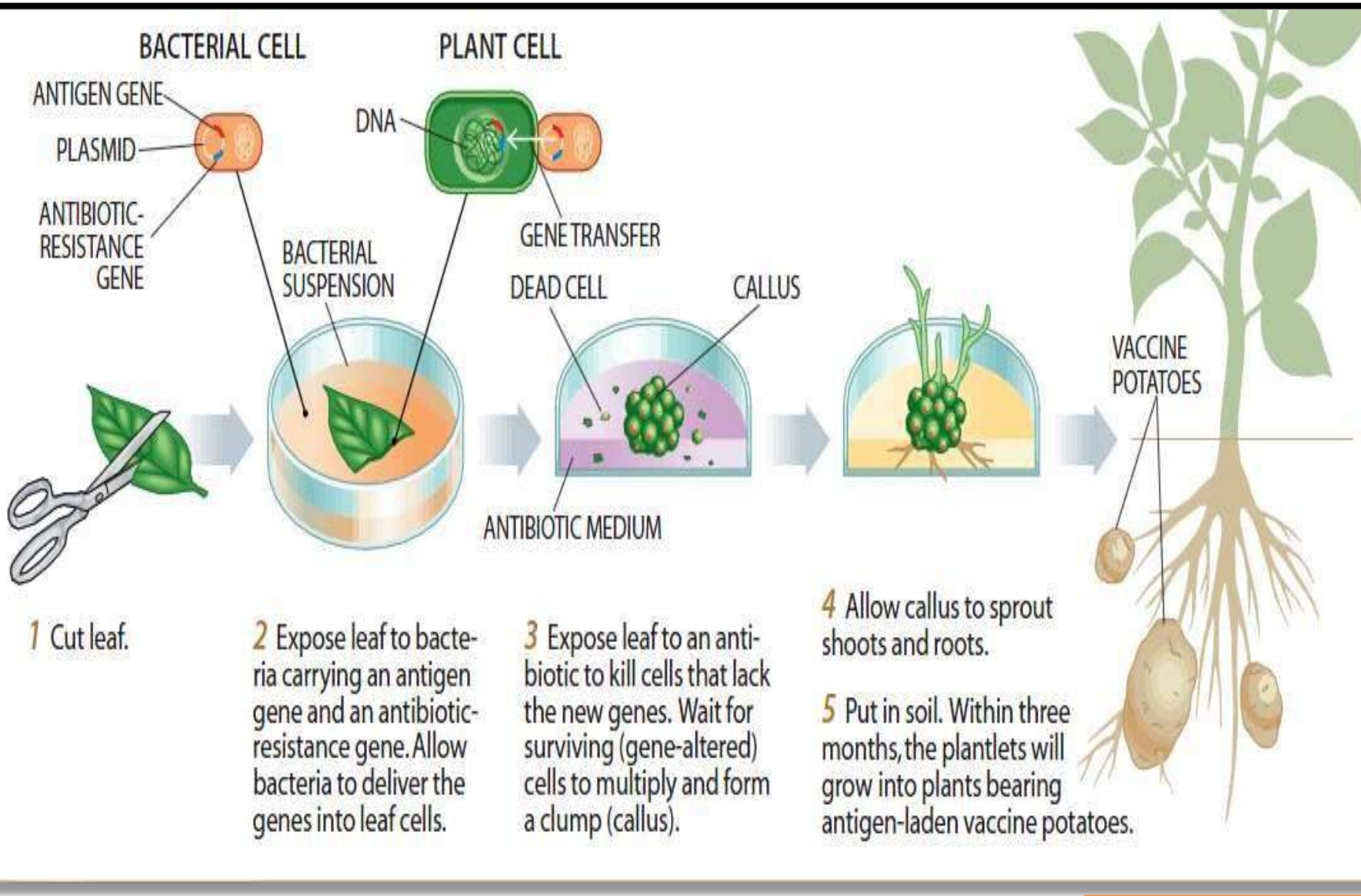
Method for transformation of DNA /gene into plant

1. Plasmid vector carrier system :
   Agrobacterium tumefaciens method.

2. Micro projectile bombardment method.

2. Electroporation method.

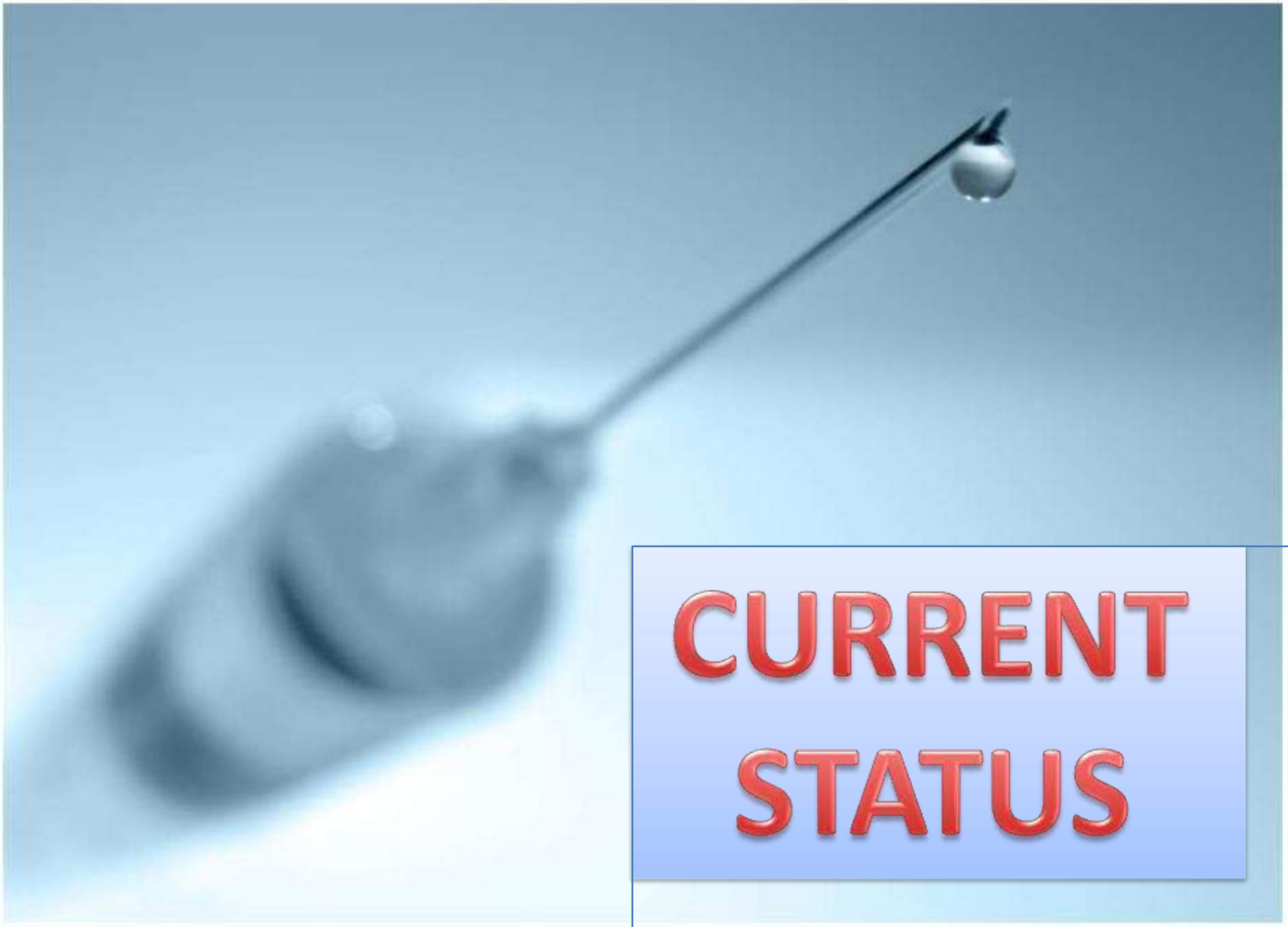
Agrobacterium tumefaciens method



William, 2000

Plants used for edible vaccine

1. Tobacco
2. Potato
3. Banana
4. Tomato
5. Rice
6. Lettuce
7. Soybean
8. Alfalfa
9. Muskmelon
10. Carrot
11. Peanuts
12. Wheat
13. Corn


CURRENT
STATUS

CLINICAL TRIALS

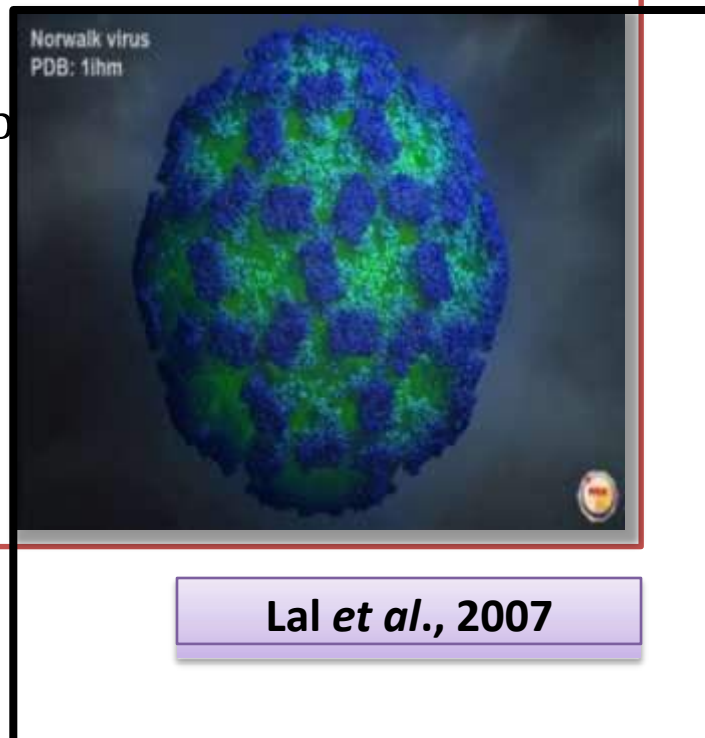
1. ETEC

- Boyce Thompson Institute, USA.
- Accomplished the first published successful human trial in 1997.
- Eleven volunteers were fed raw transgenic potatoes expressing LT-B.
- Ten (91%) of these individuals developed neutralizing antibodies, and six (55%) developed a mucosal response.

Lal *et al*., 2007

2. Norwalk virus

- Transgenic potato expressing norwalk virus antigen showed seroconversion.
- Nineteen (95%) out of 20 people fed with transgenic potato expressing norwalk virus antigen showed seroconversion .
- Attempts are underway to engineer bananas and p
  expressing norwalk virus.

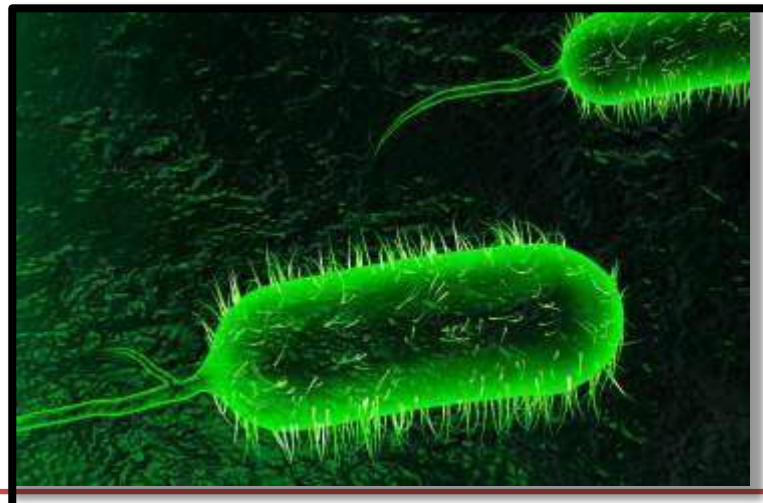


Lal *et al.*, 2007

3. Cholera

- Transgenic potato with CT-B gene of *Vibrio cholerae was* shown to be effective in mice.

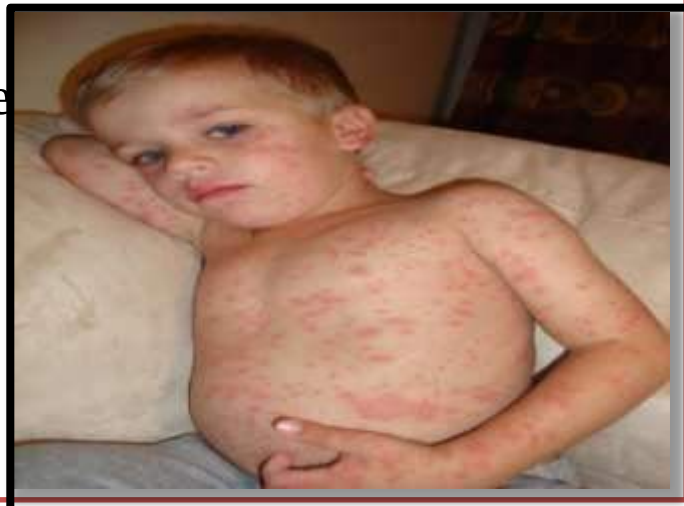
- Eating one potato a week for a month with periodic boosters was said to provide immunity.



Lal *et al*., 2007

4. Measles

- Mice fed with tobacco expressing MV-H could attain antibody titers five times the level considered protective for humans.
- MV-H edible vaccine does not cause atypical measles, which may be occasionally seen with the current vaccine.
- Transgenic rice, lettuce and baby food against me developed.

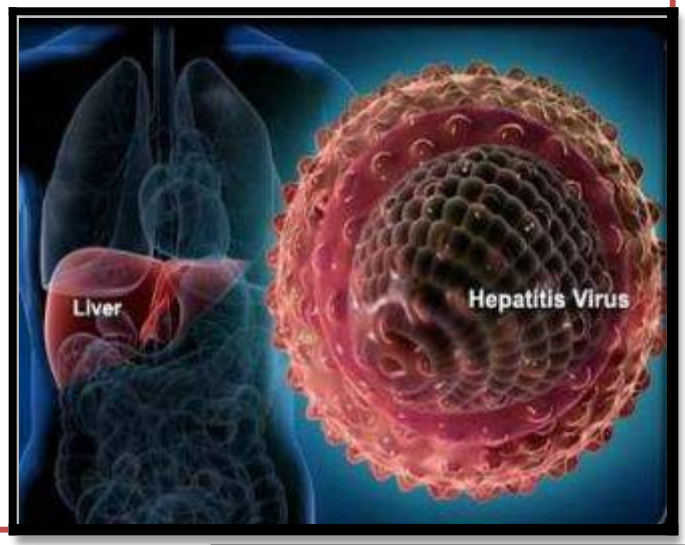


Mishra *et al.*, 2008

5. Hepatitis B

- For hepatitis B, parenteral VLPs could invoke specific antibodies in mice.
- First human trials of a potato based vaccine against hepatitis B have reported encouraging results.
- The amount of HBsAg needed for one dose could be achieved in a single potato.
- When cloned into CaMv , plasmid HBsAg

  subtype showed higher expression in roots as compared to leaf tissue of the transgenic potato.



Mishra *et al*., 2008

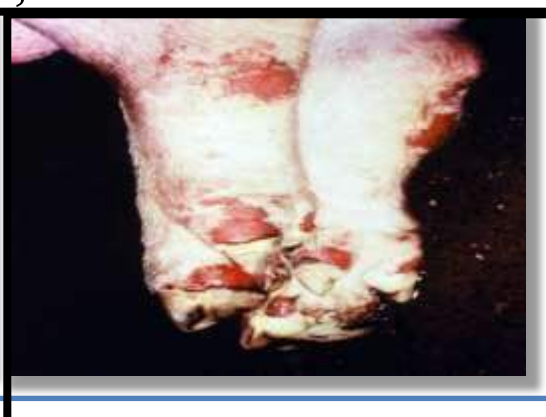
1. Newcastle disease

- NDV is highly infectious, affecting domestic poultry and wild birds.
- NDV transmission occurs through direct contact with secretions or discharge of infected birds, and contact with fomites.
- The world‟s first regulatory approval for a PMV was against NDV.
- The HN protein from NDV was expressed in a tobacco cell system and found to retain the size and immunoreactivity.

Ling *et al.*, 2010

2.Foot-and-mouth disease

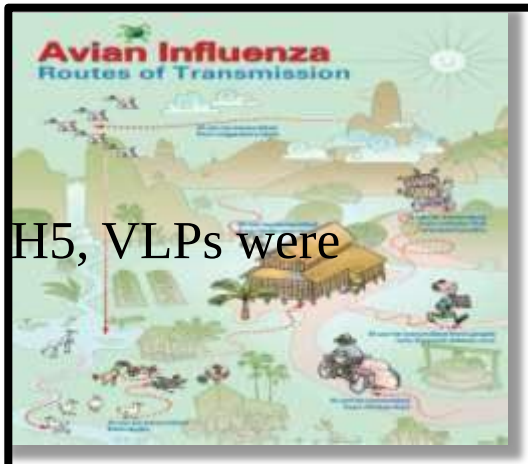
- Foot-and-mouth disease (FMD) is one of the most contagious viral diseases of wild ruminant and domestic animals.
- The causative pathogen, FMD virus (FMDV).
- FMDV is a single-stranded, positive-sense RNA virus, possessing four capsid proteins VP1 , VP2 , VP3 and VP4 .
- The VP1 protein is the critical determinant for vaccination against FMD with the induction of VP1-neutralizing antibodies required for immunity.
- Studies have shown the potential of using VP1 capsid protein as a subunit PMV candidate, in potato, tobacco, and tomato.



Ling *et al*., 2010

3.Avian influenza

- There are three influenza viral types A, B and C with distinct pathogenicity and genome properties.
- Influenza type A virus is endemic in aquatic birds. It is contagious not only to avian species but also to a variety of mammals. Influenza types B and C infect mainly humans and are generally less lethal.
- High accumulation of VLPs made from HA antigen was observed to be immunogenic.
- Mice immunized intramuscularly with doses of purified H5, VLPs were protected against influenza virus.



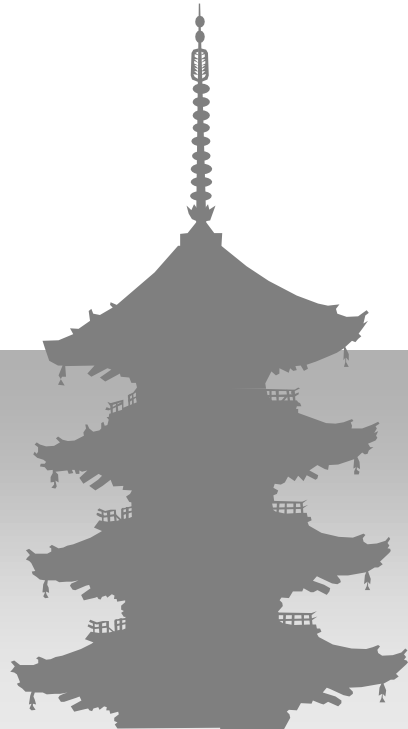
Ling *et al.*, 2010

SAFETY ISSUE

- Allergenic and toxic potential of plant components. (e.g. glycans, nicotine)
- . Potential for interference.
- . Production of oral tolerance.
- . Risk of a typical measles. (in plants with cloned measles virus genes)?
- . Health and environmental risks of GMO.
- . Prevention of misuse/overuse.

UNIT 3

NITROGEN FIXATION



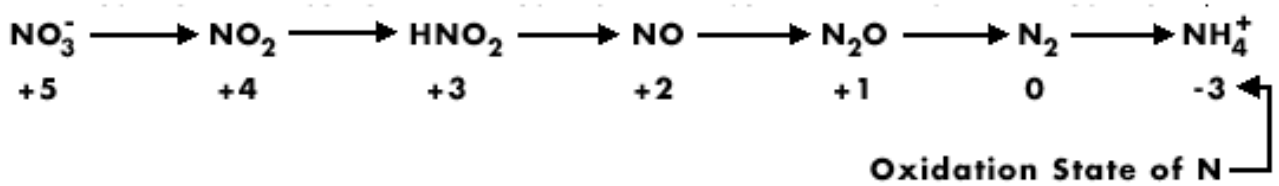
Nitrogen in the Biosphere

•Nitrogen is required in large amounts as an essential component of proteins, nucleic acids, and other cellular constituents.

•Nitrogen is the most abundant element in our atmosphere - nearly 79% in the form of N_2 gas.

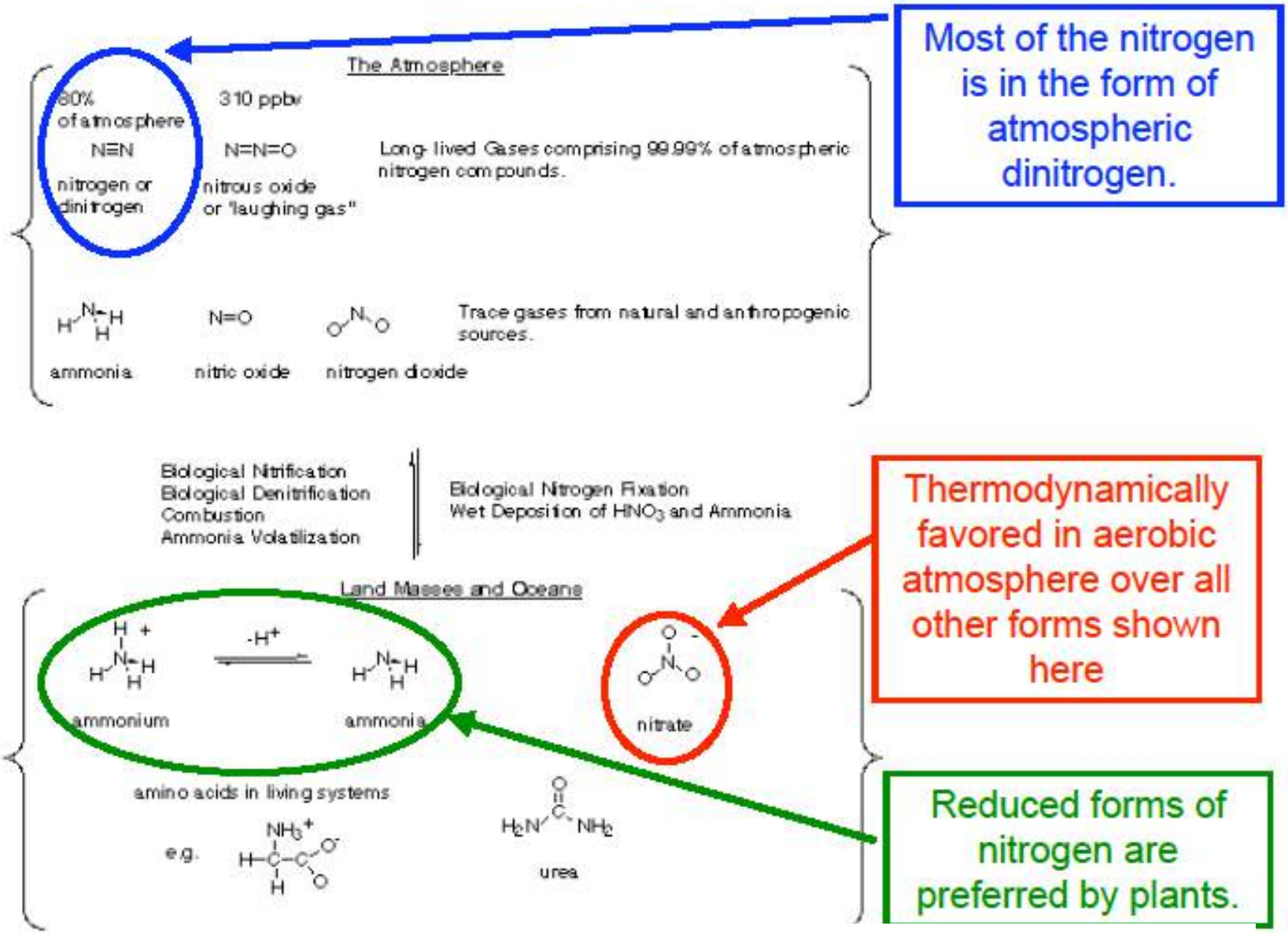
•In order for nitrogen to be used for growth it must be "fixed" (combined) in the form of ammonium (NH_4^+) or nitrate (NO_3^-) ions.

•Plants are able to use nitrogen in the form of nitrate (NO_3^-) or ammonia (NH_3^+), but these compounds are present in limited supply in the soil.



Nitrogen Fixation

- The growth of all organisms depend on the availability of Nitrogen (e.g. amino acids)
- Nitrogen in the form of Dinitrogen (N_2) makes up 80% of the air we breathe but is essentially inert due to the triple bond (N N)
- In order for nitrogen to be used for growth it must be "fixed" (combined) in the form of ammonium (NH_4) or nitrate (NO_3) ions.



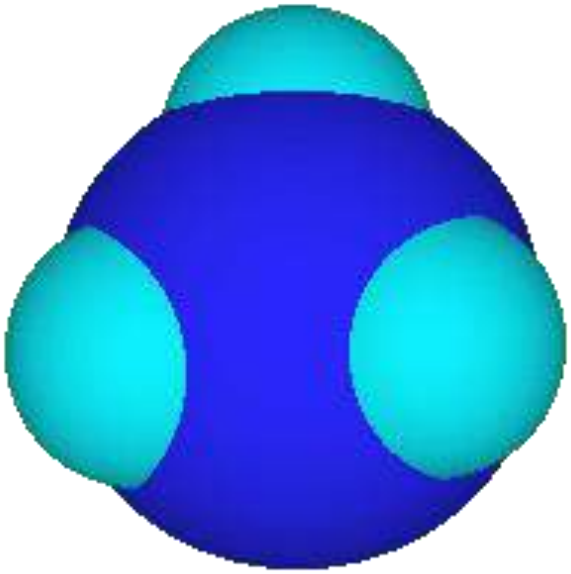
http://chem-faculty.ucsd.edu/trogler/CurrentNitroWeb/Section1/section2.shtml

Nitrogen Fixation

- **The nitrogen molecule (N_2) is quite inert. To break it apart so that its atoms can combine with other atoms requires the input of substantial amounts of energy.**

- **Three processes are responsible for most of the nitrogen fixation in the biosphere:**
- atmospheric fixation
- biological fixation
- industrial fixation

Industrial Fixation



- **Under great pressure, at a temperature of 600oC, and with the use of a catalyst, atmospheric nitrogen and hydrogen (usually derived from natural gas or petroleum) can be combined to form ammonia (NH_3).**
- **Ammonia can be used directly as fertilizer, but most of its is further processed to urea and ammonium nitrate (NH_4NO_3).**

Nitrogen Fixation Process

Energetics

- $N \equiv N$
- Haber-Bosch (100-200 atm, 400-500°C, 8,000 kcal kg^{-1} N)
- Nitrogenase (4,000 kcal kg^{-1} N)

Properties of dinitrogen which makes it inert

- Dinitrogen - two N atoms connected by triple bond
- Breaking the N N bond is difficult - high dissociation energy of 942 kJ mol^{-1}
- Breaking first bond requires 540 kJ mol^{-1}
- Very weak base – no interaction with even strong acids
- Non-polar

Initial hydrogenation is highly endothermic for N_2

$N_2 + H_2 \longrightarrow N_2H_2$ H = 213.5 kJ mol^{-1}

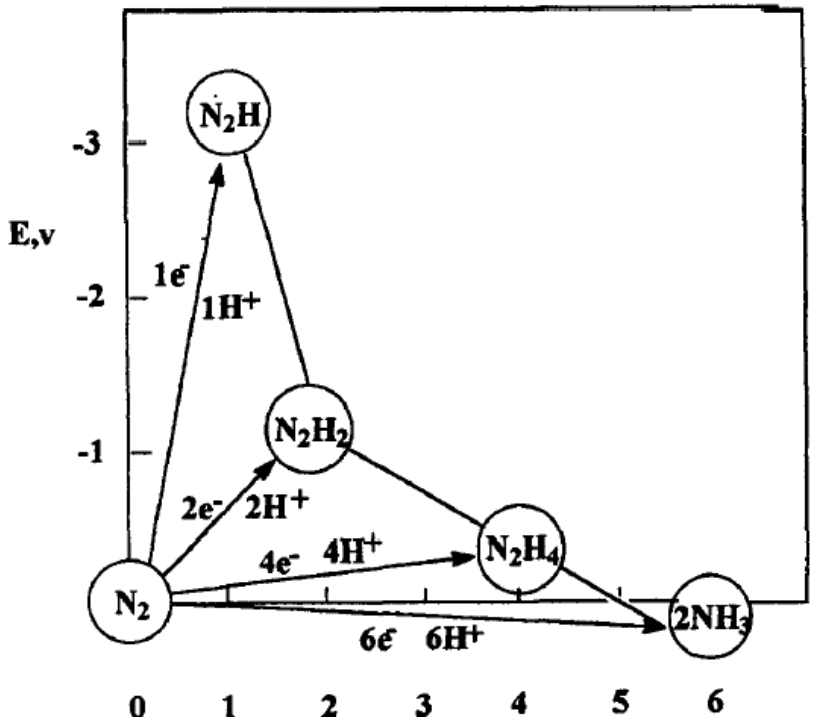
$2\,C + H_2 \longrightarrow C_2H_2$ H = -175.8 kJ mol^{-1}

Other important properties

Property \ Gas	Nitrogen	Carbon	Oxygen	Argon
Ionization potential (eV)	14.3	11.256	13.614	15.755
Electron affinity (eV)	0.073	1.595	1.461	0
Solubility in water (mole/cm^3)	0.083	Insoluble	0.153	0.140

- High ionization potential and low electron affinity - difficult to reduce and oxidize
- Solubility very less - reactions in solution phase - difficult

Stepwise redox potentials



Redox potential dependence on the number of electrons transferred

- Initial two electron transfer requires higher potential
- NH_3 formation - six electron process - less probable

Chatt J, Camara L M P, Richards R L, *New Trends in the Chemistry of Nitrogen Fixation*, Academic Press, (1980)

Biological fixation of dinitrogen

- Enzyme nitrogenase
- Present in soil bacteria, root nodules and algae
- Two decades of research - mechanism not established
- Enzyme contains Mo and Fe
- Proposed mechanism - complexation of N_2 to metal ions
- Reduces bond strength - breaking 1st bond easier

Limitations with biological route:

- Nitrogenase - sensitive to O_2 – requires O_2 free environment
- Sensitive to environmental conditions - temperature, pH
- Cannot be used for large scale N_2 fixation

Biological Fixation

The ability to fix nitrogen is found only in certain bacteria.

- **Some live in a symbiotic relationship with plants of the legume family (e.g., soybeans, alfalfa).**
- **Some establish symbiotic relationships with plants other than legumes (e.g., alders).**
- **Some nitrogen-fixing bacteria live free in the soil.**
- **Nitrogen-fixing cyanobacteria are essential to maintaining the fertility of semi-aquatic environments like rice paddies.**

Biological Fixation cont.

- **Biological nitrogen fixation requires a complex set of enzymes and a huge expenditure of ATP.**

- **Although the first stable product of the process is ammonia, this is quickly incorporated into protein and other organic nitrogen compounds.**

- **Scientist estimate that biological fixation globally adds approximately 140 million metric tons of nitrogen to ecosystems every year.**

Some nitrogen fixing organisms

- **Free living aerobic bacteria**
  - ***Azotobacter***
  - ***Beijerinckia Klebsiella***
  - ***Cyanobacteria* (lichens)**
  -
- **Free living anaerobic bacteria**
  - ***Clostridium***
  - ***Desulfovibrio***
  - ***Purple sulphur bacteria***
  - ***Purple non-sulphur bacteria***
  - ***Green sulphur bacteria***
- **Free living associative bacteria**
  - ***Azospirillum***
- **Symbionts**
  - ***Rhizobium* (legumes)**
  - ***Frankia* (alden trees)**

Some nitrogen fixing organisms

Free living				Symbiotic	
Aerobes		Anaerobes		Leguminous plants	Non leguminous plants
Heterotrophs	Phototrophs	Heterotrophs	Phototrophs		
Azotobacter spp. *Klebsiella* *Beijerinckia* *Bacillus polymyxa* *Mycobacterium flavum* *Azospirillum lipoferum* *Citrobacter freundii* Some Methylotrophs	various *Cyanobacteria*	*Clostridium* spp. *Desulfovibrio* *Disulfotomaculum*	*Chromatium* *Chloribium* *Rhodospirillum* *Rhodopseudomonas* *Rhodomicrobium* *Rhodobacter* *Heliobacterium*	soybeans, clover, locust, etc In association with a bacterium of the genus *Rhizobium* or *Bradyrhizobium*	*Alnus*, *Myrica* *Ceanthus* *Comptorinia* *Casurina* in assocation with actinomycetes of the genus *Frankia*

Genetics of Nitrogenase

Gene	Properties and function
nifH	Dinitrogenase reductase
nifDK	Dinitrogenase
nifA	Regulatory, activator of most *nif* and *fix* genes
nifB	FeMo cofactor biosynthesis
nifEN	FeMo cofactor biosynthesis
nifS	Unknown
fixABCX	Electron transfer
fixK	Regulatory
fixLJ	Regulatory, two-component sensor/effector
fixNOQP	Electron transfer
fixGHIS	Transmembrane complex

Types of Biological Nitrogen Fixation

Free-living (asymbiotic)

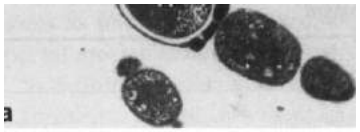
- Cyanobacteria
- *Azotobacter*

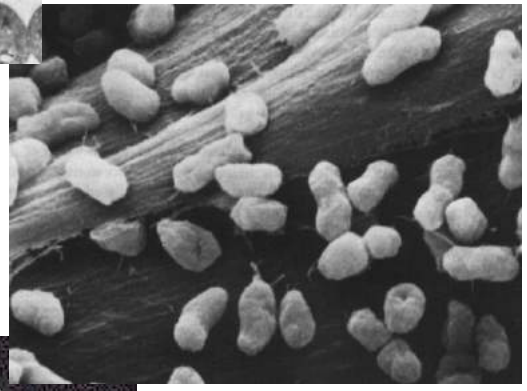
Associative

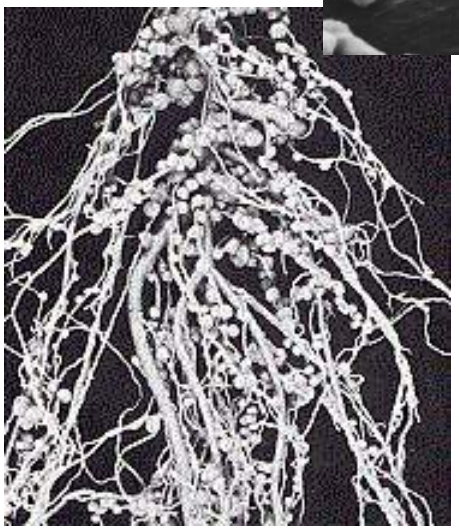
- Rhizosphere–*Azospirillum*
- Lichens–cyanobacteria
- Leaf nodules

Symbiotic

- Legume-rhizobia
- Actinorhizal-*Frankia*







Free-living N_2 Fixation

Energy

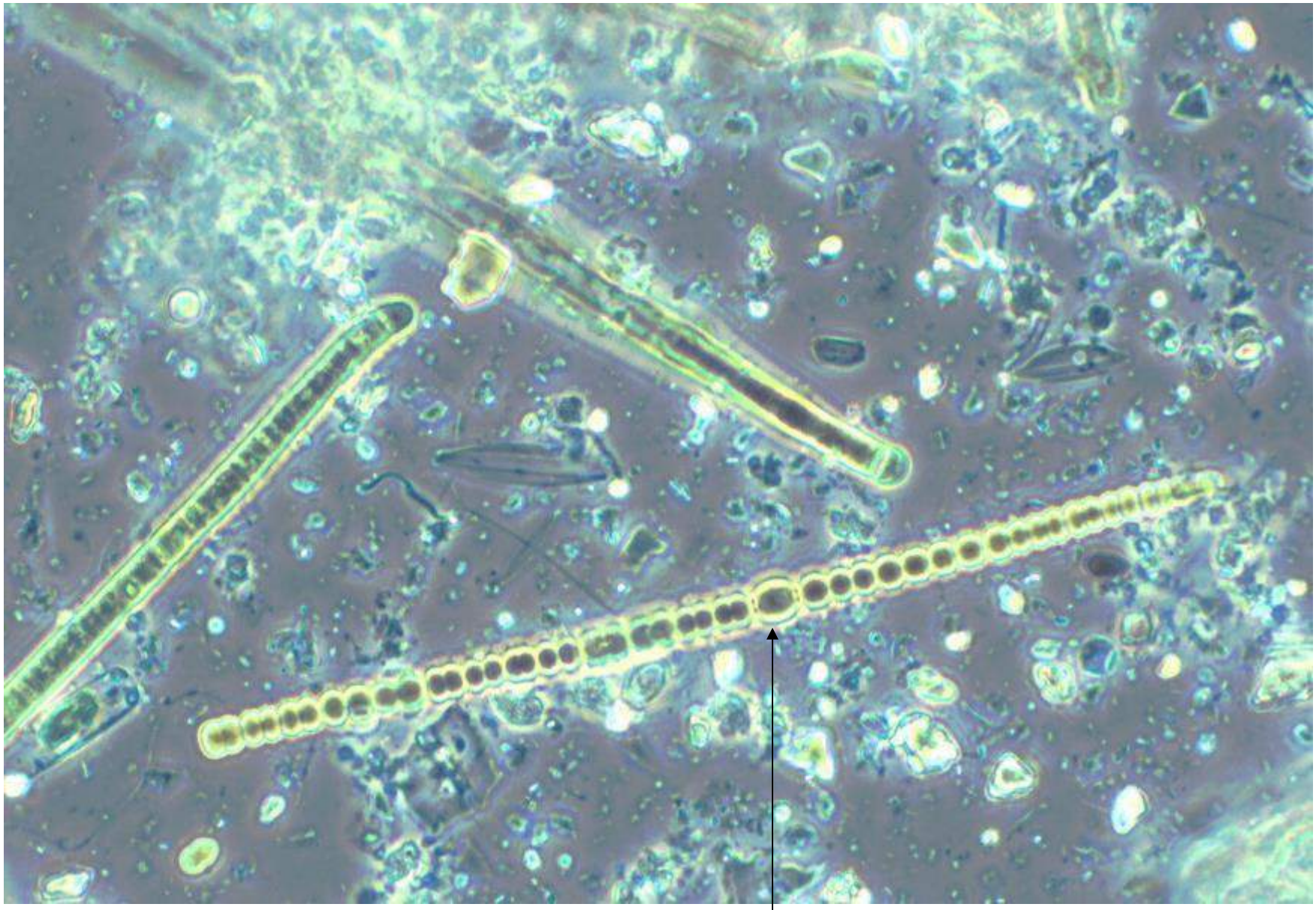
- 20-120 g C used to fix 1 g N

Combined Nitrogen

- *nif* genes tightly regulated
- Inhibited at low NH_4^+ NO_3^-(1 μg g^{-1} soil, 300 μM) and

Oxygen

- Avoidance (anaerobes)
- Microaerophilly Respiratory
- protection Specialized cells
- (heterocysts, Spatial/temporal vesicles)
- separation
- Conformational protection


Heterocyst

Associative N_2 Fixation

- Phyllosphere or rhizosphere (tropical grasses)
- *Azosprillum*, *Acetobacter*
- 1 to 10% of rhizosphere population
- Some establish within root
- Same energy and oxygen limitations as free-living
- *Acetobacter diazotrophicus* lives in internal tissue of sugar cane, grows in 30% sucrose, can reach populations of 10^6 to 10^7 cells g^{-1} tissue, and fix 100 to 150 kg N ha^{-1} y^{-1}

Phototrophic N_2-fixing Associations

- Lichens–cyanobacteria and fungi
- Mosses and liverworts–some have associated cyanobacteria
- *Azolla-Anabaena (Nostoc)*–cyanobacteria in stem of water fern
- *Gunnera-Nostoc*–cyanobacteria in stem nodule of dicot
- *Cycas-Nostoc*–cyanobacteria in roots of gymnosperm

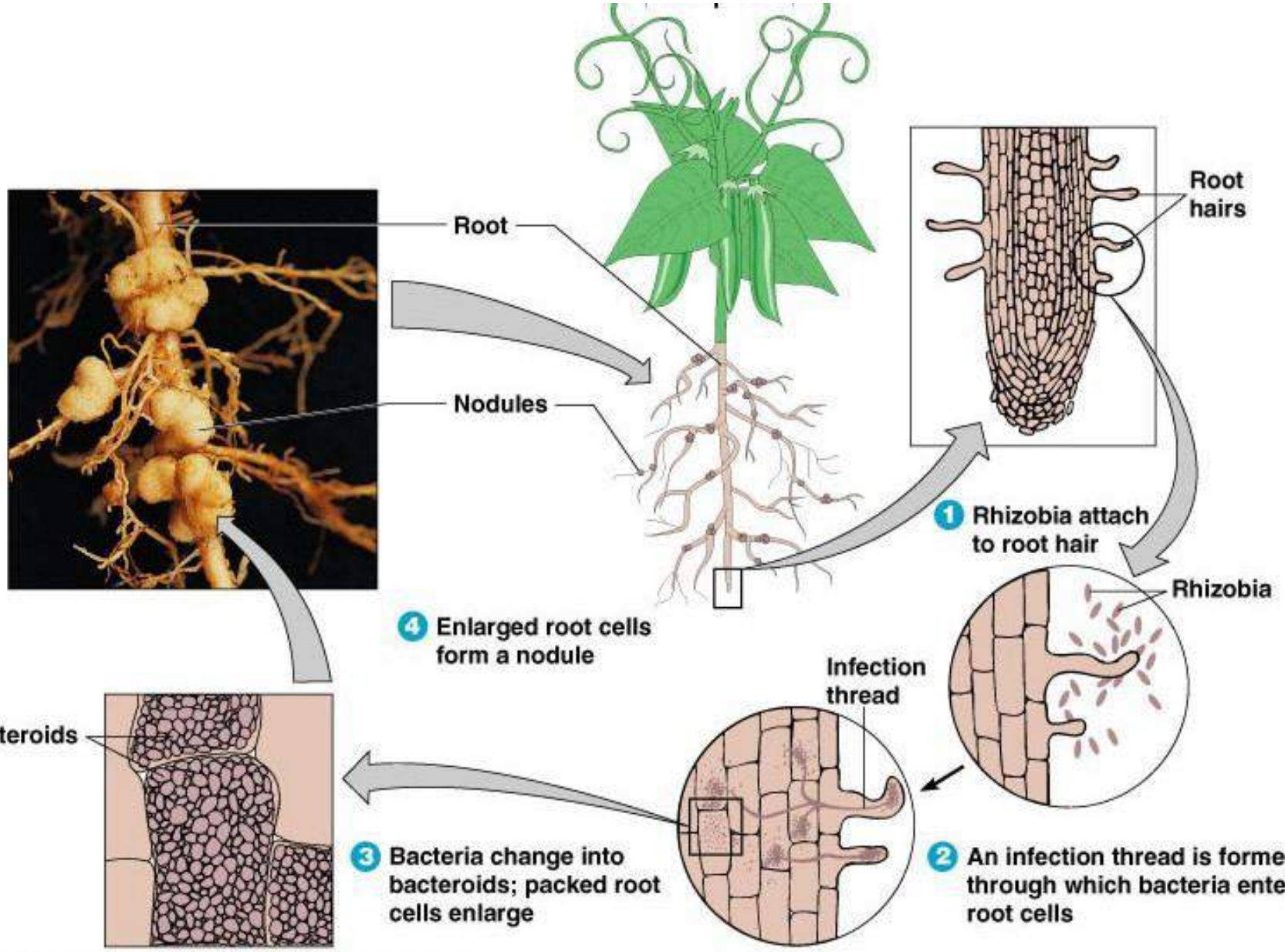
Actinorhizal Plant Hosts

Family	Genera
Betulaceae	*Alnus*
Casuarinaceae	*Allocasuarina*, *Casuarina*, *Ceuthostoma*, *Gymnostoma*
Myricaceae	*Comptonia*, *Myrica*
Elaeagnaceae	*Elaeagnus*, *Hippophaë*, *Shepherdia*
Rhamnaceae	*Ceanothus*, *Colletia*, *Discaria*, *Kentrothamnus*, *Retanilla*, *Talguenea*, *Trevoa*
Rosaceae	*Cercocarpus*, *Chamaebatia*, *Cowania*, *Dryas*, *Purshia*
Coriariaceae	*Coriaria*
Datiscaceae	*Datisca*

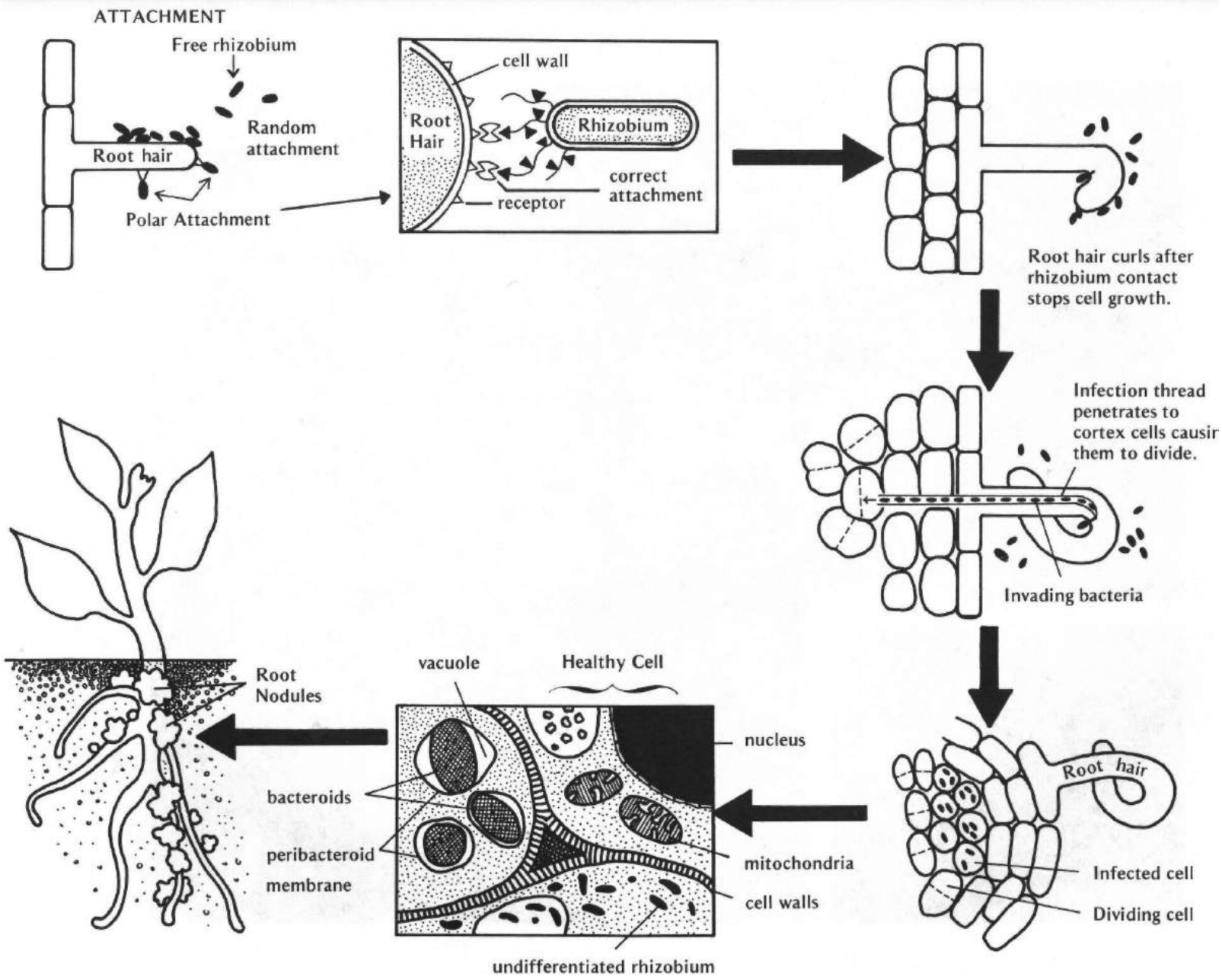
Legume-*Rhizobium* Symbiosis

- The subfamilies of legumes (Caesalpinioideae, Mimosoideae, Papilionoideae), 700 genera, and 19,700 species of legumes
- Only about 15% of the species have been evaluated for nodulation
- *Rhizobium*
  - Gram -, rod
  - Most studied symbiotic N_2-fixing bacteria
  - Now subdivided into several genera
  - Many genes known that are involved in nodulation (*nod*, *nol*, *noe* genes)

Formation of a Root Nodule

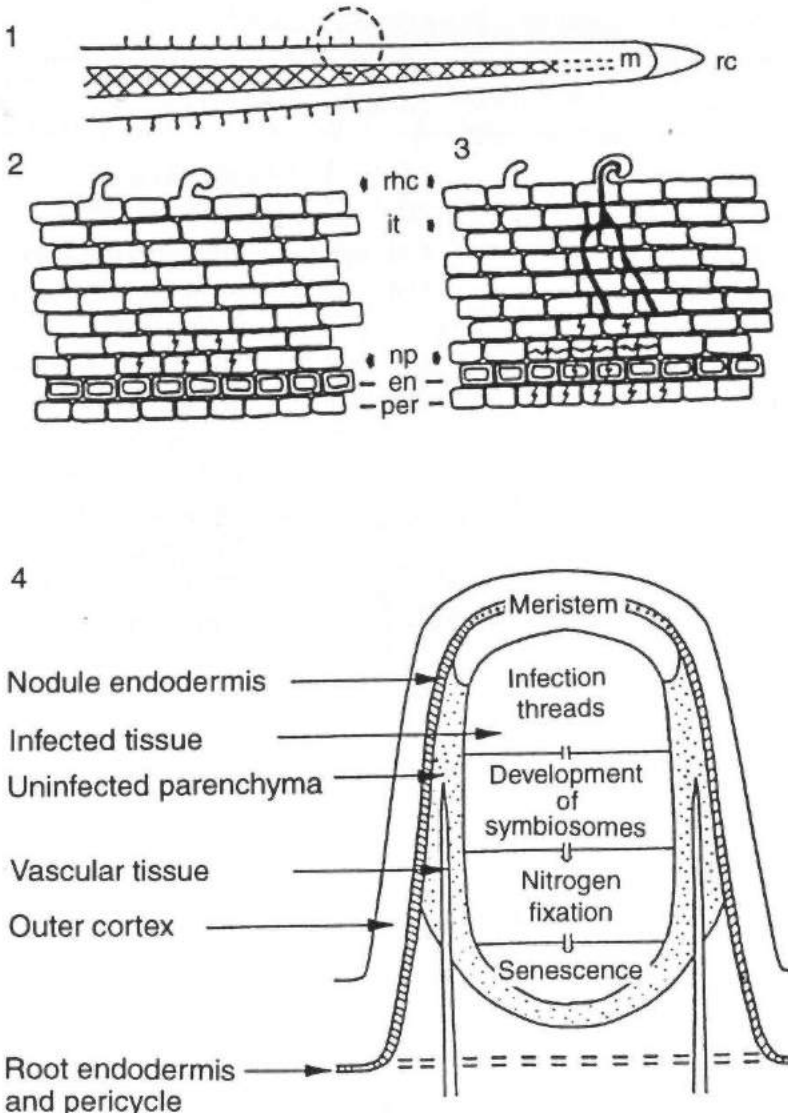


Nodulation in Legumes



Infection Process

- Attachment
- Root hair curling
- Localized cell wall degradation
- Infection thread
- Cortical cell differentiation
- Rhizobia released into cytoplasm
- Bacterioid differentiation (symbiosome formation)
- Induction of nodulins



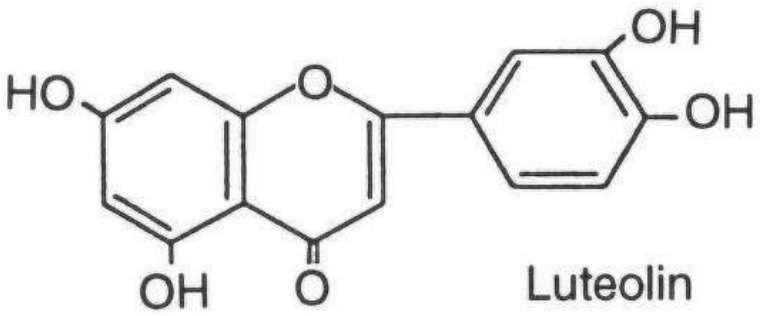
Role of Root Exudates

General

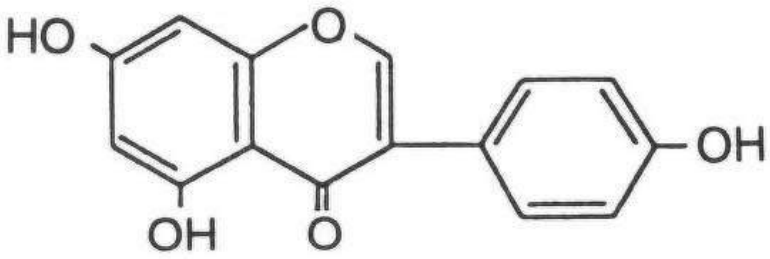
- Amino sugars, sugars

Specific

- Flavones (luteolin), isoflavones (genistein), flavanones, chalcones
- Inducers/repressors of *nod* genes
- Vary by plant species
- Responsiveness varies by rhizobia species

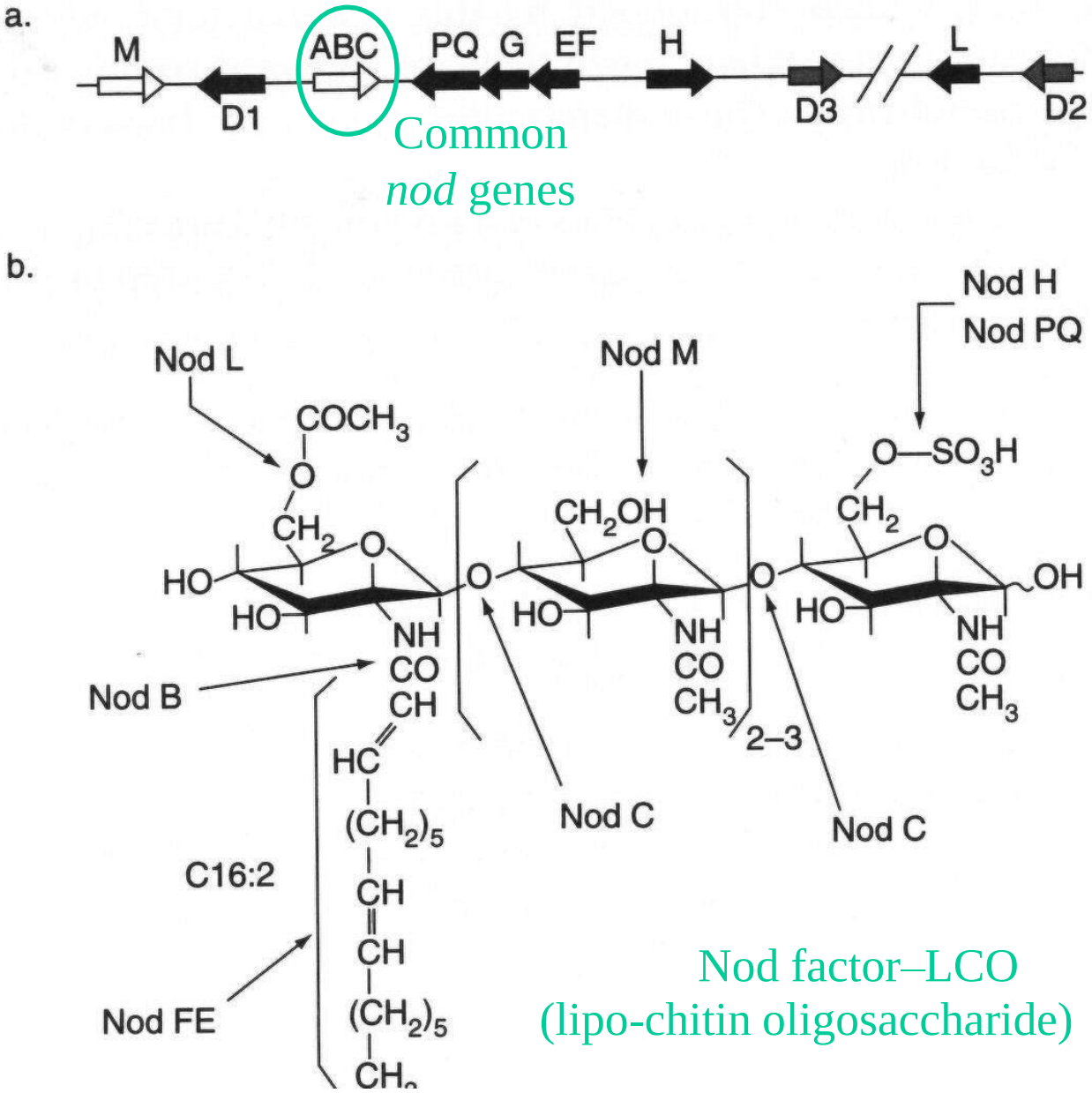


Luteolin



Genistein

nod Gene Expression



Nodule Metabolism

Oxygen metabolism

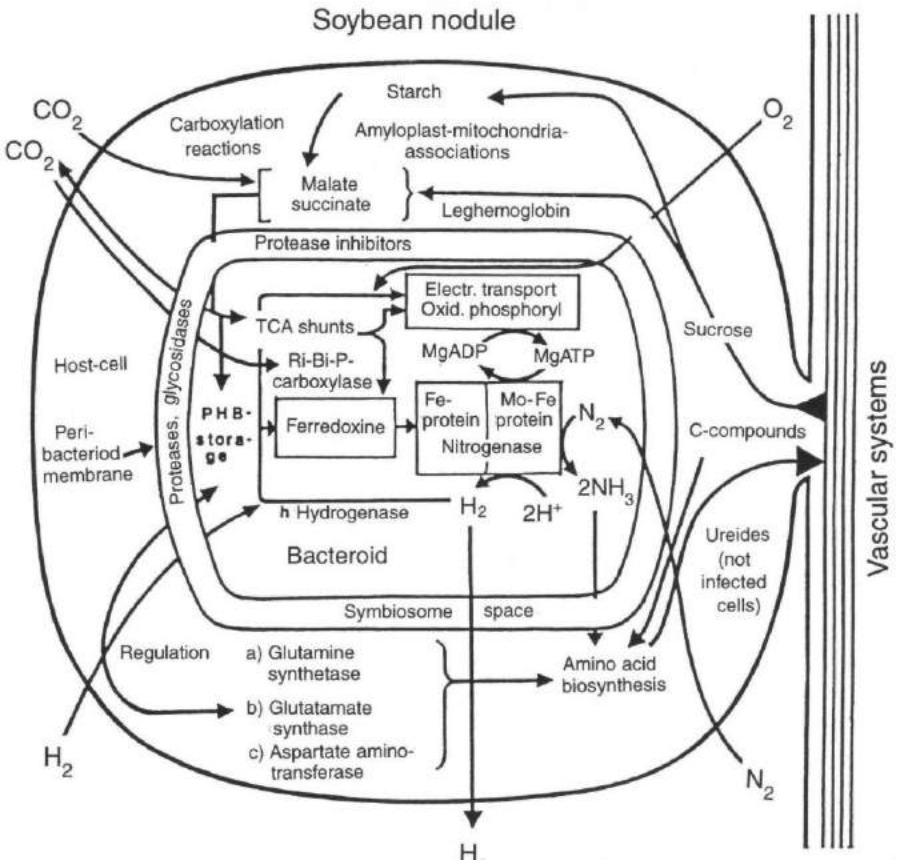
- Variable diffusion barrier
- Leghemoglobin

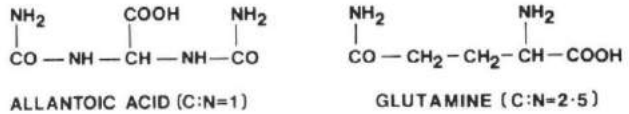
Nitrogen metabolism

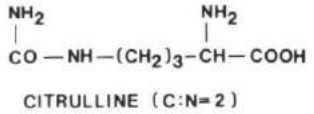
- NH_3 diffuses to cytosol
- Assimilation by GOGAT
- Conversion to organic-N for transport

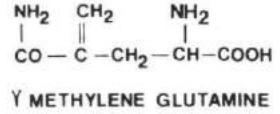
Carbon metabolism

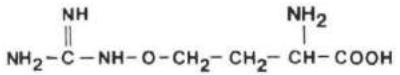
- Sucrose converted to dicarboxylic acids
- Functioning TCA in bacteroids
- C stored in nodules as starch









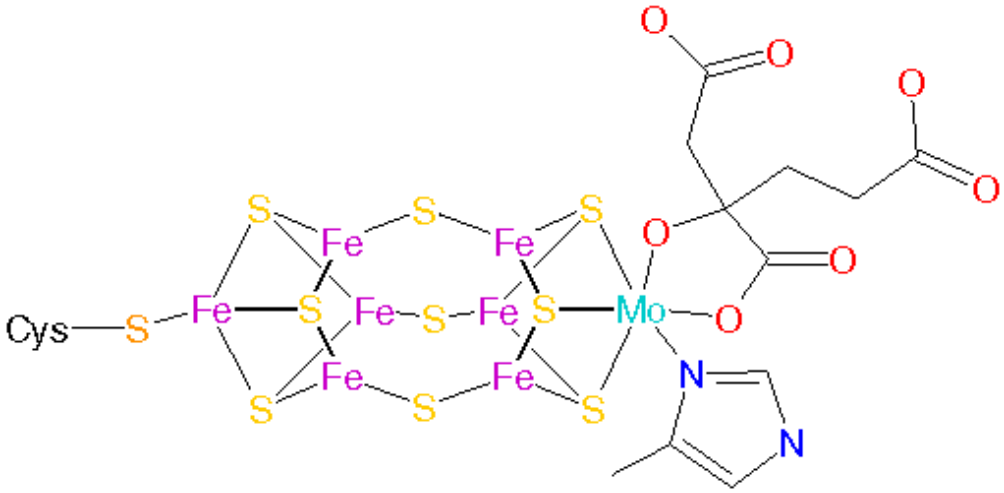


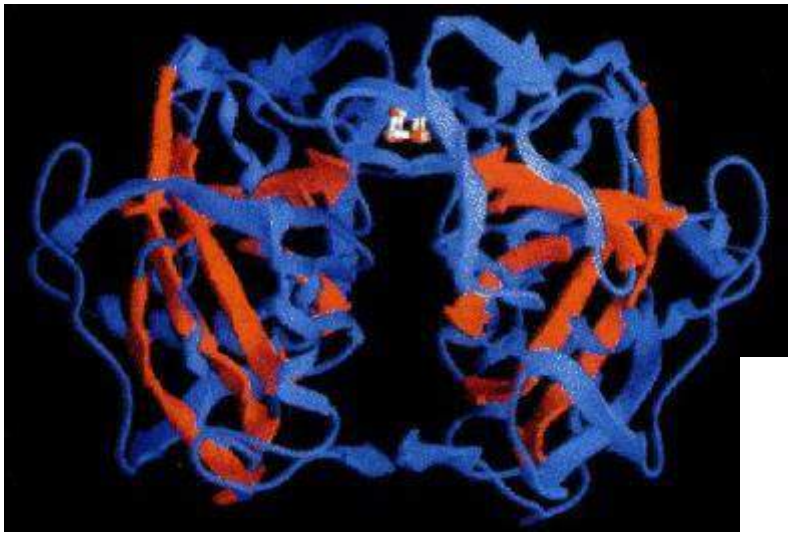
Nitrogen Fixation

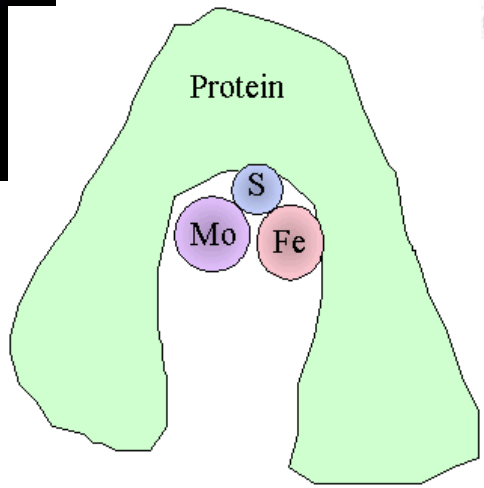
- All nitrogen fixing bacteria use highly conserved enzyme complex called **Nitrogenase**

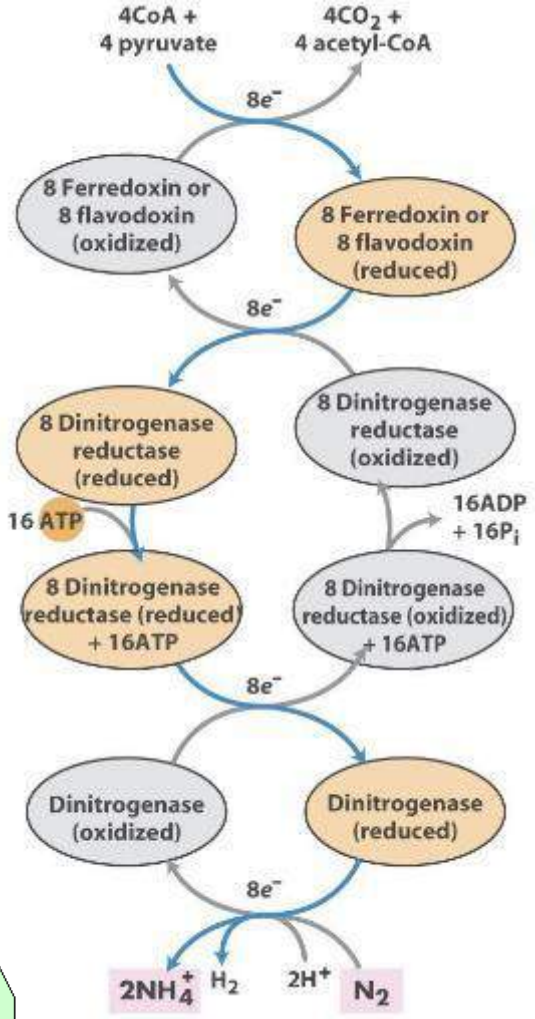
- Nitrogenase is composed of of two subunits: an iron-sulfur protein and a molybdenum-iron-sulfur protein

- Aerobic organisms face special challenges to nitrogen fixation because nitrogenase is inactivated when oxygen reacts with the iron component of the proteins

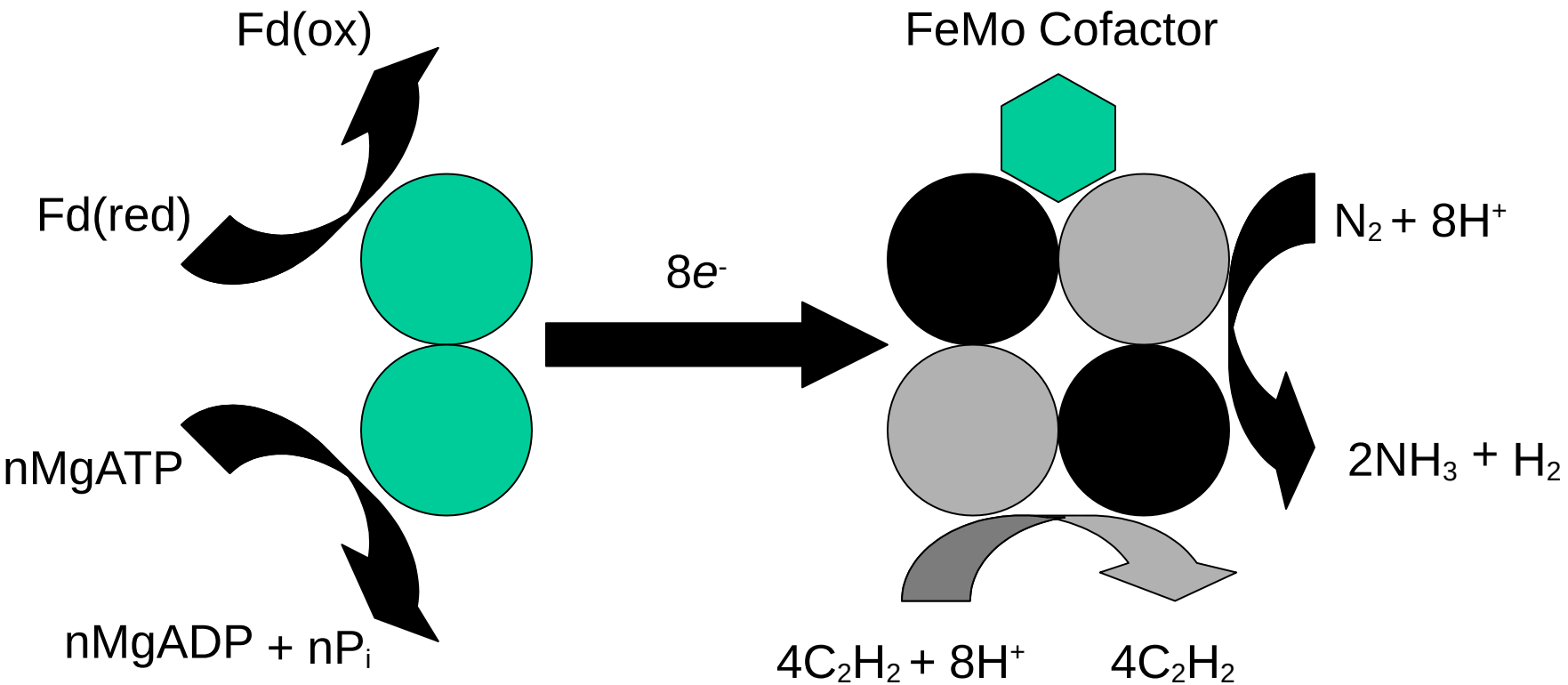

Cys
S
Fe
S
Fe
S
Fe
S
Fe
S
Fe
S
Fe
S
Fe
S
Mo
O
O
O
O
O
O
O
N
N



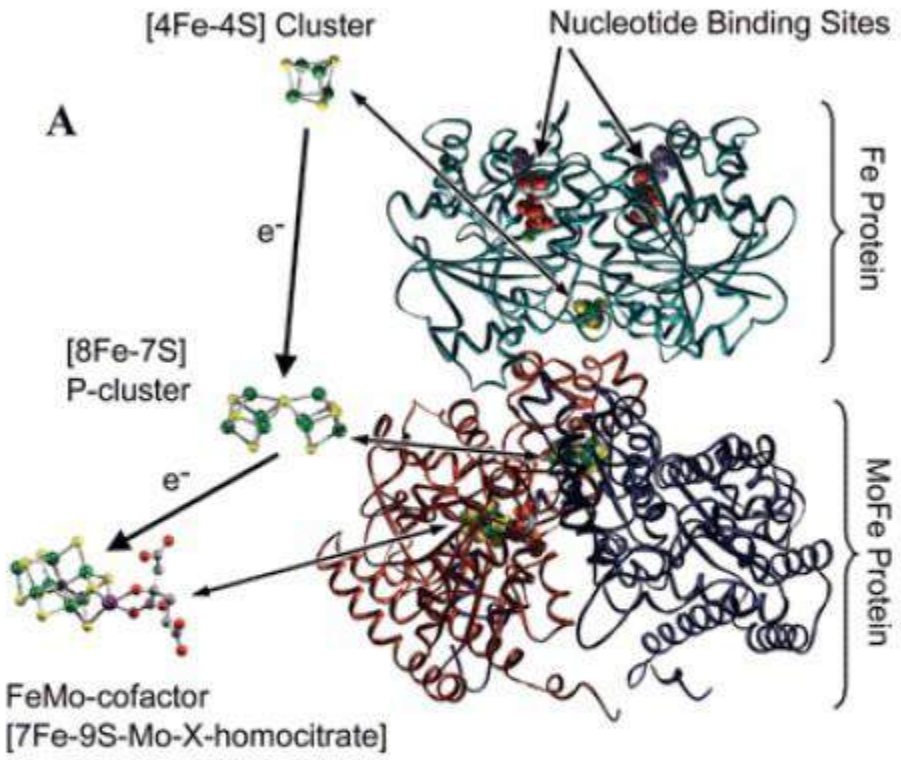

Protein
S
Mo
Fe


4CoA +
4 pyruvate
4CO2 +
4 acetyl-CoA
8e−
8 Ferredoxin or
8 flavodoxin
(oxidized)
8 Ferredoxin or
8 flavodoxin
(reduced)
8e−
8 Dinitrogenase
reductase
(reduced)
8 Dinitrogenase
reductase
(oxidized)
16 ATP
16ADP
+ 16Pi
8 Dinitrogenase
reductase (reduced)
+ 16ATP
8 Dinitrogenase
reductase (oxidized)
+ 16ATP
8e−
Dinitrogenase
(oxidized)
Dinitrogenase
(reduced)
8e−
2NH4+
H2
2H+
N2

Nitrogenase

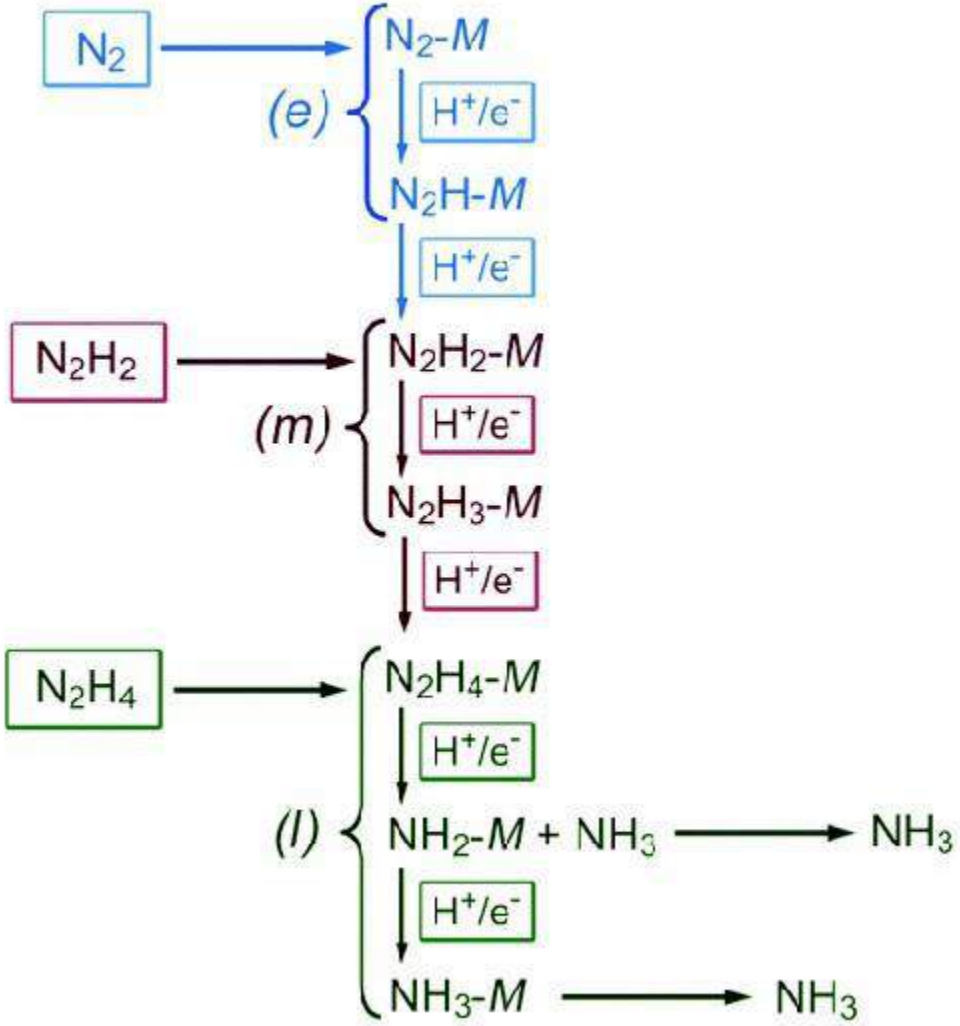


$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16MgADP$



Nitrogenase proteins and cofactors. (A) Shown is one half of the nitrogenase complex, with one αβ pair of the MoFe protein $\alpha_2\beta_2$ tetramer and one bound Fe protein dimer. The location of bound nucleotides and the three metal clusters are also shown.

Barney BM, et al., Dalton Trans. 2006, 2277-84.


N2
N2-M
(e)
H+/e-
N2H-M
H+/e-
N2H2
N2H2-M
(m)
H+/e-
N2H3-M
H+/e-
N2H4
N2H4-M
H+/e-
(l)
NH2-M + NH3
NH3
H+/e-
NH3-M
NH3


Is Your Food
Transgenic?
AMamasStory.com

TRANSGENIC PLANTS

- **TRANSGENESIS**:-is the process of introducing an *Exogenous gene* called a *Transgene* into a living organism, so that the organism will exhibit a new property and transmit that property to its offspring.

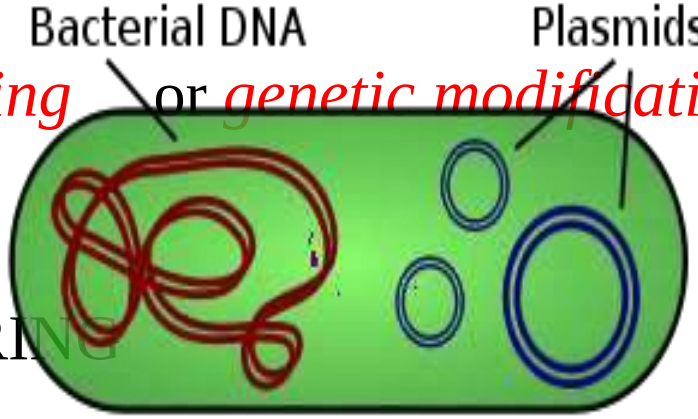
- **HISTORY**

In 1983 the first genetically engineered plant - Michael W Bevan, Richard B Flavell and Mary Dell Chilton.

They infected tobacco with *Agrobacterium* transformed, with an antibiotic resistance gene and through tissue culture techniques were able to grow a new plant containing the resistance gene.

In 2000, vitamin A-enriched golden rice developed with increased nutrient value.

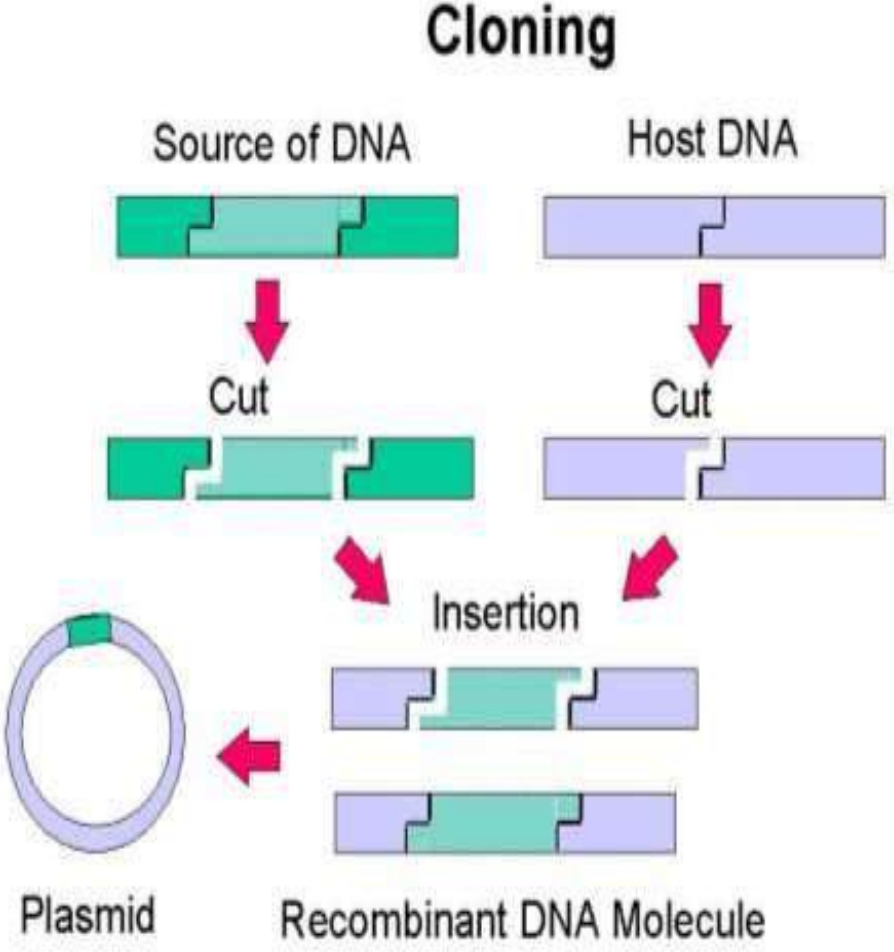
- **TRANSGENIC PLANTS:** Genetically modified plants in which foreign/source genes have been introduced/inserted into desired/targeted plants
- Generation of transgenic plants are referred as ***Transformation*** (i.e., uptake of foreign DNA by plant cells.) and this technique is known as *Transformation technique*
- It is also known as *genetic engineering* or *genetic modification*



- 3 STEPS OF GENETIC ENGINEERING
  a. Isolation of gene
  b. Finding a vector
  c. Placing the desired gene in the vector

- Digesting DNA by means of Restriction Endonuclease and clone those genes. Plasmid DNA and DNA synthesis through reverse transcriptase (c-DNA) Binary vector- plasmid

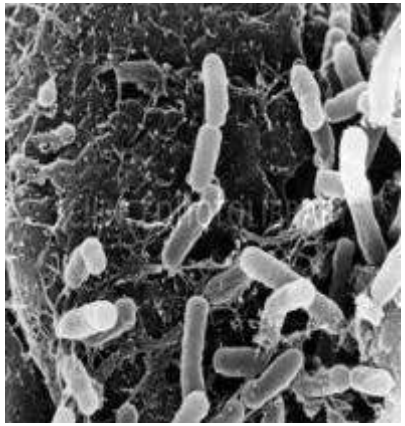
- Inserting the DNA into the vector-open plasmid and then introduce the foreign DNA and now plasmid is ready for introduction into the host cell



TRANSFORMATION TECHNIQUE

AGROBACTERIUM MEDIATED GENE TRANSFER (INDIRECT)

Used for engineering DICOTS



DIRECT GENE TRANSFER

For MONOCOT 2 methods are used

- Chemical
- Physical
  - Micro/Macro injection
  - Biolistics
  - Electroporation

Or the methods used for producing transgenic plants can be categorized as……

I. INDIRECT

a) BIOLOGICAL

- Agrobacterium mediated Virus
- mediated

II. DIRECT

2. PHYSICAL

- Gene gun/biolistics
- Micro/Macro injection
- Electrophoresis Pressure
- Laser mediated Using pollen tubes
- Silica/ carbon fibers (fiber mediated
- DNA delivery)

3. CHEMICAL

Artificial lipids (lipofection)

- PEG
- Proteins Dendrimers Dextran

TRANSFORMATION TECHNIQUE

I. INDIRECT GENE TRANSFER

a) USE OF AGROBACTERIUM SPECIES

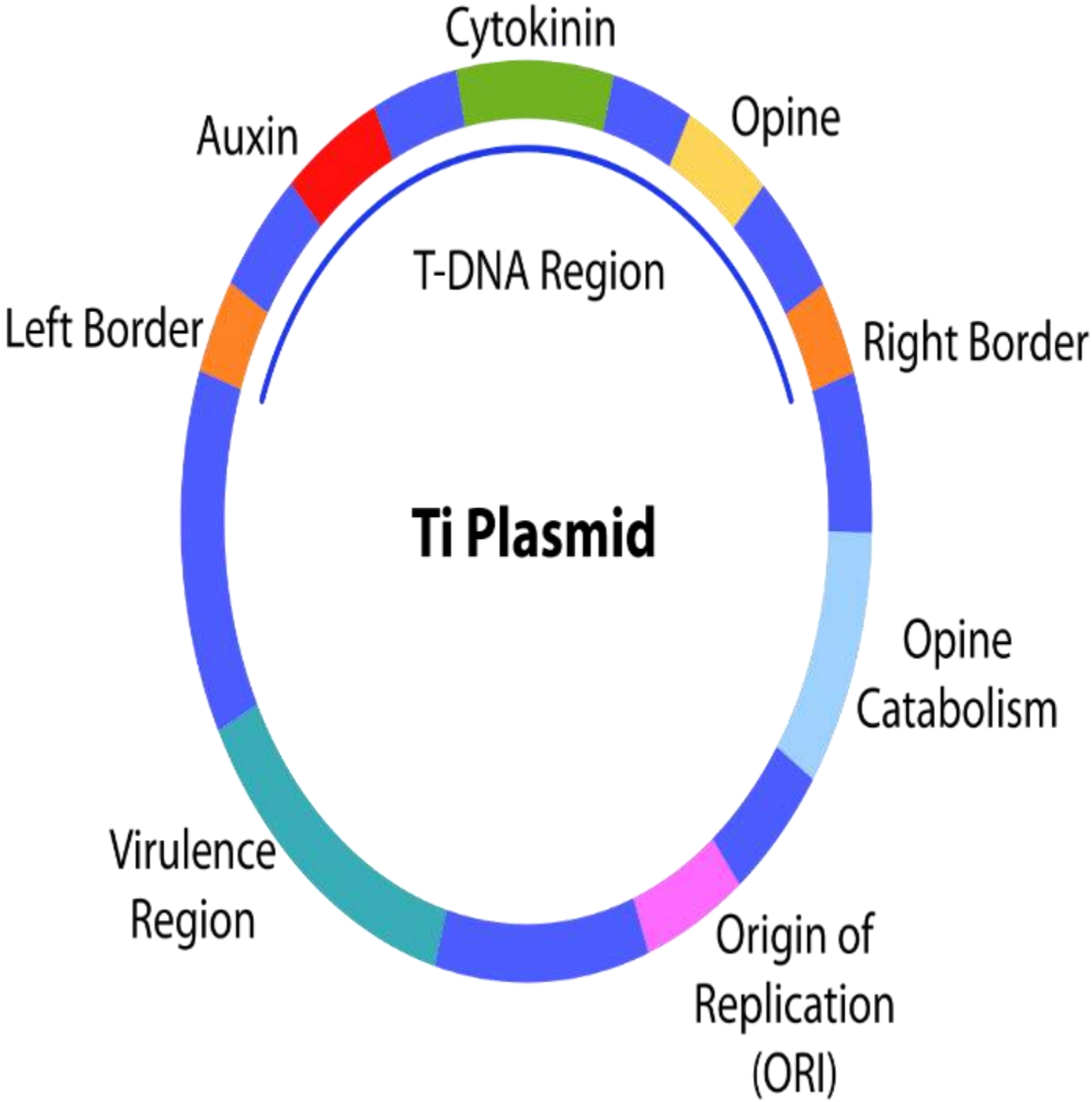
Agrobacterium-a self styled natural genetic engineer

- *A. Tumeifaciens* , *A. Righogenes* & *A. vitis* are 3 gram negative soil bacteria often found near the soil level
- *A. Tumeifaciens* : causes crown gal disease *A. Riahogenes* : cause se

- *Agrobacterium*-mediated T-DNA transfer is widely used as a tool in Biotechnology.
- *Agrobacterium* mediated transformation is the easiest and most simple plant transformation.
- It contain Ti and Ri plasmid
- It has an ability to integrate new genetic material called as T- DNA into plants
- Foreign gene used for inserting into the Ti-plasmid has similar function to the already present gene but with different DNA sequenc
es

Ti-PLASMID(pTi)

- Plasmid is a small DNA molecule within a cell-Replicate independently
- Ti plasmid –Tumour inducing plasmid of *Agrobacterium tumefaciens/A.species* which aids in the development of modified plants
- The Ti plasmid is lost when *Agrobacterium* is grown above28 °C -such cured bacteria do not induce crown galls-become avirulent


Cytokinin
Opine
Auxin
T-DNA Region
Left Border
Right Border
Ti Plasmid
Opine
Catabolism
Virulence
Region
Origin of
Replication
(ORI)

pTi is a circular DNA, contains;

- T-DNA (has gene for phytohormones)
- Virulence region(has gene for T-DNA transfer)
- Origin of replication
- Opine catabolism (has gene for opine utilization)

T-DNA

- Is the transferred DNA of the tumor-inducing (pTi) plasmid of some species of *Agrobacterium*
- This T-DNA is responsible for crown gall formation in plants
- The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the *right border* and terminated at the left border and requires the *vir* genes of the Ti plasmid.

- The bacterial T-DNA is about 24,000 base pairs long and contains genes that code for enzymes synthesizing opines and phytohormones.

T-DNA

- Auxin & cytokinin gene induces cell division & proliferation
- Opine synthesize opine-amino acid
- LB &RB are required for transfer

VIR REGION

- Transfer the T-DNA to plants
- **Acetosyringone(AS) –**flavonoid released by wounded plant cells, activate vir genes.
- Vir region organized into 8 operons- vir A-H
- Has approximately 25 genes

OPINES

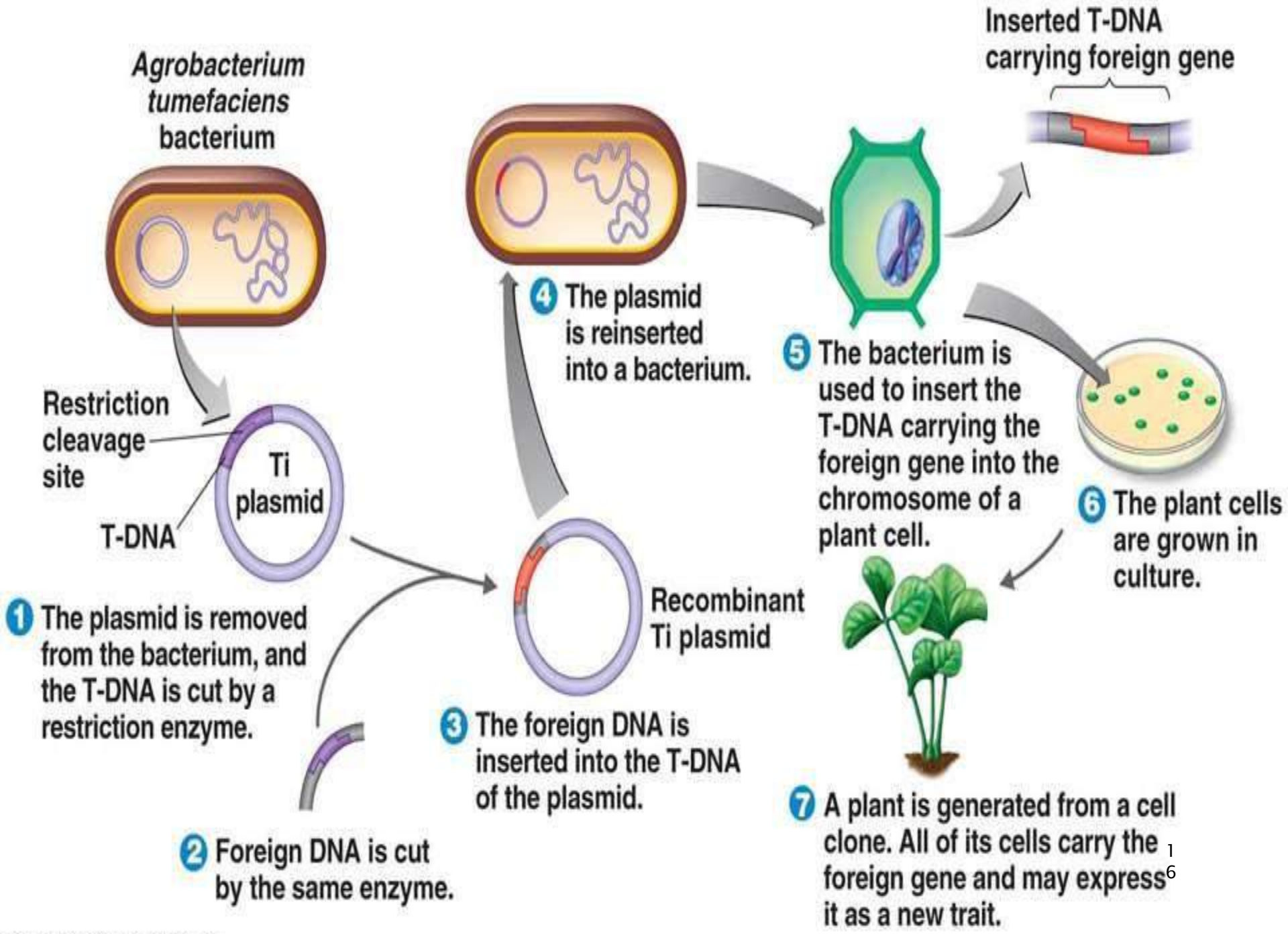
- Derivatives of amino acid synthesized by T-DNA
- pTi are categorized based on the type of *opine* produced by their genes- *octopine, nopaline, agropine, succinamopine and leucinopine*

LEFT & RIGHT BORDER SEQUENCE

- Required for T-DNA integration
- RB enable LB to produce single stranded DNA

PROCEDURE

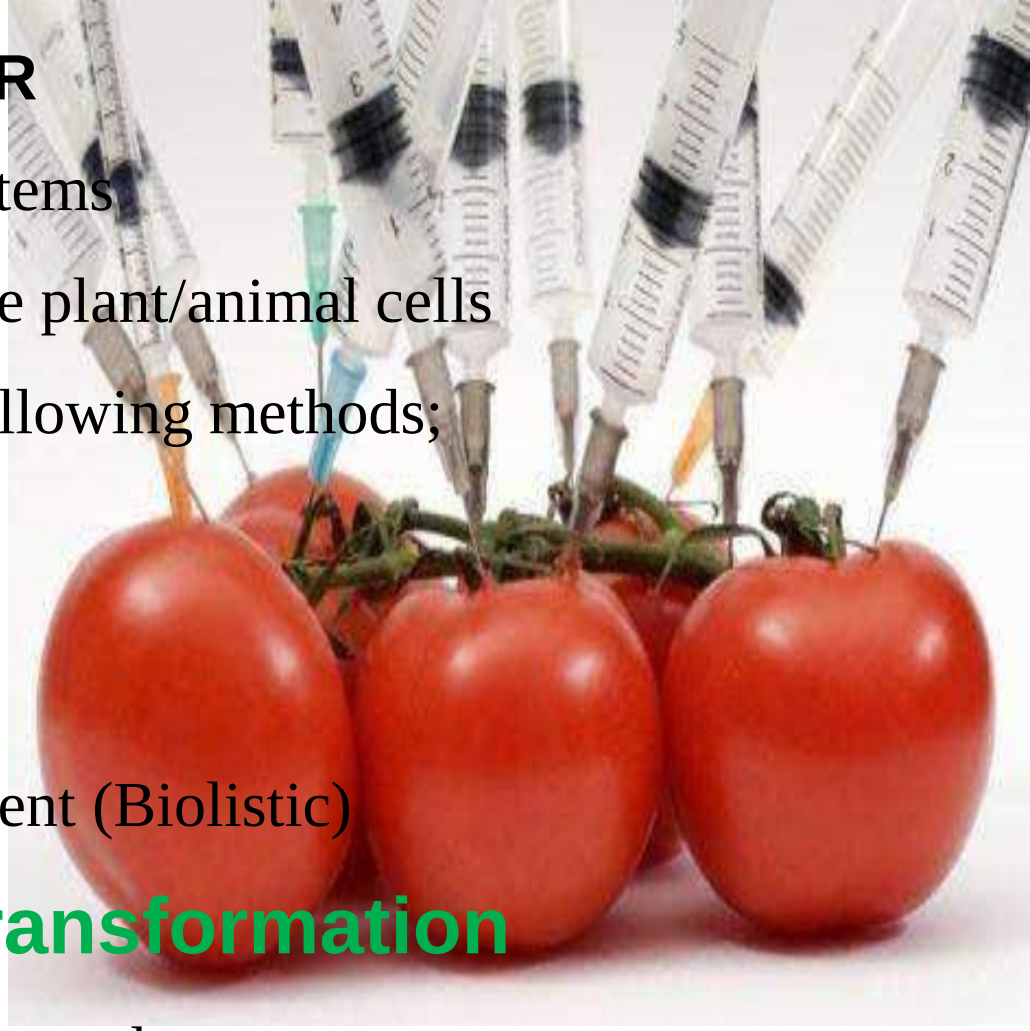
- Plant tissue (often leaves) are cut into small pieces, e.g. 10x10mm, and soaked for 10 minutes in a fluid containing suspended *Agrobacterium*.
- The bacteria will attach to many of the plant cells exposed by the cut.



b) VIRAL TRANSFORMATION

- **Viral transformation** is the change in growth, phenotype, or indefinite reproduction of cells caused by the introduction of inheritable material.
- Through this process, a virus causes harmful transformations of an in vivo cell or cell culture. The term can also be understood as DNA transfection USING A VIRAL VECTOR
- In order for a cell to be transformed by a virus, the viral DNA must be entered into the host cell. The simplest consideration/ e.g; is viral transformation of a bacterial cell. This process is called lysogeny.
- A bacteriophage(Entero bacteriophage/lambda phage) lands on a cell and pins itself to the cell. The phage can then

II. DIRECT GENE TRANSFER



- Is a Vector less DNA transfer systems
- Naked DNA is introduced into the plant/animal cells
- DNA can be introduced by the following methods;
    a. Chemical
    b. Microinjection
    c. Electroporation
    d. Particle bombardment (Biolistic)

a) Chemical-induced transformation

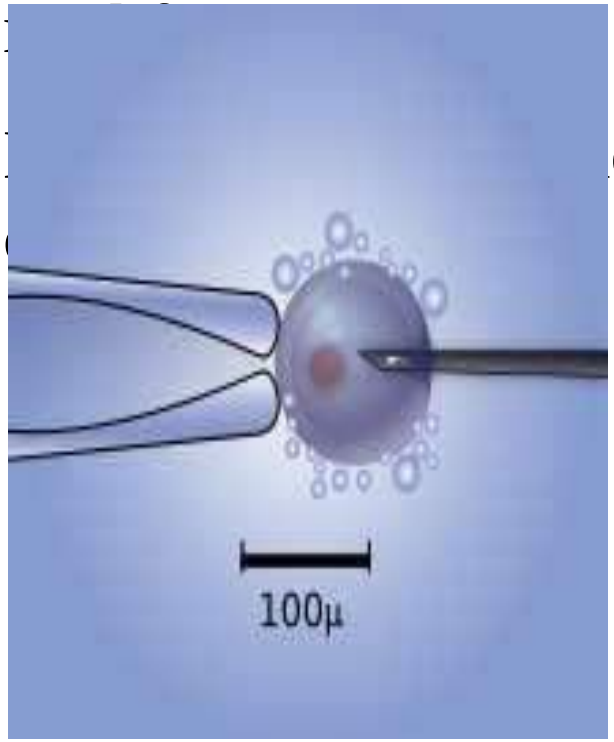
- Usually one cell lacking walls are used
- Protoplast are incubated with a solution of DNA and PEG (in case of PEG mediated transfer)
- Catechol was the most potent, inducing transformation at concentrations of 1– 30 µm followed by hydroquinone (3–30

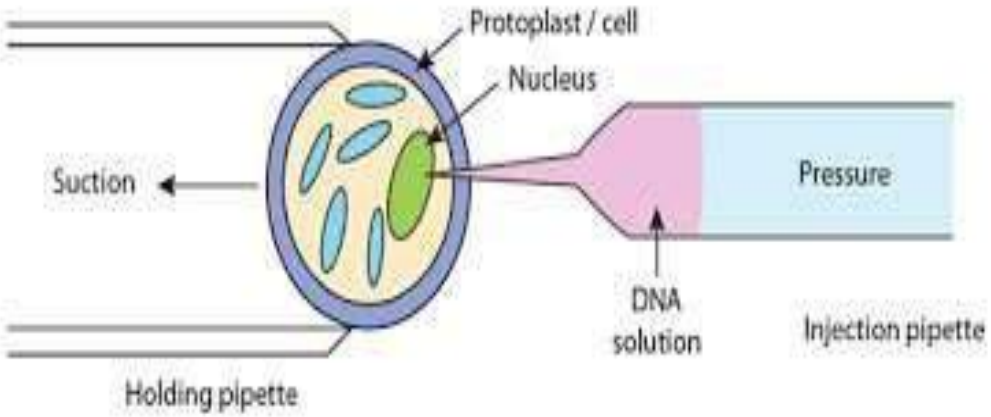
b) Micro injection

- Introduction of cloned genes into plant cells by means of *very fine needles or glass micropipettes,(dia:0.5-10μm).*
- The microinjection technique is a direct physical approach, and therefore host- range independent, for introducing substances under microscopical control into defined cells without damaging them.
- Is a limited technique only one cell can be injected at a time
- these two facts differentiate this technique from other physical approaches, such as biolistic transformation and macroinjection.
- different parameters affecting the DNA transfer via nature of microinjected DNA, and cell cycle stage, etc.

Advant ages

- Frequent stable integration of DNA is far better when compared to other methods
- Method is effective in transforming primary cells as well as cells in established cultures
- The DNA injected in this process is subjected to less extensive
- on o io.





c) Electroporation

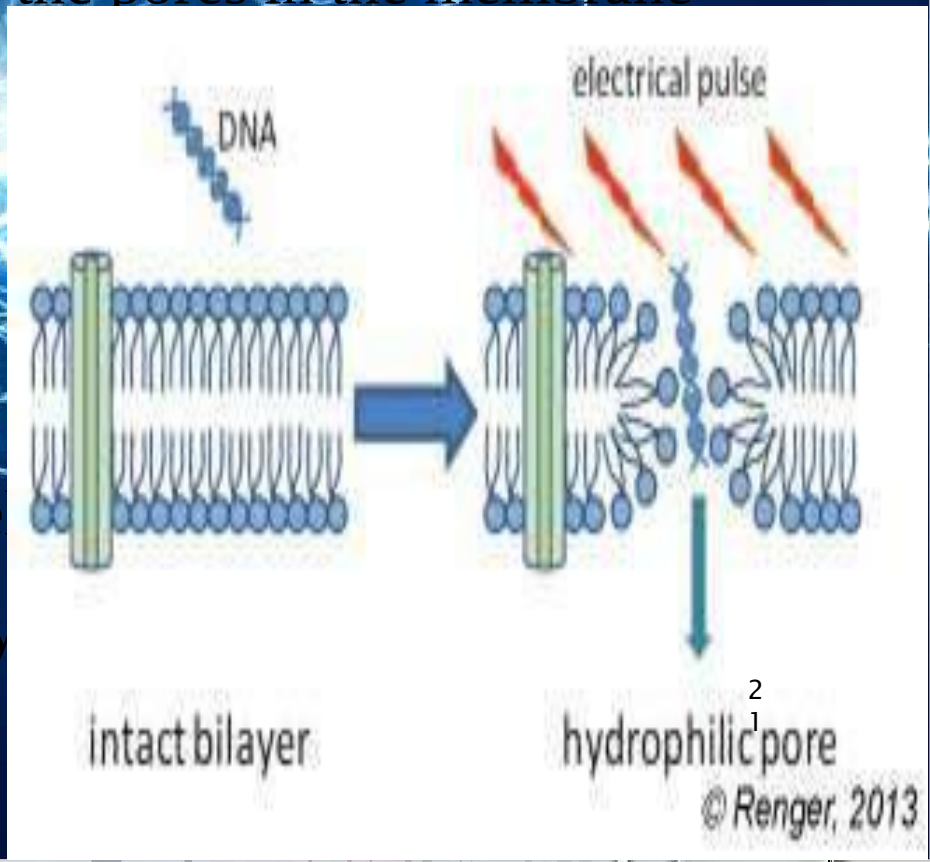
- Uses electrical pulses (high intensity electric field) to produce transient pores in the plasma membrane (destabilizes the membrane) there by allowing DNA in to the cells
- These pores are known as electro-pores
- When the electric field is turned off, the pores in the membrane reseal, enclosing the DNA inside.

Advantages

- Easy to perform
- High efficiency
- Don't alter biological structures/ ce
- Can be used for wide range of cell ty

Disadvantages

- Cell mortality (if using sub-optimal conditions)

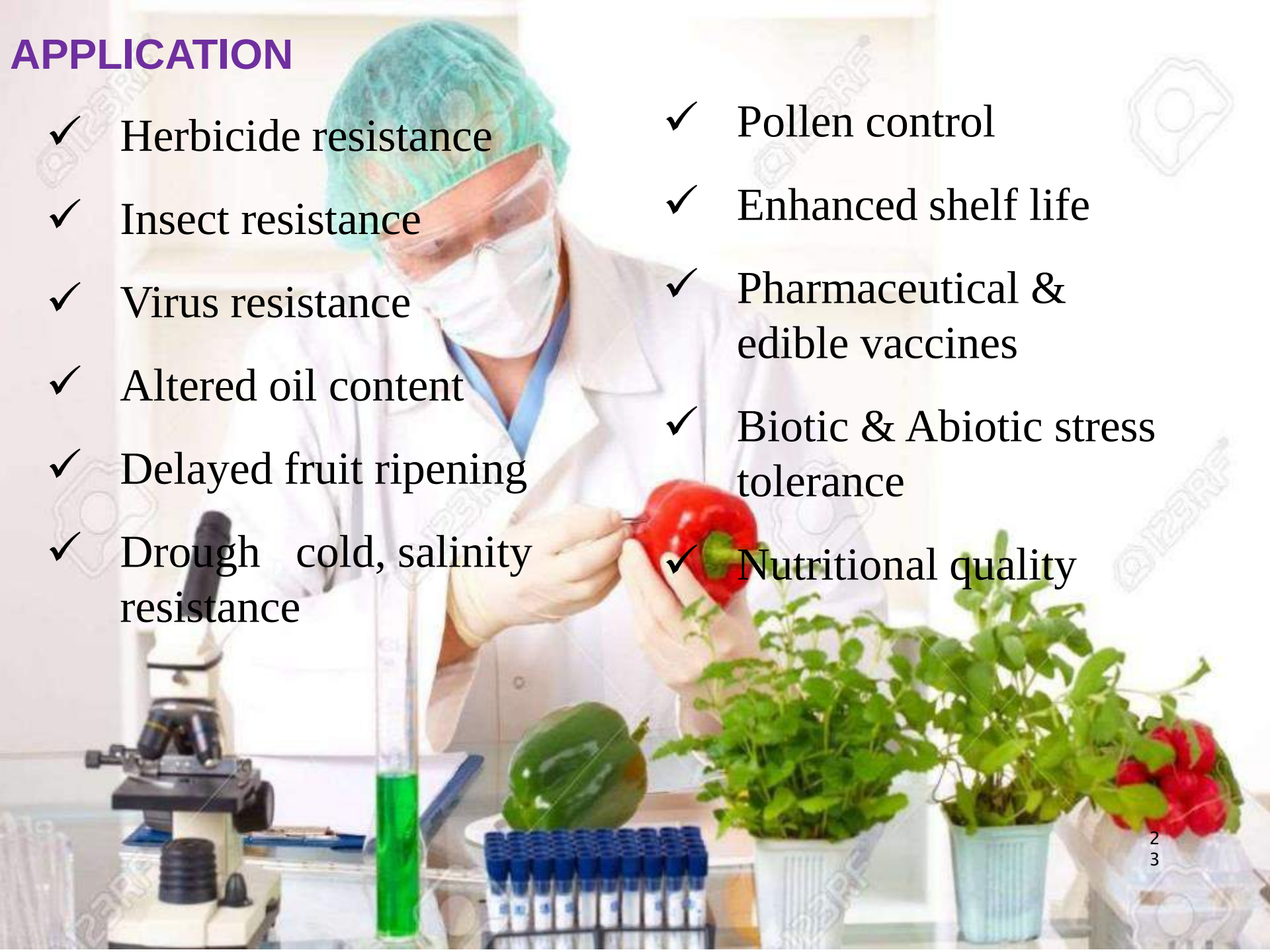


d) Gene Gun Method

- A biolistic particle delivery system, originally designed for plant transformation,
- Device for delivering exogenous DNA (transgenes) to cells
- It was invented and used by **John C Sanford**, **Ed Wolf** and **Nelson Allen** at **Cornell university**, and **Ted Klein** of **Dupont**, between 1983 and 1986, to transform epidermal cells of Allium cepa.
- Thi cereal t

APPLICATION

- ✓ Herbicide resistance
- ✓ Insect resistance
- ✓ Virus resistance
- ✓ Altered oil content
- ✓ Delayed fruit ripening
- ✓ Drough cold, salinity resistance
- ✓ Pollen control
- ✓ Enhanced shelf life
- ✓ Pharmaceutical & edible vaccines
- ✓ Biotic & Abiotic stress tolerance
- ✓ Nutritional quality



1. HERBICIDE RESISTANCE

a. Bromoxynil Resistance

i. A gene encoding the enzyme Bromoxynil nitrilase (BXN) is transferred from *Klebsiella pneumoniae* bacteria to plants.

ii. Nitrilase inactivates the bromoxynil before it kills the plant.

a. Sulfonylurea

i. Kills plants by blocking an enzyme needed for synthesis of the amino acids valine, leucine, and isoleucine.

ii. Resistance generated by mutating a gene in tobacco plants, and transferring the mutated gene into crop plants.

2. INSECT RESISTANCE

- The Bt toxin isolated from *Bacillus thuringiensis* has been used in plants. The gene has been placed in corn, cotton, and potato, and has been marketed.
- Alkaline protein degrades gut wall of lepidopteran larvae
  - Tobacco hornworm
  - Gypsy moth larvae
  - Corn borer catepillars
  - Cotton bollworm catepillars
- Sprayed onto plants – but will wash off

3. VIRUS RESISTANCE

- Chemicals are used to control the insect vectors of viruses, but controlling the disease itself is difficult because the disease spreads quickly.
- Plants may be engineered with genes for resistance to viruses, bacteria, and fungi.
- Virus-resistant plants have a *viral protein coat gene* that is overproduced, preventing the virus from reproducing in the host cell, because the plant shuts off the virus' protein coat gene in response to the overproduction.
- Coat protein genes are involved in resistance to diseases such as cucumber mosaic virus, tobacco rattle virus, and potato virus x.

4. ALTERED OIL CONTENT

- Oil content in plants are altered by modifying an enzyme in the fatty acid synthesis pathway (oils are lipids, which fatty acids are a part of).
- Varieties of canola and soybean plants have been genetically

5. DELAYED FRUIT RIPENING

- Allow for crops, such as tomatoes, to have a higher shelf life.
- Tomatoes generally ripen and become soft during shipment to a store.
- Tomatoes are usually picked and sprayed with the plant hormone ethylene to induce ripening, although this does not improve taste
- Tomatoes have been engineered to produce less ethylene so they can develop more taste before ripening, and shipment to markets.

6. POLLEN CONTROL

- Hybrid crops are created by crossing two distantly related varieties of the same crop plant.
- The method may generate plants with favorable traits, such as tall soybean plants that make more seeds and are resistant to environmental pressures.
- For success, *plant pollination* must be *controlled.* This is usually done by removing the male flower parts by hand before pollen is released. Also, sterilized plants have been genetically engineered with a gene

RISKS OF GMO/GMCs……

- Can be dangerous & cause allergies

  E.g.. Soybean containing gene of

- Indirectly promote Antibiotic resistance (Resistance of microbes)
- Weed shows herbicide resistance & resistance to viral disease
- Change in chemistry of soil
- Genetically engineered plant cross pollinate non-engineered plants

EXAMPLES OF GM CROPS…….

1. Soybeans.

Normal Rice

2. Corn
3. Canola.
4. Cotton.
5. Papaya, rice,
6. Tomato,
7. Sugar beet, and
8. Red heart chicory.
9. Golden rice.

Transgenic technology produced a type of rice that accumulates *beta-carotene* in rice grains. Once inside the body, beta-carotene is converted

Golden Rice