SOUTH VALLEY UNIVERSITY

FACULTY OF SCIENCE

DEPARTMENT OF BOTANY AND MICROBIOLOGY

INDUSTRIAL MICROBIOLOGY

An Introductory Course

for

B. Sc. Students

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أهداف المقرر ونواتج التعلم المستهدفة

اسم المقرر: الميكروبيولوجيا الصناعية (الرمز الكودي: 304ن+406 ن)

هدف المقرر

إجادة التعرف على أسس الصناعات الميكروبيولوجية ودور الكائنات الدقيقة بها وفهم المشكلات المرتبطة بهذه الصناعات ومحاولة إيجاد حلول لها

نواتج التعلم المستهدفة

1- المعلومات والمفاهيم:

أ1- أن يسمى الطالب فوائد الكائنات الدقيقة فى العمليات الصناعية و الظروف المختلفة لتنمية الكائنات الدقيقة وأنواع مفاعلات التخمر المختلفة وأن يحدد دور الكائنات الدقيقة المختلفة فى عمليات التخمر وانزيماتها وتكاثرها وظروف تنميتها.والمنتجات الممكنة .الخ

أ2- أن يصف الطالب فوائد بكتريا حمض اللاكتيك فى الصناعات الغذائية والمشاكل التى تسببها الكائنات الدقيقة في الصناعات الغذائية وحلولها ويحدد طرق إعادة تدوير المواد الطبيعية والمخلفات الغذائية ودور الميكروبات بها وفوائدها

أ3- أن يحدد الطالب دور الكائنات الدقيقة فى الصناعات الغذائية وفسادها ومراقبة جودتها وطرق الحفظ والصناعات الغذائية وغير الغذائية المختلفة ويذكر الطالب دور الميكروبات فى تخمرات الكتلة الحيوية والصناعات المختلفة القائمة عليها

2- المهارات الذهنية:

ب1- أن يميز الطالب طرق رفع كفاءة الكائنات الدقيقة لزيادة المنتج وتحسين نوعيته

ب2- أن يوضح الطالب كيفية تحسين الصناعات الميكروبية والتغلب على المشكلات التى تطرأ أثناء تطوير الاختبارات من مرحلة المعمل الى التصنيع النهائى

ب3- أن يقارن الطالب بين مجموعات الانزيمات الهامة للميكروبات الصناعية وإنتاج الأحماض العضوية والفيتامينات والكحول والبلاستك الحيوى والمضادات الحيوية والأمصال والوقود الحيوى

ب4- أن يستنتج الطالب دور انتقاء السلالات والبيولوجيا الجزيئية وظروف التخمر فى المنتجات المختلفة

ب5- أن يميز الطالب بين الضرر الذى تسببه الكائنات الدقيقة للمواد المختلفة وطرق اختبارها واستخدامات الميكروبات فى معالجة هذه المواد.

3- المهارات المهنية:

ج1-أن يستخدم الطالب بعض المتخمرات واللقاحات الميكروبية مثل الخميرة وأنواع البكتريا والطحالب والظروف المثلى المحيطة بها فى العمليات الصناعية المختلفة

ج2- أن يتناول الطالب طرق فحص الألبان المتخمرة وبكتريا حمض اللاكتيك وتقدير نواتج التخمر الأخرى مثل الانزيمات وانتاج الجبن والخبز والأسمدة الحيوية..الخ

ج3- أن يستخدم الطالب الأدوات والأجهزة المناسبة لفحص واختبار المنتجات الميكروبية

ج4- أن يتناول الطالب تأثير المضادات الحيوية والمواد الحافظة وتأثير العوامل المختلفة وطرق حفظ الأغذية على نمو الميكروبات

ج5- أن يصمم الطالب طرق عزل الكائنات الدقيقة من الأوساط المختلفة واستخداماتها فى التحلل البيولوجى للمواد

4- المهارات العامة:

أن يكون الطالب قادرا على:

د1- المناقشة والتواصل مع الآخرين ثم الفهم والاستنتاج

د2- المشاركة بفاعلية فى العمل الجماعى ووضع الخطط المختلفة

PART (1)

GENERAL INTRODUCTION

INDUSTRIAL MICROBES AND FERMENTATION

In general, industrial microbiology is concerned with all aspects of business that relate to microbiology. In a more restricted sense, industrial microbiology is concerned with (i) employing microorganisms to produce a desired product, and with (ii) preventing microbes from diminishing the economic value of various products. This dual purpose is clearly seen in the food industry, a major area of industrial microbiology.

Various commercial products of economic value made by microbes are (i) medicines i.e. pharmaceuticals, including antibiotics, steroids, vaccines, and vitamins, (ii) organic acids, (iii) amino acids, (vi) enzymes, (v) alcohols, (vi) organic solvents and (vii) synthetic fuels. In addition to these, quite recently potential of microbes could also be used in the recovery of metals from ores (bioleaching), recovery of petrol, and single cell protein production.

The term fermentation in industrial microbiology is used in a wider sense to include any chemical transformation of organic compounds carried out by using microbes and their enzymes. Production methods in industrial microbiology bring together the raw materials (substrates), microorganisms (specific strains or microbial enzymes) and a controlled favourable environment (created in a fermentor) to produce the desired substance. The essence of an industrial process is to combine the right organism, an inexpensive substrate, and the proper environment to produce high yields of a desired product.

The used microbe in industrial microbiology should be originally isolated from nature, but increasingly "improved" by genetic manipulation via mutagenesis and selection or recombinant DNA technology or protoplast fusion (fungi)

To be useful in industrial microbiology, an organism must:

1. -produce usable substance(s) or effect(s)
2. -be available in pure culture
3. -be genetically stable, but amenable to genetic manipulation
4. -produce spores or other reproductive structures to allow easy inoculation
5. -grow rapidly and produce product quickly in large- scale culture

6. -grow in such a way that the cells are easily separated from the product
7. -not be harmful to humans or agricultural plants and animals, etc.

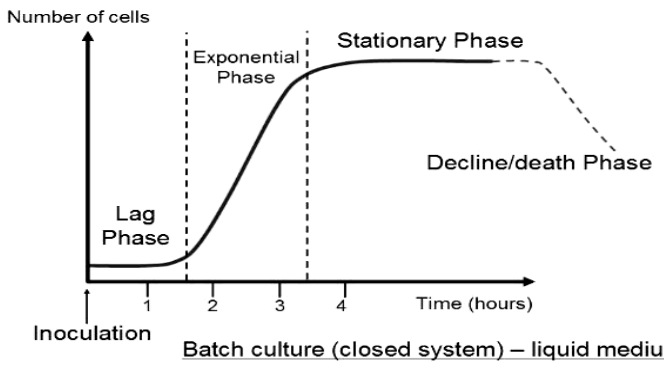
PRINCIPLES OF MICROBIAL GROWTH

- Growth of microorganisms is the increase in cell mass and number resulting in a complicated series of enzyme- catalyzed biological steps.
- The quantity of biomass or specific cellular components can be determined gravimetrically (by dry weight, wet weight, DNA or protein) or numerically for unicellular systems (by number of cells).
- **Doubling time** is the time required for doubling the weight of biomass while **generation time** is the time required for doubling the cell numbers.
- Average doubling times increase with the increasing cell size and complexity, e. g.: bacteria 0.25-1 h; yeast 1-2 h; mold fungi 2-6.5 h; plant cells 20-70 h and animal cells 15-48 h.

In normal practice an organism will seldom have ideal conditions for unlimited growth. Growth is usually dependent on a limiting factor such as an essential nutrient. As the concentration of this factor drops, the growth potential of this organism will decrease.

- In biotechnological processes there are three main ways of growing microorganisms in the bioreactor, namely batch, semi- continuous and continuous culture.
- In **batch culture** the microorganisms are inoculated in a fixed volume of medium and, as growth takes place, nutrients are consumed and products (biomass, metabolites) accumulate.

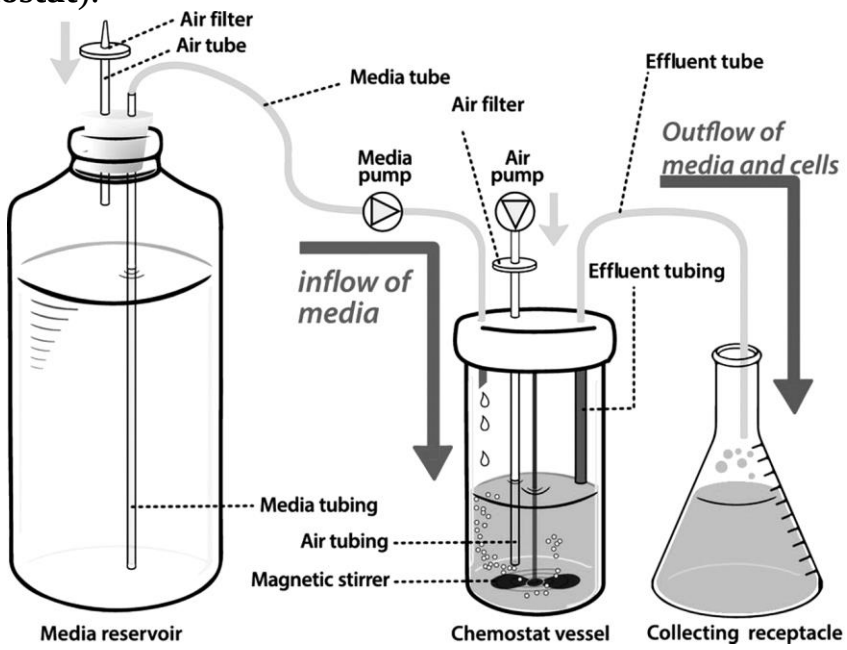
Bacteria - Population Growth Curve



Batch culture (closed system) – liquid medium

This will enforce changes to cell metabolism, and eventually, cell multiplication ceases because of the exhaustion of nutrients and accumulation of toxic waste products. There are many factors that control the growth of microorganisms in batch culture and are related to growth phases. However, in order to maintain high growth ratio (i. e. exponential growth) there are many ways to increase the yield by various substrate feed methods, namely:

1) Gradual addition of concentrated nutrient components (**fed batch**).
2) Addition of fresh medium to the culture (perfusion) and withdrawal of old consumed medium and dead cells (**continuous culture** or **chemostat**).



- In contrast to batch culture, continuous culture gives approximately balanced growth with little fluctuation of nutrients, metabolites, cell numbers and mass. This will permit cells to grow in a steady state conditions at a constant rate. Generally, batch cultivation methods represent the dominant form of industrial usage for many reasons.

Growth Conditions

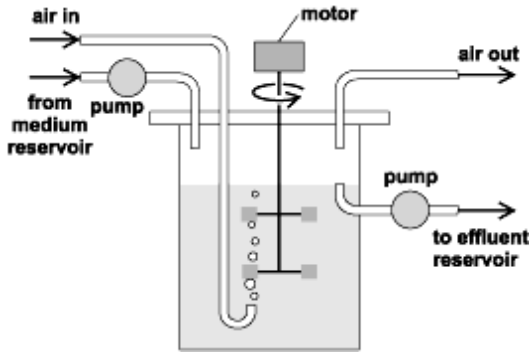
- Composition of growth medium- generally the cheapest sources of carbon (molasses, whey, grains), nitrogen (ammonia and ammonium salts), phosphorous, trace minerals and other growth factors (either of which may be used to regulate product generation)
- Other considerations- aseptic conditions with maintained temperature, pH, oxygen concentration at optimal levels in the

microenvironment in which each individual cell is growing and metabolizing

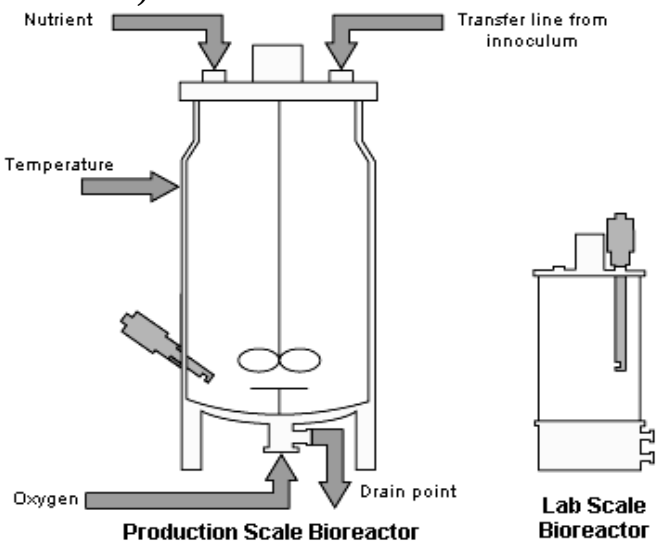
FERMENTORS

Many types (lift-tube, solid state, fixed bed, fluidized bed, dialysis, continuous) and many sizes according to the purpose, cost, ..etc.

- **Aerobic fermentor** - stainless steel cylinder with temperature control (cooling jacket *vs*. internal coils) and aeration system (sparger and impeller plus baffles) with process control and monitoring devices (real- time acquisition of data provides for "on- line" control of temperature, pH, pO_2, pCO_2, cell concentration, foaming, product concentration.
- **Anaerobic fermentor** - essentially the same as aerobic, but does not need aeration.

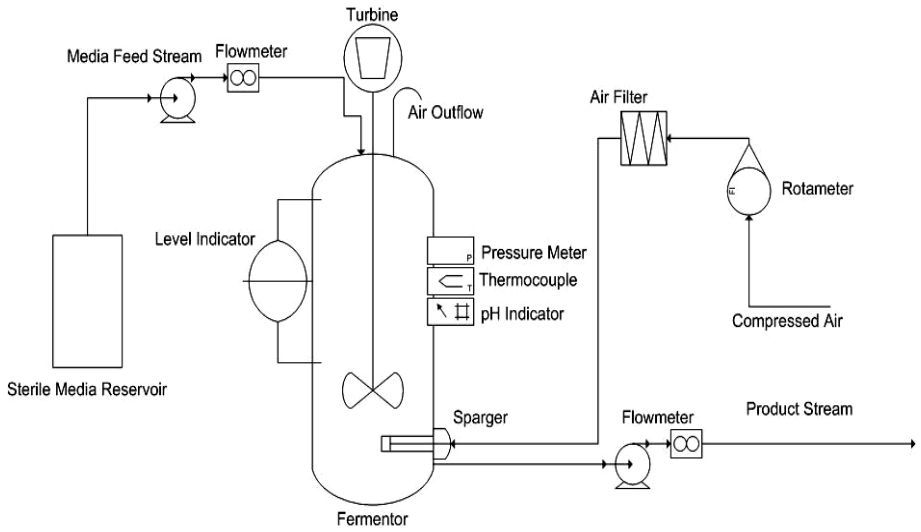


- **<u>Scale-up of fermentation process</u>**: carried out by biochemical engineers in conjunction with microbiologists
  - Steps - laboratory flask (0.1-1 L) --> laboratory fermentor (5-10 L) --> pilot plant (300-3000 L) --> commercial fermentor (10,000-400,000 L or more).



- **Practical considerations** - surface/volume ratio, uniformity of mixing (maintain appropriate conditions, especially oxygen transfer rate, at level of individual cell - consider microenvironment).

The fermentor or bioreactor and its role in industry will be discussed in details later.



A diagram representing an industrial fermentor

MICROBIAL FERMENTATION PRODUCTS

Microbial products through fermentation can be produced during different phases of microbial growth, and therefore, are divided into:

- **Primary metabolites**

Metabolites formed in parallel with growth, during trophophase (example - alcoholic fermentation).

- **Secondary metabolites**

Metabolites formed after growth has occurred, during idiophase with the following characteristics:

- Formed by only a few organisms
- Not essential for growth and reproduction
- Dependent upon growth conditions and frequently produced from several intermediate products formed during trophophase; may require an inducer produced during trophophase
- Often produced as a group of related structures
- Possible to induce overproduction (not possible with primary metabolites)

Example - antibiotic production

QUESTIONS

1- What are the main targets of using microbes in industry?
2- What are the main characteristics of a microbes to be used in industry ?
3- What are the different feeding mechanisms involved for having high yield from industrial microbes?
4- Describe the main components of a fermentor and how to scale-up the process?
5- What is the chemostat?
6- What are the two kinds of products that can be differentiated by microbial growth phase? give one example on each?
7- Discuss in brief the differences between aerobic and anaerobic fermentors.
8- Compare between different kinds of bacterial culturing?

PART (2)

BIOPROCESSING AND FERMENTATION

INTRODUCTION

- In the beginning, the fermentation technology or bioprocessing technology was derived partly from the use of microorganisms for the production of food such as yoghurt, cheese, pickles, sauce and sausages and other beverages such as beers, wines and spirits.
- Parallel to these processes was the identification of the role of microorganisms in removing unwanted and unhealthful wastes that resulted in the worldwide service industries involved in water purification, waste treatment and management.
- There are many factors that are critical for successful bioprocessing.
- Although the traditional bioprocessing technologies are for food and beverages, there are many modern technologies that are derived from microbial fermentation such as:

(1) The production of primary metabolites such as acetic acid, glycerol, lactic acid, acetone, organic and amino acids, vitamins and polysaccharides.

(2) The production of secondary metabolites (that have no obvious role in the producer organisms) such as penicillin, streptomycin, cephalosporin, etc.

(3) The production of many industrially useful enzymes, e. g. extracellular such as amylases, pectinases and proteinases, and intracellular enzymes such as invertase, restriction endonuleases, etc. Some important food products are summarized in the following Table.

Food	Raw Material	Fermentor
Pickles	Cucumber	*Leuconostoc mesenteroides* *Lactobacillus*
Chocolate	Cacao bean	*Saccharomyces cerevisiae* *Candida rugosa* *Kluyveromyces marxianus*
Bread	Flour	*Saccharomyces cerevisiae*
Coffee	Coffee bean	*Erwinia dissolvens*
Sauerkraut	Cabbage	*Leuconostoc plantarum*
Soy sauce	Soya bean	*Aspergillus oryzae*

- More recently, bioprocessing technology is using higher plant and animal cells to produce many important products. Plant cell culture is used largely for secondary product fermentation such as flavours, perfumes and drugs while mammalian cell culture techniques are concerned with vaccines, antibody formation and the production of

protein molecules such as interferon, etc. The future market for these bioproducts are largely guaranteed because most of these products cannot be produced economically by other chemical processes.

- It is possible to make further economic production by genetically engineered organisms to higher or unique productivities but with some disadvantages (the Table below).

Advantages and disadvantages of producing organic compounds by biological rather than chemical processes

Advantages	Disadvantages
Complex molecules such as proteins cannot be produced by chemical processes	Can be easily contaminated with foreign unwanted microorganisms, etc.
Bioconversions give higher yields	The product is usually present in a complex mixture requiring separation
Biological systems operate at lower temperatures, near neutral pH, etc.	Need to provide, handle and dispose large volumes of water
Much greater specificity of catalytic reactions	Bioprocesses are usually extremely slow when compared with conventional chemical processes
Can achieve exclusive production of an isomeric compounds	

- The bases are similar in all fermentation processes whatever the organism, the medium used or the end product. In all examples, large numbers of cells are grown under defined conditions. The organisms must be cultivated and motivated to form the desired product by physical/ technical containment system (**bioreactor**), and the correct medium composition and environmental growth conditions. Optimizing the bioprocess involves both the biosystem and the technical system.
- The exploitation of the organism potential to form the proper product requires a detailed knowledge of the biochemical mechanisms of product formation. The same apparatus, with modifications, can be used to produce an enzyme, an antibiotic, and an amino acid or single cell protein.
- In the simplest form, the bioprocess can be seen as mixing the appropriate microorganism with nutrient broth and allowing the components to react (e. g. yeast cell with sugar solution to form alcohol).

The main function of bioreactors is to minimize the cost of producing a product or service.

THE BIOREACTOR/ FERMENTOR

History

Humans have used fermentation from the beginning of recorded history to provide products for everyday use. For many centuries, most microbial processing was to preserve or alter food products for human consumption.

The natural yeasts that caused the fermentation added some vitamins and other nutrients to the bread or beverage. Lactic acid bacteria fermented milk to yogurt and cheeses, extending the life of milk products. Other food products were preserved or enhanced in flavor by fermentation, such as pickled vegetables and the fermentation of tea leaves and coffee beans.

The idea that microbes were responsible for fermentations was not introduced until 1857, when Louis Pasteur published a paper describing the cause of failed industrial alcohol fermentations. He also quantitatively described microbial growth and metabolism for the first time and suggested heat treatment (pasteurization) to improve the storage quality of wines.

The first aseptic fermentation on a large scale was the acetone–butanol fermentation, which both Britain and Germany pursued in the years preceding World War I. After the beginning of the war, the focus of the process in Britain became acetone, which was used for munitions manufacture. As the process was developed and scaled up, it was found that the producing by *Clostridium acetobutylicum*, was contaminated by competing bacteria introduced from the raw materials. Thus, the culture medium had to be sterilized and the process run under aseptic conditions. All penetrations on the reaction vessel were steam sealed to prevent contamination.

In the 1920s and 1930s, the emphasis in fermentation shifted to organic acids, primarily, lactic acid and citric acid. Previously, fungi had been grown on solid media or in the surface of liquid media. This set the stage for the large- scale production of penicillin, which was discovered in 1929 in Britain, developed in the 1930s, and commercialized in 1942 in the United States. It was the first "miracle" drug, routinely curing bacterial infections that had previously caused serious illness or death. Scientists discovered a new production culture and developed a submerged culture fermentation. This led to significant increases in productivity per

unit volume and the ability to greatly increase the scale of production by using stirred tank bioreactors. The success of penicillin inspired pharmaceutical companies to launch massive efforts to discover and develop many other antibiotics in the 1940s and 1950s. Most of these fermentations were highly aerobic, requiring high aeration and agitation.

In the 1960s, amino acid fermentations were developed in Japan. Initially, L-glutamic acid, as monosodium glutamate, was produced as a flavor enhancer. Using cultures derived from glutamic acid bacteria, production of other amino acids followed. Amino acids are used in foods as nutrients, sweeteners, and flavor enhancers, and in animal feeds to increase the efficiency of low protein feeds.

History of the major developments in biotechnology.

Development	Year*
Fermented beverages	**5000 BC**
Pasteur's discovery of yeast	**1857**
First medium designed for culturing bacteria	**1860**
Trickling filter for wastewater	**1868**
Anaerobic digester	**1881**
Production of citric acid using mold	**1923**
Production of penicillin in a petri dish	**1928**
Production of penicillin in small flasks	**1942**
Hixon and Gaden paper on oxygen transfer	**1950**
Air sterilization in fermentors	**1950**
Continuous media sterilization	**1952**
Aiba, Humphrey, and Millis biochemical engineering textbook on bioreactor design	**1965**
Continuous airlift reactor for production of yeast	**1969**
Advances in instrumentation and computer control	**1970**
Progress in airlift bioreactor design	**1973**
Recombinant DNA technology	**1973**
Insect cells grown in suspension culture	**1975**
Large-scale cell culture to produce interferon	**1980**
Insulin produced using bacteria	**1982**
Bioreactors for fragile cell cultures	**1988**
Textbook on plant cell biotechnology	**1994**
Textbook on protein engineering	**1996**
Textbook on tissue engineering	**1997**

* The dates are approximate and are indicative of periods of time when advances were progressing from initial studies to published works or commercial use.

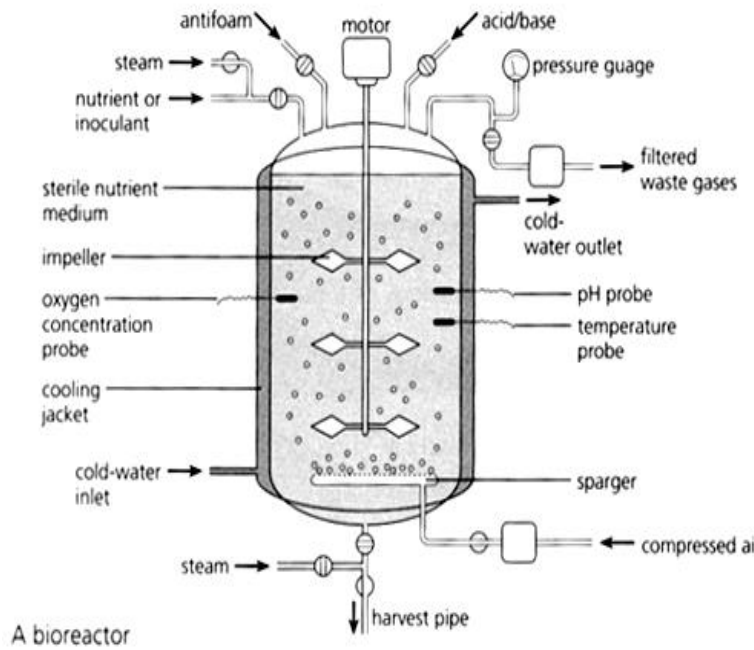
Commercial production of enzymes for use in industrial processes began on a large scale in the 1970s as well. Microbial enzymes

account for 80% of all enzymes in commercial use, including grain processing, sugar production, juice and wine clarification, detergents, and high fructose corn syrup. The discovery of the tools of genetic engineering expanded the possibilities for products made by fermentation.

Insulin was the first genetically engineered fermentation commercialized, developed in 1977. Since then, many genetically engineered products have been produced on a large scale.

TYPES OF BIOREACTORS

- The most suitable containment for each biotechnological process must be designed to give the correct environment for optimizing growth and productivity.
- Bioreactors range from simple (stirred or non- stirred) open containers to complex, aseptic, integrated systems involving varying levels of advanced computer inputs.
- **Bioreactors are of two distinct types**: **1) <u>non- aseptic systems</u>** where it is not absolutely essential to operate with entirely pure cultures (e. g. brewing, effluent disposal systems). **2) <u>aseptic conditions</u>** is essential for successful productivity (e. g. vitamins, polysaccharides). This type is more difficult to operate, maintain and design. Open and closed systems are illustrated below.



A bioreactor

- Within the bioreactor the microorganisms are suspended in the aqueous nutrient medium containing the necessary substrates for growth and product formation. All nutrients including oxygen must be provided to diffuse into each cell, and waste products such as heat, CO_2 and waste metabolites removed.
- Fermentation reactions are multiphase, involving a gas phase (containing N_2, O_2, and CO_2), one or more liquid phases (aqueous medium and liquid substrate) and solid microphase (the microorganisms and possibly solid substrates). All phases must be kept in close contact to achieve rapid mass and heat transfer.
- In a perfectly mixed bioreactor all reactants entering the system must be immediately mixed and uniformly distributed to ensure homogeneity inside the bioreactor.

GUIDELINES FOR OPTIMIZING A BIOREACTOR

1. The bioreactor should be designed to exclude entrance of contaminating microorganisms as well as containing the desired organisms.
2. The culture volume should remain constant (no leaking or evapouration).
3. The dissolved oxygen level must be maintained above critical levels of aeration and culture agitation for aerobic organisms.
4. Environmental parameters such as temperature, pH, etc., must be controlled; and the culture volume must be well mixed.

- Fermentation technologists seek to achieve a maximization of culture potential by accurate control of the bioreactor environment. But still there is a great lack of true understanding of just what environmental conditions will produce an optimal yield of organism or product. There is also a lack of good sensor probes that will allow on- line analysis to be made on the chemical components of the fermentation process.
- As fermentation systems were developed, two design solutions for the problems of aeration and agitation have been developed. The first approach uses mechanical aeration and agitation devices, with high power requirements; the standard example is the continuously stirred tank reactor (CSTR), widely used throughout conventional laboratory and industrial fermentations. Such bioreactors ensure good gas/ liquid mass transfer, reasonable heat transfer, and good mixing of the bioreactor contents. The second main approach to aerobic bioreactor

design uses air distribution (with low power consumption) to create forced and controlled liquid flow in a recycle or loop bioreactor. In this way the contents are subjected to a controlled recycle flow, either within the bioreactor or an external recycle loop. Thus, stirring has been replaced by pumping, which may be mechanical or pneumatic (using compressed air).

- The stirred tank bioreactor system is still the most widely used but most new designs are dominated by the recycle principles.
- In almost all fermentation processes performed in a bioreactor there is generally a need to measure specific growth- related and environmental parameters, record them and then use the information to improve and optimize the process.
- Bioreactor control measurements are made in either an on- line or an off- line manner. 1) In on- line measurement, the sensor is placed directly with the process stream. 2) In an off- line measurement, a sample is removed aseptically from the process stream and analyzed.
- Bioreactor processing is limited by a shortage of reliable instruments capable of on- line measurement for important variables such as DNA, RNA, enzymes and biomass. Off- line analysis is essential for these compounds but the results cannot be obtained until several hours after sampling. Therefore, they cannot be used for immediate control purposes. However, on- line measurement is readily available for temperature, pH, dissolved oxygen and CO_2 analysis.

SCALING- UP

- This process is the important step for transferring bench- top fermentations to mass production. Scale- up would be very simple if all parameters affecting bacteria remained the same. Numerous empirical and semi- empirical relationships are often used to correlate variables such as shear rates and oxygen mass transfer with physical parameters such as impeller speed and reactor dimensions. One of the most important and often overlooked factors in scale- up is power consumption. When scaling- up a process, it is important to factor in the power costs of operating large fermenters**.** Fermentation processes are normally developed in three stages or scales**:**

(1) Initial stage: basic screening procedures are carried out using simple microbiological techniques, such as Petri dishes, Erlenmayer flasks, etc.

(2) Pilot investigation: to determine the optimal operating conditions in a volume of 5 to 200 liters.

(3) Final stage: application of the pilot study to the plant (production and final economic realization).

- Throughout these stages or scales, the environmental conditions should be kept at optimum all the time. These environmental conditions involve both chemical factors (e.g. substrate concentration) and physical factors (e.g. mass transfer ability, mixing ability, power). In particular, the physical factors create problems when the process is moving from one scale to another. It is this area that requires all the skills of chemical or process engineer.

DOWNSTREAM PROCESSING

- Extraction and purification of the end- product is very important after growing the required cells in the bioreactor. These processes are called downstream processes.
- The design and efficient operation of downstream processing are vital elements in getting the required products into commercial use. Improvement in downstream processing will benefit the overall efficiency and costs of the process.
- Downstream processing will be primarily concerned with initial separation of the bioreactor broth into a liquid phase and a solids phase and subsequent concentration and purification of the product.
- Processing will normally involve more than one stage (see the Table below).
- Methods used may include distillation, centrifugation, filtration, ultrafiltration, solvent extraction, adsorption, selective membrane technology, reverse osmosis, molecular sieves, electrophoresis and affinity chromatography (see the Figure below). It is this area where several complications appear to represent a barrier for economic extraction process of the product(s) may be due to faults in extraction by the designer or chemist or, more probably, because the extraction process requires so much energy rendering the process uneconomic.
- Final product of the downstream purification stages should have some degree of stability for commercial distribution. Stability is best achieved for most products by using some form of drying. Practically, drying is achieved by spray- drying, fluidized- bed drying or by freeze drying. The method of choice is product and cost dependent.

- Products sold in the dry form include organic acids, amino acids, antibiotics, polysaccharides, enzymes, and many others. Care must be taken to avoid microbial contamination and deterioration and, for proteinaceous products, to avoid denaturation. This is why the storage conditions are very important for each of the products according to the recommendations provided.

Downstream processing operations

Process	Operations
Separation	Filtration Centrifugation Flotation Disruption
Concentration	Solubilization Extraction Thermal processing Membrane filtration Precipitation
Purification	Crystallization Chromatography
Modification	Drying

- Purity and stability of the end- product of the downstream processes are the hallmarks of most valuable biotechnological products.

QUESTIONS

1- Discuss in brief the main categories of food and non- food products of bioprocessing technology?
2- What are the main types of bioreactors and its components?
3- Give any examples on the advantages and disadvantages of producing organic compounds by biological or chemical methods?
4- How to optimize the bioreactors for better processing?
5- How to monitor the bioreactor process on-line and off- line
6- What is scaling- up and what are the initial stages for the scaling- up process?
7- Downstream processing is a very important step in industrial microbiology. Explain?
8- What could be the methods used for downstream processing? explain in no more than 5 lines.

PART (3)

SAFETY IN BIOTECHNOLOGY

INTRODUCTION

- The main areas of consideration for safety in biotechnology are:

(1) Pathogenicity: potential ability of living organisms and viruses (natural and genetically engineered) to infect humans, animals and plants to cause disease.

(2) Toxicity and allergy associated with microbial production.

(3) Other medically relevant effects: increasing environmental pool of antibiotic- resistant microorganisms.

(4) Problems associated with the disposal of microbial biomass and the purification of effluents from biotechnological processes.

Generation times or time until toxin formation by some psychrotrophic pathogens during growth in food. Adapted from Snyder (1996).

Pathogen	Temperature ° C	° F	Generation time (h)	Food
Listeria monocytogenes	0	32	110.0	Corned beef
	3	37	37.6	Roast beef
	4	39	36.0	Milk
	5	41	43.0	Raw cabbage
	5	41	44.0	Cooked meat
	5	41	33.2	Ham
	10	50	21.7	Lettuce
	10	50	8.2	Corned beef
Yersinia enterocolitica	0	32	67.4	Imitation crab legs
	0	32	44.0	Oysters
	3	37	18.0	Boiled shrimp
	7	45	10.3	Cooked beef
	10	50	12.0	Imitation crab legs
Escherichia coli	10	50	5.2	Culture medium
Pathogen	**Temperature ° C**	**° F**	**Time to toxin formation (h)**	**Food**
Clostridium botulinum type E	3.3	38	744	Beef stew
	3.3	38	964	Fish
	4.0	39	644	Fish
	4.4	40	1320	Crabmeat
	5.0	41	426	Fish
	6.0	43	456	Beef stew
	7.0	45	243	Fish
	9.0	48	163	Fish
	10.0	50	138	Fish

(5) Safety aspects associated with contamination, infection or mutation of process strains.

(6) Safety aspects associated with the industrial use of microorganisms containing *in vitro* recombinant DNA.

FOOD SAFETY

Food safety includes all practices involved with protecting food from the risk of contamination, including harmful bacteria, poisons and

foreign objects, preventing any bacteria present in the food multiplying to a level that would result in food poisoning, or the early spoilage of the food, and destroying any harmful bacteria in the food thorough cooking or processing.

A good standard of food safety depends on foodworkers knowing how the job is done, why it should be done, and then by doing it properly. **High risk foods** are those perishable foods which can support the growth of harmful bacteria and are intended to be eaten without further treatment such as cooking, which would destroy such organisms.

PROBLEMS OF ORGANISM PATHOGENICITY

- Most organisms used by industry are harmless and many are used directly for the production of human and animal food.
- Only a small number of dangerous microorganisms have been used by industry in the manufacture of vaccines or diagnostic reagents, e.g. *Bordetella pertussis* (whooping cough), *Mycobacterium tuberculosis* (tuberculosis) and the virus that causes foot- and mouth- disease.
- A classification of the degree of potential hazard of microorganisms has been drawn up by the European Federation of.

European classification of microorganisms according to their pathogenicity

CLASS 1	Microorganisms that have never been identified as causative agents of disease in human beings and that offer no threat to the environment.
CLASS 2	Microorganisms that may cause human disease and might offer a hazard to laboratory workers. They are unlikely to spread in the environment. Prophylactics are available and treatment is effective.
CLASS 3	Microorganisms that offer a severe threat to health of laboratory workers but comparatively small risk to the population at large. Prophylactics are available and treatment is effective.
CLASS 4	Microorganisms that cause severe illness in human beings and offer a serious hazard to laboratory workers and to people at large. In general, prophylactics are not available and no effective treatment is known.
GROUP E	This group contain microorganisms that offer a more severe threat to the environment than to people. They may be responsible for heavy economic losses. National and international lists and regulations concerning these microorganisms already exist in contexts other than biotechnology (e. g. agricultural purposes).

- In the recent years recombinant DNA techniques have been the most successful ones for genetic alteration of microorganisms. Also, these techniques are the cause of much concern to the public. This natural worry has been ameliorated by several evidences:

(1) Risk assessment studies have failed to demonstrate that host cells can acquire novel hazardous properties from DNA donor cells.

(2) Considerable experimentation has shown no observable hazard.

However, care must be adopted when using recombinant DNA molecules.

PROBLEMS OF BIOLOGICALLY ACTIVE PRODUCTS

- Vaccines and antibiotics are examples of biologically active products. care must be taken to prevent their undistinguishing dispersal.
- Contaminants in other safe processes may produce toxic molecules that could become incorporated into final products, leading to food poisoning
- Production of formulation against allergic reactions must be guarded.
- Overuse of antibiotics in agriculture could lead to carry- over into human food, resulting in possible development of antibiotic resistance in human disease organisms. Many countries now restrict the use of antibiotics in agriculture.
- Biotechnology must always be subjected to regulations for its successful application. The potential risks of biotechnology are manageable be proper regulations.

SAFETY AND PUBLIC ACCEPTANCE OF NEW FOODS

- The public have a negative attitude to excessive manipulation of foods, in particular, to genetic engineering of foodstuffs (GM or genetically modified foods).
- The food industry is highly conservative and slow to welcome new technological changes. The ultimate full acceptance of new biotechnology in food sector will depend on many interacting factors, e. g. economics, consumer acceptance, regulatory procedures, and the types of technology

At present, new biotechnology is having a greater impact in developed nations. It is hoped that these new approaches can also be brought to the advantage of the developing countries where the food needs are greatest.

QUESTIONS

1- What is the main consideration for safety in biotechnology? Give examples on two of these considerations
2- What are the high risk foods? Why they are considered high so?
3- How to avoid the pathogenicity of microorganisms in industry?
4- Can the pathogenic microbes be used in industry .Give an examples?
5- What is the classification of hazards caused by microorganisms set by the European Federation of Biotechnology?
6- What are the main considerations involving the biologically active products?
7- What is the response of public to the genetically modified or new biotechnology products?

PART (4)

FOOD QUALITY

INTRODUCTION

- The impact of biotechnology on the food and beverage industries can be divided into two categories:

1. **Agronomic**: Increased animal and plant yield, extended growth range and environments from which the farmers will mainly benefit.
2. **Non- agronomic:** Improving plants and microorganisms to provide benefits to the food producer, retailer or consumer.

- New developments in biochemical engineering could also be of advantage to those industries using mechanical (e. g. grinding), physical (e. g. membrane separation, cooking) and chemical (e. g. hydrolysis, salting) methods.

Some processed beverages, foods and ingredients

Alcoholic beverages			
Beers, wine and spirits.			
Non- alcoholic beverages			
Tea, coffee and cocoa			
Food and food ingredients			
Cheese	Sauerkraut	Enzymes	Vitamins
Bread	Soy sauce	Flavours	Biopolymers
Vinegar	Pickles	Organic acids	Sweeteners
Yoghurt	Amino acids	Mushrooms	

FOOD AND BEVERAGE FERMENTATION

- Fermented foods and beverages have a significant role in all societies and result from the action of microorganisms or enzymes on a wide range of agricultural or animal materials with the desired biochemical changes and improvement of the final product.
- All food fermentations are classified as indigenous, native to a country or culture according to the climate and raw material available in different geographical regions.
- Reasons for the development of such fermentations are mainly because of the need to preserve the basic organic components from spoilage and for the enhancement in the final product.
- The nutritional value of these fermentations, especially in the developing world, is inestimable and modern fermentation practices are providing increased control and ensuring product safety.
- Fermented foods can be divided into nine groups, namely beverages, cereal products, dairy products, fish products, fruit and vegetable

products, legumes, meat products, starch crop products, and miscellaneous products according to the geographical area.

Production of fermented foods according to geographical areas.

Region	Importance	
	Major	**Minor**
Europe	Dairy, beverage cereals, meat	Legumes, starch crops
North America	Beverages, dairy, meat	Fish, legumes, Starch crops
Africa south of Sahara	Starch crops, cereals, beverages	Dairy
South America	Beverages, dairy	Legumes
Middle East	Dairy	Legumes, meat
Indian subcontinent	Cereals, legumes	Meat
East Asia	Fish, legumes	Dairy
South East Asia	Fish, legumes	Dairy
Oceania	Dairy	Legumes
North Africa	Dairy	legumes

FOOD SPOILAGE

Food decays or goes off, due to the microorganisms that always exist in food;- they are not necessarily the bacteria that cause food poisoning.

The signs that food is spoiling are:

Odour - "off odours" are smells (sometimes like rotten eggs) that are produced when bacteria break down the protein in food, (usually fatty foods). This process is called putrefaction. Taints due to flavour change may also occur.

Sliminess - Food becomes slimy as the bacterial population grows. Moulds may also form slimy whiskers.

Discolouration - Foods can become discoloured by microbial growth. Some moulds have coloured spores that give the food a distinctive colour, for example, black pin mould on bread, or blue and green mould on citrus fruit and cheese.

Souring - Foods go sour when certain bacteria produce acids. A common example is when milk sours from the production of lactic acid.

Gas - Bacteria and yeasts often produce gaseous by-products that can affect food. You may have noticed meat becoming spongy, or packages and cans swelling or having a popping or fizzing sound on opening.

The involved microorganisms

Microorganisms differ from one another in appearance and activity, and providing suitable nutrients growth occurs:

- At temperatures between -7 to around 70°C.
- Over a pH range from 0 to 11.
- In the presence or absence of oxygen.
- At water activities above about 0.6.

Spoilage of any particular food will be by those organisms most suited to the conditions in and around that food.

The three main groups of concern are Bacteria, viruses and fungi (yeasts/ moulds).

Bacterial contamination (vehicles and routes)

Clean food can be contaminated by bacteria from four main sources-

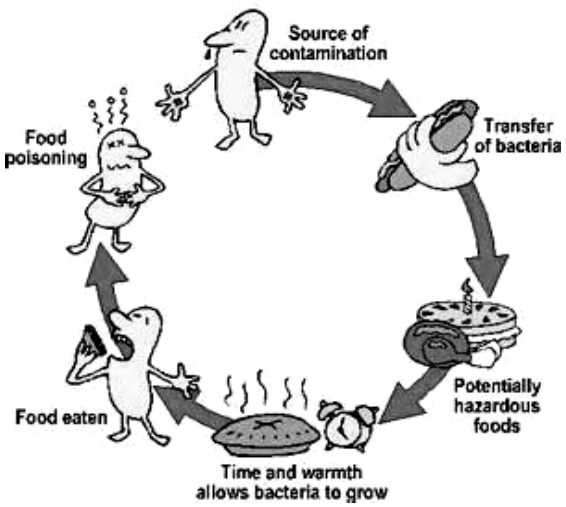
- The people present in the workplace and their clothing.
- Other food that is already contaminated.
- Dirty kitchen or work premises and equipment.
- Insects and vermin.

Sometimes, harmful bacteria pass directly from the source to high risk food, but usually they rely on other things to transfer them to food. These things are called **Vehicles**.

Indirect contamination using an intermediate vehicle is the most common, eg.- the movement of bacteria from the intestine of a food handler to food via their hands, after using the toilet.

Where contamination is passed from raw food to high risk food via for example, a cutting board, this is known as cross contamination.

The path that bacteria use to move from the source to the food, is known as the **Route**.



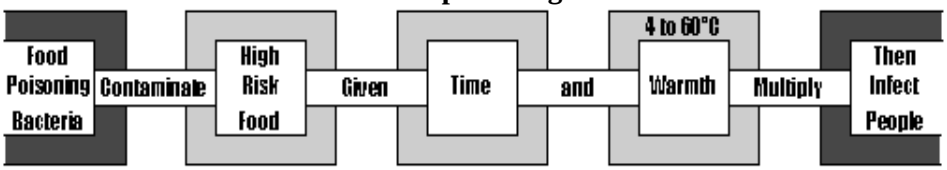
The ten main reasons for food poisoning

1. Inadequate cooling/refrigeration, food left at room temperature.
2. Too long between preparation and consumption.
3. Inadequate reheating.
4. Inadequate cooking.
5. Cross-contamination from raw to high risk/ready to eat foods.
6. Infected food handlers.
7. Inadequate hot holding temperatures.
8. Inadequate hand washing.
9. Contaminated raw foods and ingredients.
10. Improper cleaning of equipment and utensils.

Prevention of food poisoning

In most cases of food poisoning a chain of events takes place, and if we are to reduce the incidence of illness, this chain must be broken.

The food poisoning chain



There are three main ways of breaking the food poisoning chain

- Protecting food from contamination.
- Preventing any bacteria present in the food from multiplying.
- Destroying those bacteria that are present in the food.

FOOD PRESERVATION

Most preservatives today are actually fungistatic in their action. That means they prevent the growth of fungi, moulds and yeasts. They have little effect on bacteria but using a combination of preservatives, with antibacterial properties, can give good all round protection. Food preservatives help to control the spread of bacteria which can cause life threatening illnesses such as salmonellosis or botulism.

Preservatives are commonly used in the following foods:

- low fat spreads
- cheeses, margarine, mayonnaise and dressings
- bakery products
- dried fruit preparations

Safety for preservatives

Food preservatives have to be safe for human consumption. They can stop the food- decay microbes from growing but must not harm

the cells of the human body. There are also maximum levels of preservatives allowed, so that high concentrations of preservatives in food are not permitted.

Preservatives Action

Preservatives are often present in nature but they are in such small quantities that it makes them difficult to obtain. To obtain commercially- useful amounts of the preservative, they are produced as synthetic copies of the natural products.

Typically, preservatives attack the enzymes inside the microbes and this stops their cell reactions. Some can disrupt the microbe's cell wall so that substances cannot enter or leave the cell. These two processes kill or seriously slow the growth of food-spoiling microbes.

The Range of Preservatives

There are many substances which have permitted use as preservatives. The list below shows some important preservatives:

Sorbic acid‣(E200-203)
Benzoic acid‣(E210-213)
Sulfur dioxide‣(E220-228)
Potassium and‣sodium nitrate(E249 and E250)
Propionic acid‣(E280-283)

Sorbic acid and its salts (E200-203)

Sorbic acid (and its salts) are naturally occurring substances and are among the most important food preservatives for industrialized countries. Sorbic acid has two main advantages:

- it is effective over a wide range of foods and beverages
- it gives no taste or flavour to products

Sorbic acid is used in beverages, dairy products, fish and seafood, fat- based products, fruit and vegetable products, baked goods and confectionery products.

Benzoic acid and its salts (E210-E213)

A widely used preservative that is important in developing countries. It is only used in acidic situations which include non- alcoholic beverages, products prone to spoilage by bacteria and fruit-based products.

Sulfur dioxide and sulfites (E220-E228)

This preservative was known to the Romans, Ancient Greeks and Egyptians. These preservatives are multifunctional food ingredients which act as preservatives, antioxidants and colour stabilizers. They have a much more pronounced antibacterial effect than other

preservatives and are used when control of bacterial growth is essential. Sulfur dioxide is used in a wide range of products including some dried fruits, tinned crabmeat, sausage meat and jams.

Potassium nitrite and sodium nitrite (E249 and E250)

Synthetic additives capable of performing many functions in food preparation. They act as preservatives, stabilizers and flavours. There are health concerns about their use. However, without their contribution there would undoubtedly be many more deaths from botulism which is caused by the bacterium *clostridium botulinum*. Potassium and sodium nitrite are particularly important in the preservation of cured meat products.

Propionic acid and its salts (E280-E283)

The propionates are other examples of naturally occurring preservatives. They work better in the more alkaline conditions of bakery products and may be used, for example, to delay the green mould growth on bread.

PERSONAL HYGIENE

Good personal hygiene reduces the chance of contamination of food.

- Hands must be washed before and after handling food.
- If unwell, do not handle food until cleared by a doctor.
- The hair, nose and mouth must not be touched during food preparation.
- Suitable light coloured protective clothing should be worn.
- Cuts and abrasions should be covered with waterproof bandages and if on the hands suitable gloves worn.
- Rings and other jewellery should not be worn as they can harbour dirt and bacteria and could themselves fall into the food being prepared.

FOOD QUALITY CONTROL

The general purpose of quality control is to ensure that a maximum amount of the product being processed reaches the desired level of quality with minimum variation and that this is achieved as economically as possible. The products of natural raw materials are never exactly the same, so control is necessary to keep product quality within the standards set. Raw materials should be purchased from reliable suppliers who hold a current food manufacturer's registration.

Quality control generally involves inspections of three kinds of materials:

- Raw materials

- Materials in process
- Finished product

If effective raw material and process controls are not put in place and only examination of the finished product is done, then quality control stops being a control and becomes merely an inspection.

FOOD SAFETY PROGRAMS

The introduction of the Control at source, Good Manufacturing Practices (GMP), Hazard Analysis of Critical Control Points (HACCP) and Quantitative Risk Analysis (QRA) have made food production more controlled in a cost- efficient way. GMP and HACCP protocols should verify for instance that only raw materials without pathogens are used in production, and that processing conditions are well defined to prevent the growth of harmful microorganisms present in the manufacturing lines. QRA is a stepwise analysis of health risks associated with a particular type of food product. In QRA an estimation is made of the probability of negative health effects after consumption of products.

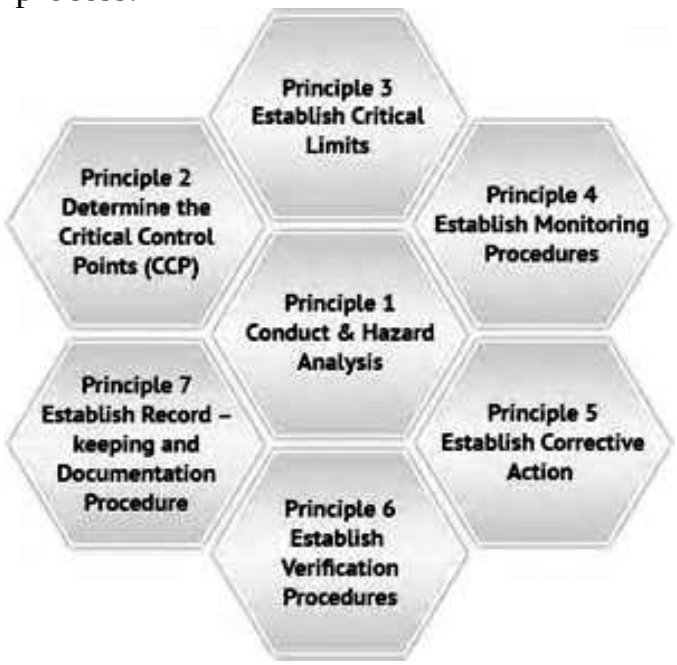
It has been estimated that less than 1 % of the microorganisms are culturable, and not all microorganisms, are detected by the same media. There are microorganisms associated with food (*Escherichia coli*, *Salmonella enteritidis*, *Shigella* spp., *Vibrio vulnificus*, *Campylobacter jejuni*, *Pseudomonas* spp.) which are known to have viable but non- culturable forms (VBNC), and they remain potentially virulent during the dormant phase. Some form spores or other dormant stages when environmental conditions are unfavourable and can start to grow when environmental conditions changes. Rapid and reliable fingerprinting methods should be developed for finding contamination sources.

Hazard Analysis Critical Control Point- (HACCP)

It is recommended that every food business adopt the HACCP approach to identify all potential hazards and control them. Setting up a HACCP system will involve the following -

- **Set up a HACCP team** - of those people who fully understand the product.
- **Draw up flow charts** - that define all stages in the preparation process, from raw materials through to consumption or sale.
- **Identify all potential hazards** - (eg. physical, chemical, bacterial)

- **Identify the critical control points** - consider all preventive measures and decide which are needed to eliminate or reduce potential hazards to acceptable levels.
- **Determine target levels and tolerances for control points** - (eg. time).
- **Establish monitoring systems for critical control points** - (eg. work out who should act and when, where and what action should be taken).
- **Establish a recording and documentation system.**
- **Review the HACCP system** - annually and when changes are made to any process.



QUESTIONS

1- What are the main food and beverage fermentations in industry?
2- Does the geographical area have any impacts on the industrial food products? Explain?
3- What are the signs of food spoilage? Are they recoverable?
4- Why food spoilage can happen in any environment?
5- What are the mechanisms of food spoilage?
6- What are the main reasons of food poisoning that can be avoided?
7- What is the food poisoning chain and how it can be broken?
8- What is the personal hygiene and its importance in food processing?
9- Give short notes on each of the three main microorganism groups responsible for food poisoning?
10- What is quality control in food industry?
11- What is the process involved in HACCP programs?

PART (5)

MICROBIOLOGICAL INDUSTRIES

I. FOOD AND RELATED PRODUCTS

INTRODUCTION

The involvement of microbes in food products, as food production is the major microbial industry, is summarized in the following. The industrial processes itself will be detailed later.

a) Dairy products - fermentation of milk generates lactic acid, which precipitates milk proteins and prevents other microbial growth. Products include (for example):

- **Buttermilk** (from skim milk) - *Streptococcus diacetylactis*
- **Yogurt** - *Streptococcus thermophilus* and *Lactobacillus bulgaricus*
- **Sour cream** (from cream) - *Streptococcus diacetylactis*
- **Acidophilus milk** - *Lactobacillus acidophilus*
- **Cheeses** (lactic acid fermentations; 2000 varieties approximately, 20 types).

Examples of the involved microbes are as follows:

- **Soft** - cottage (e. g. *Streptococcus lactis*), cream (e. g. *S. thermophilus, Lactobacillus bulgaricus*, mozzarella (*S. thermophilus, L. bulgaricus*), **Semi-soft**- blue (Roquefort - *S. lactis, S. cremoris*; *Penicillium roqueforti*), **Hard**- cheddar (*Streptococcus lactis, S. cremoris, L. plantarum*), Swiss (*S. lactis, S. thermophilus; Propionibacterium shermanii),* **Very hard**- parmesan (goat milk fermented and flavored by *Streptococcus lactis, S. cremoris, S. thermophilus; Lactobacillus bulgaricus*).

b) Meat products (salami, summer sausage) - *Pediococcus cerevisiae* and *Lactobacillus plantarum*

c) Baked goods- Baker's yeast (*Saccharomyces cerevisiae*) aerobically generates carbon dioxide for breads and pastries

d) Miscellaneous foods

- **Coffee** (coffee beans) - *Erwinia dissolvens, Saccharomyces spp*.
- **Pickles** (cucumbers) - *Leuconostoc mesenteroides* together with *Lactobacillus plantarum*
- **Vinegar** (apple juice, wine) - *Acetobacter* or *Gluconobacter*

e) Microbes as a direct food source

1- Single-cell protein - *Candida* grown on sulfite waste "liquors" from paper manufacturing

- approximately 50% protein, but also 16% nucleic acid

2- Spirulina (*cyanobacterium* - photosynthetic)
3- Mushrooms - *Agaricus campestris bisporus*

f) Food additives and supplements

- **Amino acids** - used as food additives or starting materials in the chemical industry. Examples: glutamic acid (MSG), phenylalanine and aspartic acid, lysine, tryptophan
- **Vitamins** - food supplements for humans and animals. Examples: vitamin B1, riboflavin (*Saccharomyces*); ascorbic acid (*Acetobacter*)
- **Brewer's yeast** (*Saccharomyces carlsbergensis*)- used as vitamin B-rich food additive (relatively high in nucleic acids, though)
- **Organic acids (e. g. Citric acid)** (*Aspergillus niger*) - food and beverage additive

MICROORGANISMS IN FOOD

The microorganisms present in products are dependent on the microbiological quality of raw materials, hygienic conditions in the production, and how the product has been stored after the production. Scientists have listed 36 bacteria genera, 16 mould genera, 14 yeast genera, and five protozoa species associated with food. Several important microorganisms implicated with food- borne diseases include:

Gram-negative rod shaped bacteria

The most common spoilage microorganisms are *Pseudomonas* spp., particularly in aerobically stored foods with a high a_w and relatively neutral pH conditions (meat, fish, poultry, milk and dairy products). Other gram-negative rod shaped bacteria include *Aeromonas* spp., *Photobacterium* spp., and *Vibrio* spp.

Gram-positive spore forming bacteria

Gram-positive, spore forming bacteria are capable of surviving the high temperatures used in food production including species of *Bacillus* spp. and *Clostridium* spp. For the food industry, especially strains which are able to grow in refrigeration temperatures may create problems. Only a few of the *Clostridium* spp. can grow in cold storage conditions and *Bacillus* spp. are considered to be a more important bacteria causing spoilage and food-borne diseases.

Lactic acid bacteria

The lactic acid bacteria (LAB) group contain several species in the following genera: *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Carnobacterium, Enterococcus, Oenococcus,*

Streptococcus, *Tetragenococcus*, *Vagococcus*, and *Weissella*. LAB have a dual role in food industry. While the production of certain products relies on the actions of LAB, the production process of other products or the final products themselves (e.g. vacuum- packed meat) are spoiled by their growth. In some products LAB ferment sugars to form lactic acid, slime, and CO_2 and as a consequence lower the pH.

Other gram-positive bacteria

Gram-positive spoiling bacteria include *Brochothrix thermosphacta* which also spoils vacuum- packed fresh meat products and *Micrococcus* spp. which spoil products with high salt concentration. Many of the strains are thermoduric surviving pasteurisation processes.

Yeasts and moulds

Yeasts and moulds are able to utilise a variety of substrates (sugars, pectines, organic acids, proteins and lipids) and furthermore they are relatively tolerant to low pH, low a_w, low temperatures and various preservatives. These microorganisms have also a dual role in food production, while *Penicillium roqueforti* is used in the production of blue mould (roquefort) cheese, some other cause disease by producing mycotoxins and some other strains cause spoilage of products.

Staphylococcus aureus: Currently, the genus *Staphylococcus* consists of 33 species. The members of the genus *Staphylococcus* have been found to be common to all animals (in skin and in mucosal membranes). Therefore, their presence in food manufacturing lines have been considered as an indicator of poor hygienic conditions. For instance in one food factory, 44% of the workers were found to harbour enterotoxic staphylococci in their noses. *S. aureus* has been considered to be one of the most important bacteria causing food poisoning.

FOOD INDUSTRIES

COFFEE, TEA AND COCOA

- In Asia, Africa, India and South America, non- alcoholic fermented beverages are derived from coffee, tea and cocoa plants. These beverages have gained high commercial value.
- Tea is derived from the enzymatic activity of microorganisms after crushing the leaves, while for coffee and cocoa the pulp surrounding the beans is removed in part by natural fermentation with bacteria,

yeast and fungi, which is critically important for full aroma and flavour development.

- The dried products, namely tea leaves and coffee and cocoa beans, can then be shipped to any other part in the world and the final beverage is formed by the addition of water.

PROCESSING OF COFFEE

Is the method converting the raw fruit of the coffee plant (cherry) into the commodity green coffee. Coffee (*Coffea arabica* and *C robusta*) originated from Ethiopia. The main producers of coffee today are Colombia, Brazil, Angola, and Indonesia, in that order. It takes from three to five years of growth before the coffee tree is ready to bear fruit. The fruits grow slowly, taking from 8 to 12 months to reach maturity (when they are bright red in colour). Each coffee fruit or berry contains two seeds covered by pulp.

Coffee berries

There are two methods of processing coffee: the **wet method** and the **dry method**. In the wet method, the fruits are passed through a pulping machine which removes the pulp leaving by mucilage which is removed by pectinolytic enzymes of microbiological origin. The coffee may also be dried by exposure to sunlight. When dry, the fruits are dehulled to remove the dry outer portions. The studies on the microbiology of coffee fermentation showed that many organisms were pectinolytic, including spore- and non-spore forming ones. Other workers found lactic acid bacteria (e.g. *Leuconostoc* and *Lactobacillus spp*.) and yeasts (*Saccharomyces spp* and *Schizosaccharomyces spp*.).

Traditional coffee drying in Panamá

Aging

All coffee, when it was introduced in Europe, came from the port of Mocha in what is now modern day Yemen. To import the beans to Europe the coffee was on boats for a long sea voyage around the Horn of Africa. This long journey and the exposure to the sea air changed the coffee's flavour. Once the Suez Canal was opened the travel time to Europe was greatly reduced and coffee whose flavour had not changed began arriving. To some degree, this fresher coffee was rejected because Europeans had developed a taste for the changes that were brought on by the long sea voyage. To meet this desire, some coffee was aged in large open-sided warehouses at port for six or more months in an attempt to simulate the effects of a long sea voyage.

Decaffeination

Decaffeination is the process of extracting caffeine from green coffee beans prior to roasting. The most common decaffeination process used in the United States is supercritical carbon dioxide (CO_2) extraction. In this process, moistened green coffee beans are contacted with large quantities of supercritical CO_2 (CO_2 maintained at a pressure of about 4,000 pounds force per square inch (28 MPa) and temperatures between 90 and 100 °C, which removes about 97 % of the caffeine from the beans. The caffeine is then recovered from the CO_2, typically using an activated carbon adsorption system.

Another commonly used method is solvent extraction, using oil (extracted from roasted coffee) or ethyl acetate as a solvent. Water extraction is also used for decaffeination. Decaffeinated coffee beans have a residual caffeine content of about 0.1 % on a dry basis.

Roasting

Coffee roasting is carried out by coffee houses and could be done at home as the old days. Nearly all coffee sold to consumers throughout the world is sold as roasted coffee.

TEA PROCESSING

A tea bush.

Plantation workers picking tea in Tanzania.

Tea is a beverage made by steeping processed leaves, buds, or twigs of the tea bush (*Camellia sinensis*) in hot water for a few minutes. The processing can include oxidation (fermentation), heating, drying, and the addition of other herbs, flowers, spices, and fruits.

Young tea leaves are harvested by hand and spread on trays to wither.

Tea withering in trays

Thereafter the leaves are rolled to squeeze out juices from the leaves and spread the juices over the surface of the leaves. This exposes the polyphenols to oxidation, and the green colour gradually begins to turn brownish. Rolling also breaks the leaves into smaller pieces. The 'fermentation' stage follows, but this is a chemical reaction involving polyphenols. After fermentation, the tea is 'fired', i.e. subjected to hot air of between 80 and 90°C. After firing, the tea is sorted and graded.

Tea is a natural source of methylxanthines such as caffeine, catechins, and theanine. It has almost no carbohydrates, fat, or protein. It has a cooling, slightly bitter and astringent taste.

Classification

The types of tea are distinguished by the processing that they undergo. Leaves of *Camellia sinensis* soon begin to wilt and oxidize if not dried quickly after picking. The leaves turn progressively darker because chlorophyll breaks down and tannins are released. The next step in processing is to stop the oxidation process at a predetermined stage by

heating, which deactivates the enzymes responsible. In black tea this is done simultaneously with drying.

Tea is traditionally classified based on the degree or period of "fermentation" (actually enzymatic oxidation) the leaves have undergone.

White tea

Young leaves (new growth buds) that have undergone no oxidation; the buds may be shielded from sunlight to prevent formation of chlorophyll. White tea is more expensive than tea from the same plant processed by other methods.

Green tea

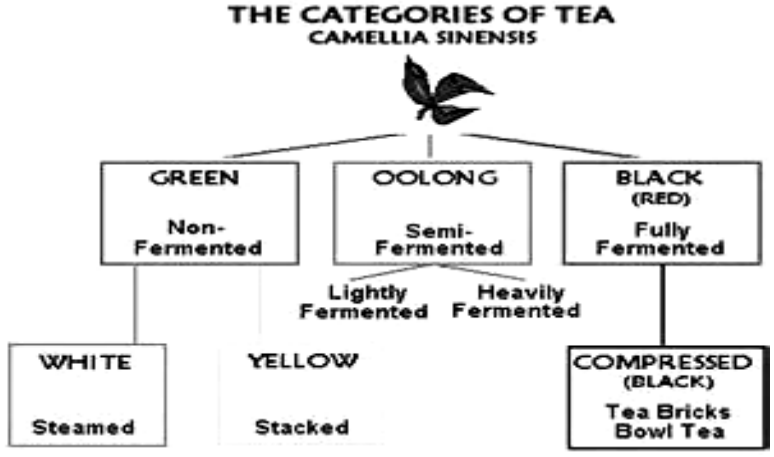
The oxidation process is stopped after a minimal amount of oxidation by application of heat, either with steam, a traditional Japanese method, or by dry cooking in hot pans, the traditional Chinese method. The tea is processed within one to two days of harvesting.

Oolong tea

Oxidation is stopped somewhere between the standards for green tea and black tea. The oxidation process takes two to three days.

Black tea/ Red tea

The tea leaves are allowed to completely oxidize. Black tea is the most common form of tea in southern Asia (India, Pakistan, Bangladesh, Sri Lanka, etc.) and in the last century many African countries. The Chinese call it *red tea* because the actual tea liquid is red. Westerners call it *black tea* because the tea leaves used to brew it are usually black. The oxidation process will take between two weeks and one month. Black tea is further classified as either *orthodox* or as *CTC* (*Crush, Tear, Curl*, a production method developed about 1932). Other types include Pu-erh, Yellow tea, herbal teas and more.



COCOA PROCESSING

Cocoa beans in a cacao pod

Cocoa (*Theobroma cacao*) is a native of South America, but today the major producers are Ghana, Nigeria, Ivory Coast, Cameroon, and Malaysia. The tree produces pods which contain from 40 to 60 seeds. The pods are opened and the seeds heaped and allowed to ferment, often in baskets which permit liquid to drain out.

During fermentation the mucilagenous outer covering of the seeds is broken down by microbial action, while the seeds themselves change from pinkish to black. It is believed that the lactic acid bacteria play important roles in the development of the aroma of cocoa.

Cocoa often refers to cocoa powder, the dry powder made by grinding cocoa seeds and removing the cocoa butter from the dark, bitter cocoa solids. Cocoa powder has a bitter flavor.

Cocoa bean fermentation

History

The cacao tree apparently originated in the foothills of the Andes in the Amazon and Orinoco basins of South America. It was introduced into Central America by the ancient Mayas, and cultivated in Mexico by the Toltecs and later by the Aztecs.

Cacao trees will grow in a very limited geographical zone, of approximately 10 degrees to the north and south of the Equator. Nearly 70% of the world crop is grown in West Africa.

The cacao plant was first given its name by Swedish natural scientist Carl von Linné (1707-1778), who called it "*Theobroma cacao*" or "food of the gods".

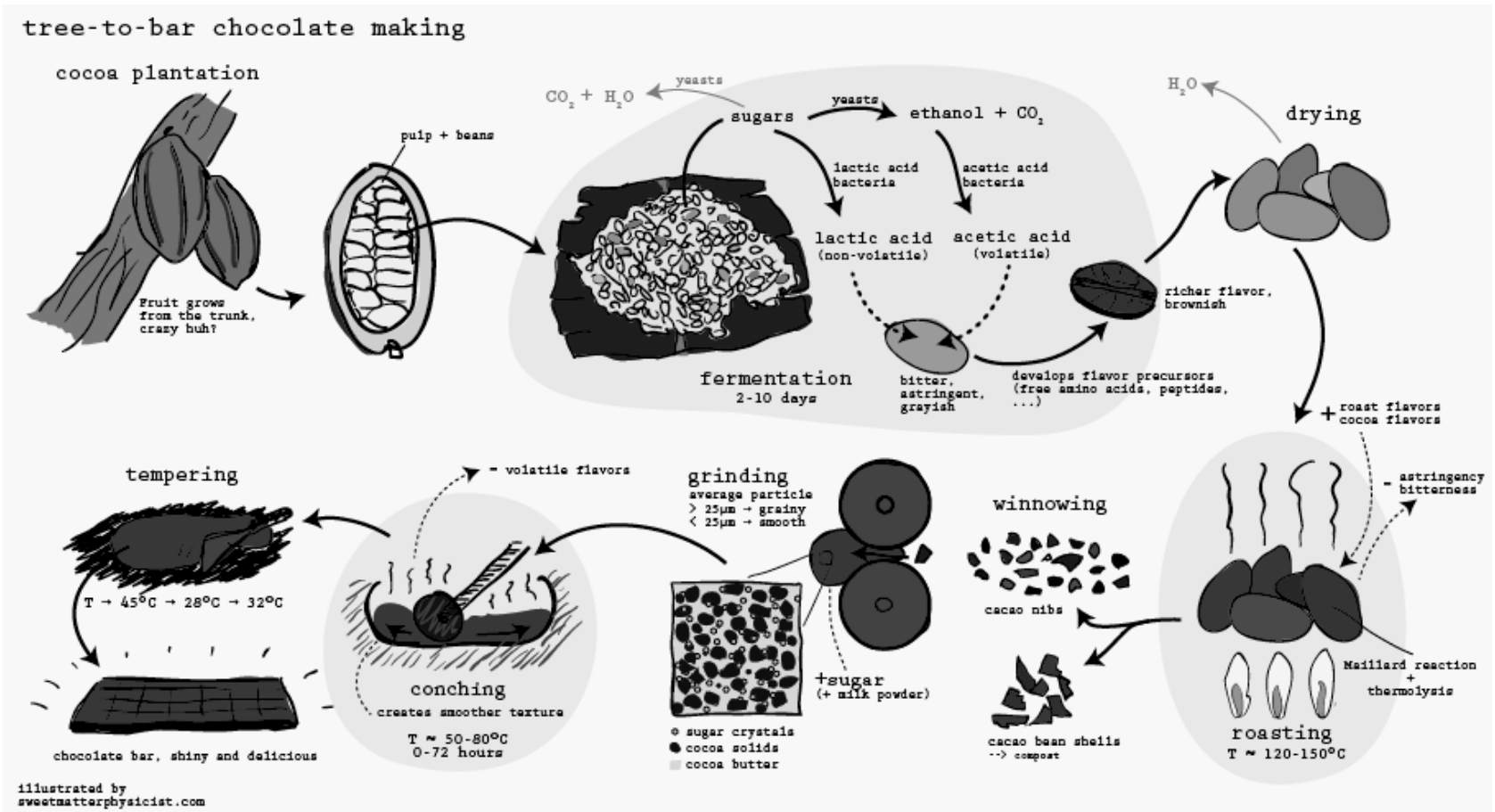
Harvesting

When the pods ripen, they are harvested from the trunks and branches of the Cocoa tree with a curved knife. The pod itself is green when ready to harvest, rather than red or orange. Normally, red or orange pods are considered of a lesser quality because their flavors and aromas are poorer; these are used for industrial chocolate. The pods are either opened on the field and the seeds extracted and carried to the fermentation area on the plantation, or the whole pods are taken to the fermentation area.

Processing

The harvested pods are opened with a machete, the pulp and cocoa seeds are removed and the rind is discarded. The pulp and seeds are then piled in heaps, placed in bins, or laid out on grates for several days. During this time, the seeds and pulp undergo "sweating", where the thick pulp liquifies as it ferments. The fermented pulp trickles away, leaving cocoa seeds behind to be collected. Sweating is important for the quality of the beans, which originally have a strong bitter taste. If sweating is interrupted, the resulting cocoa may be ruined; if underdone the cocoa seed maintains a flavor similar to raw potatoes and becomes susceptible to mildew. The liquified pulp is used by some cocoa producing countries to distill alcoholic spirits.

The fermented beans are dried by spreading them out over a large surface and constantly raking them. In large plantations, this is done on huge trays under the sun or by using artificial heat. Small plantations may dry their harvest on little trays or on cowhides. Finally, the beans are trodden and shuffled about (often using bare human feet) and sometimes, during this process, red clay mixed with water is sprinkled over the beans to obtain a finer color, polish, and protection against molds during shipment to factories in the United States, the Netherlands, United Kingdom, and other countries. Drying in the sun is preferable to drying by artificial means, as no foreign flavours such as smoke or oil are introduced which might otherwise taint the flavour.


tree-to-bar chocolate making
cocoa plantation
Fruit grows from the trunk, crazy huh?
pulp + beans
$CO_2 + H_2O$
yeasts
sugars
yeasts
ethanol + CO_2
lactic acid bacteria
acetic acid bacteria
lactic acid (non-volatile)
acetic acid (volatile)
fermentation
2-10 days
bitter, astringent, grayish
develops flavor precursors (free amino acids, peptides, ...)
richer flavor, brownish
H_2O
drying
+ roast flavors
cocoa flavors
- astringency
bitterness
roasting
T ~ 120-150°C
Maillard reaction
+
thermolysis
winnowing
cacao nibs
cacao bean shells
--> compost
grinding
average particle
> 25µm → grainy
< 25µm → smooth
+sugar
(+ milk powder)
sugar crystals
cocoa solids
cocoa butter
- volatile flavors
conching
creates smoother texture
T ~ 50-80°C
0-72 hours
tempering
T → 45°C → 28°C → 32°C
chocolate bar, shiny and delicious
illustrated by
sweetmatterphysicist.com

DAIRY PRODUCTS

Milk is an excellent food source for humans. It is full of vitamins, fats, minerals, nutrients and carbohydrates. It is rich in the protein casein which gives milk its characteristic white colour. The most abundant carbohydrate is the disaccharide lactose "milk sugar."

At room temperature, milk undergoes natural souring caused by lactic acid produced from fermentation of lactose by fermentative lactic acid bacteria. This accumulation of acid (H^+ ions) decreases the pH of the milk and cause the casein to coagulate and curdle into curds and whey. Curds are large, white clumps of casein and other proteins. Whey is the yellow liquid that is left behind after the casein has formed curds.

The important microbes for dairy product manufacturing can be divided into two groups, primary and secondary microflora. Products undergoing fermentation by only primary microflora are called unripened and those processed by both primary and secondary microflora are called ripened. During dairy product production, milk is first pasteurized to kill bacteria that cause unwanted spoilage of the milk and the final products. Primary microflora consists of certain kinds of *Lactococcus, Lactobacillus* and *Streptococcus* that are intentionally added to pasteurized milk and grown at 30°C or 37°C (temperature depends on the bacteria added). Secondary microflora include several different types of bacteria (*Leuconstoc, Lactobacillus*, and *Propionibacterium*), yeasts and molds; that are only used for some types of surface ripened and mold ripened cheeses. The various combinations of microflora determine what milk product you will end up with.

Different unripened milk products are created by using various starting materials and bacteria. During yogurt production, dry milk protein is added to milk to concentrate the milk before addition of actively growing *Streptococci* and *Lactobacilli*. Butter is produced by curdling and slight souring from *Streptococci* growing in sweet cream. *Leuconostoc* is then added so it can synthesize diacetyl, a compound that gives butter its characteristic aroma and taste. The milk is then churned to aggregate the fat globules into solid butter.

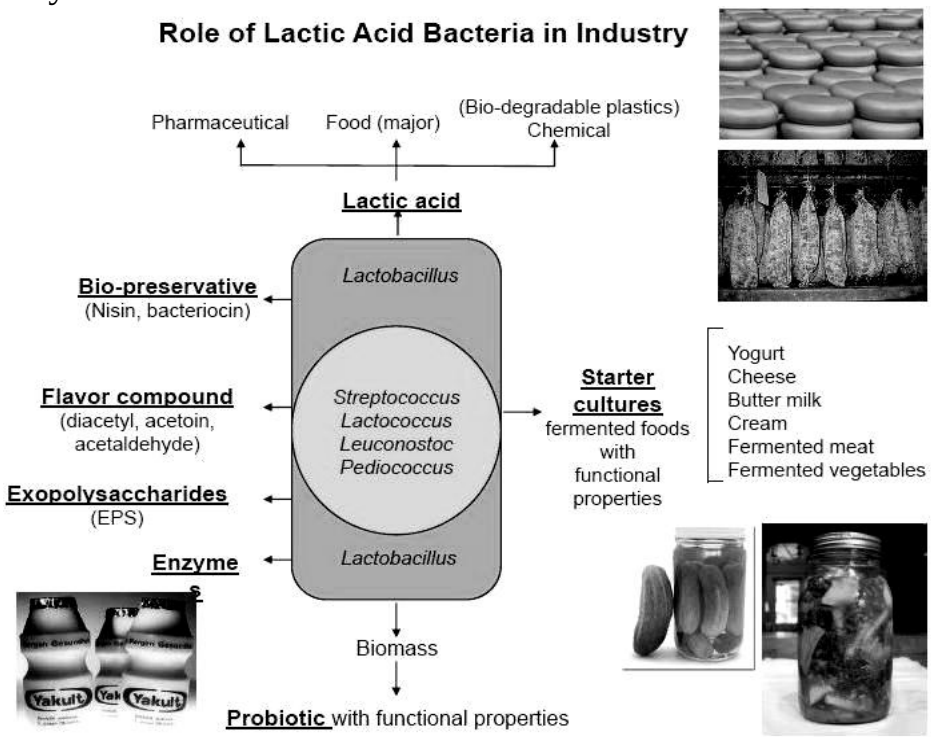
CHEESE PRODUCTION:

- In the past days, these fermentations were accidentally occurring by the natural presence of lactic acid bacteria. In the present time, an

inoculum (of pure cultured bacteria) is added to the milk for obtaining the best final product.

- The early cheese production processes arisen from the use of animal stomachs (sheep) in which the milk is heated and soured by naturally occurring bacteria and contaminated with the enzymes "*rennet*" from stomach lining. This results in the transformation of milk into solid curds and liquid whey.
- The main benefits gained from the use of lactic acid bacteria are:

1- They inhibit many undesirable bacteria while they are harmless bacteria. Therefore, they preserve milk in this way.
2- They create the required texture and flavour in the fermented milk.
3- They have beneficial health effects on intestinal microflora.



Particularly in the past, cheese was valued for its long shelf life. Due to its reduced water content, acidic pH, and inhibited bacterial growth. This causes cheese to spoil much more slowly than other milk products. The basic process steps for cheese production can be summarized as follows:

1. Acidification of the milk by the conversion of sugar lactose into lactic acid by the lactic acid bacteria.
2. Coagulation of the casein by a combination of proteolysis and acidification.

- Proteolysis is started by the rennet (chymosin or rennin enzyme from animal or fungal origin) and the coagulated caseins form a gel that entraps any fat present. The separated curd is cut into blocks, drained and pressed into shapes, matured and made into cheeses. The details of cheese production are very complicated and involve many strains of bacteria and fungi (e. g. Camembert, blue- cheese), special milks, additives and differing process techniques as seen in the Figures below.

Recent biotechnological cheese productions involve the use of recombinant DNA techniques for chymosin production and commercial use. In industry, cheese production has three major steps: curd formation, curd treatment and curd ripening.

1. <u>Curd formation</u> can use mare, ewe, cow or goat milk to produce "sour" or "sweet" curd. Sour curd is produced by fermentative lactic acid bacteria as mentioned above. Sweet curd is produced by adding an enzyme called rennin instead of bacteria to curdle the milk. The curd is separated from the whey by draining. The curd can be used directly to make unripened cheeses such as ricotta or cottage cheese or can undergo further processing to make a ripened cheese.

Cooking and stirring

Curd collection

2. <u>Curd treatment</u> consists of condensing and squeezing to form dense, hard curd. It is then molded into the desired shape, salted and mixed with different types of secondary microflora.

Adding rennet

Draining and compressing curd

Flipping curd

3. **<u>Curd treatment (Secondary microflora)</u>** for ripening the cheese and determine the final texture and aroma of each type of cheeses. For hard ripened cheeses such as Cheddar, curds are further compressed and the bacteria particular for the cheese is added. The Cheddar is wrapped in wax or plastic to prevent contamination and then incubated to allow the bacteria to do its work. For soft ripened cheeses such as Camembert and Limburger, a microbe, usually mold, is added to the surface of the cheese that produces a protein-digesting enzyme. This enzyme breaks apart the curds and causes the cheese to become creamy and spreadable.

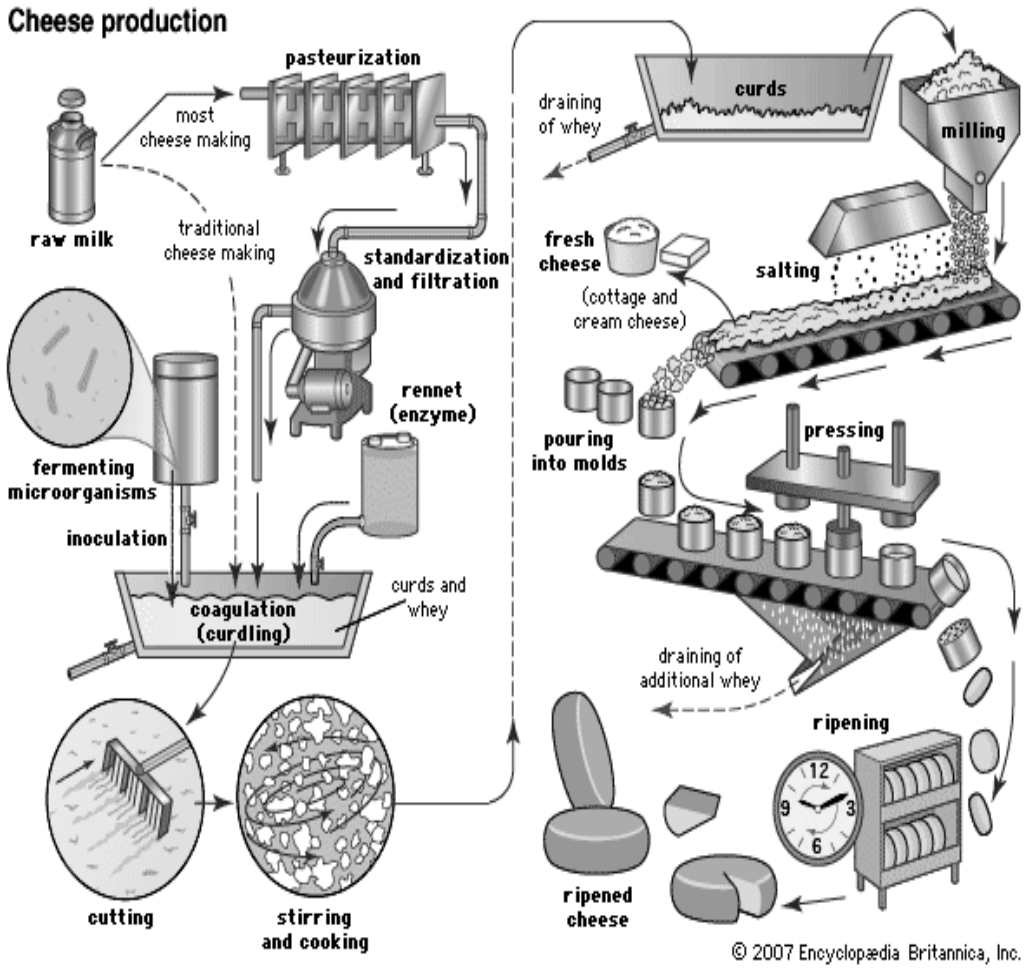
Unripened cheese

Cheese ripening

Rennet

rennet, substance containing rennin, an enzyme having the property of clotting, or curdling milk. It is used in the making of cheese and junket. Rennet is obtained from the stomachs of young mammals living on milk, especially from the inner lining of the fourth, or true, stomach (abomasum) of milk- fed calves. The preparation of rennet was formerly a part of the domestic function of making cheese; the inner membrane was kept in salt, dried, and, when rennet was needed, soaked in water. Now extract of rennet is made and sold commercially. It is usually prepared by soaking the tissues in warm, slightly salted water and straining and preserving the resulting liquid. Heat interferes with the action of rennet.

The following Figures show a schematic diagram of the cheese manufacturing process.



- Microorganisms introduced, or permitted to develop, in cheese during the ripening process to impart distinctive flavours and textures. Cheese is valuable as a source of protein, fat, insoluble minerals (calcium, phosphorus, sulfur, and iron), and, when made from whole milk, vitamin A.

Principal types of cheeses

Unripened cheeses
Low fat (cottage cheese)
High fat (cream cheese)
Ripened cheeses
1- Hard cheeses (internal ripening)
Ripened by bacteria (cheddar, Swiss)
Ripened by mould (Rouquefort and other blue cheeses)
2- Soft cheeses (external ripening)
Ripened by bacteria (Limburger)
Ripened by bacteria and mould (Camembert)

QUESTIONS

1- Give short notes on food industries and related products involving the use of microbes.

2- Mention some microbes used as direct food source.

3- Discuss in brief two common types of bacteria that cause food spoilage.

4- Write on two main processes for beverage fermentation

5- How and why coffee processing involves the process of aging?

6- Define the classes of tea based on fermentation?

7- Write on cocoa fermentation and processing. What are the end products?

8- How lactic acid bacteria is the most beneficial organisms in microbial industry? What are their benefits for human beings?

9- Discuss in brief the steps of cheese production.

10- How the process of cheese making can end with various kinds of cheeses?

11- What is the difference between primary and secondary microflora in cheese production? If the secondary microflora is not involved, will the cheese production process be completed?

PART (6)

I. FOOD AND RELATED PRODUCTS (Continued..)

CEREAL PRODUCTS

- Cereal products are the main class of food consumed by people to be fermented as solid food or alcoholic beverages.
- **Bread** is the main fermented cereal product around the world that is consumed in many forms. The kind of bread produced differs according to the geographical area.
- In Europe, wheat and rye are the main cereal flours used and usually mixed with milk or water, salt, fat, sugar and many other ingredients including the yeast *Saccharomyces cereviciae*.

BREAD

Wheat, and several related grains, make a group of proteins called glutens. These proteins have the characteristic of forming long molecular strings when they are "worked" or "kneaded" that bind the bread together in the sticky mass we call **dough**. Gluten also contributes to the delightful flavour imparted to bread during baking. Bread rises due to the activity of contaminating (or added) yeast which metabolizes the sugar in the wheat and converts it into **carbon dioxide**. Because of the **gluten glue**, the carbon dioxide is trapped within the bread which causes the bread to **rise** from the pressure of the carbon dioxide buildup. This results in the formation of many small bubbles within the bread. When the bread is baked the protein is denatured and it and the starch will harden into bread. The yeast also contributes important flavouring to the bread.

Although, our knowledge of the biology of bread making is only a little more than 100 years old, people have known for several thousand years that in order to make bread you had to add a **starter culture** of dough containing the yeast to each new batch of fresh bread dough.

- Thus, the process of bread making involves three primary steps: 1) **leavening** (CO_2 production), 2) **falvour development and texture changes** in the dough, 3) at the end of fermentation process, **baking** in an oven, giving a final product that is free from living microorganisms and with an extended shelf- life.
- Bread texture is affected by fats, emulsifiers and oxidizing agents while the speed of bread- making is affected by fats, oxidizing and reducing agents and Soya flour. The yeast enzymes have an important

role. Additional enzymes such as amylases are added to assist mixing, fermentation, baking and storage characteristics of the bread.

- In other parts of the world sour- dough breads use the yeast *Candida milleri* and the bacterium *Lactobacillus sanfrancisco* for fermentation while other species are used in other parts of the world.

Dough

Bread showing pockets left by carbon dioxide.

BAKER'S YEAST PRODUCTION

The production of baker's yeast is the largest domestic use of a microorganism for food purposes. Baker's yeast is a strain of *Saccharomyces cerevisiae*. The strain of the yeast is carefully selected for its capacity to produce abundant gas quickly, its viability during storage, and its ability to produce desirable flavour.

- The organisms are mixed with bread dough to bring about vigorous sugar fermentation. The carbon dioxide produced during the fermentation is responsible for leavening or rising of the dough.

INDUSTRIAL SIGNIFICANCE OF YEASTS

Yeasts have been exploited for thousands of years in traditional fermentation processes to produce beer, wine, and bread. The products of modern yeast biotechnologies impose on many commercially important sectors, including food, beverages, chemicals, industrial enzymes, pharmaceuticals, agriculture, and the environment. The Table below lists some of the principal industrial commodities from yeasts. *S. cerevisiae* is the most exploited microorganism known and

is the yeast responsible for producing potable and industrial ethanol, which is the world's premier biotechnological commodity.

Some industrial products of yeast

Item	Examples
Beverages	Alcoholic beverages
Food and animal feed	Baker's yeast, yeast extract, food pigments, fodder yeast and livestock growth factors
Chemicals	Fuel ethanol, carbon dioxide, glycerol, citric acid, vitamins, bioreductive catalyst in organic chemistry
Enzymes	Invertase, inulinase, pectinase, lactase, lipase
Recombinant proteins	Hormones (e. g. insulin), viral vaccines (e. g. hepatitis B), antibodies, growth factors, blood proteins (e. g. human serum albumin), enzymes(e. g. gastric lipase)

The useful physiological properties of yeast have led to their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest and largest application of this technology. Many types of yeasts are used for making many foods: Baker's yeast in bread production, brewer's yeast in beer fermentation, and for xylitol production. Yeasts are also one of the most widely used model organisms for genetics and cell biology.

It is not known when yeast was first used to bake bread. The first records that show this use came from Ancient Egypt. Researchers speculate that a mixture of flour meal and water was left longer than usual on a warm day and the yeasts that occur in natural contaminants of the flour caused it to ferment before baking.

Today there are several retailers of baker's yeast; one of the best-known is Fleischmann's Yeast, which was developed in 1868. During World War II Fleischmann's developed a granulated active dry yeast, which did not require refrigeration and had a longer shelf life than fresh yeast. The company created yeast that would rise twice as fast, cutting down on baking time. Baker's yeast is also sold as a fresh yeast compressed into a square "cake". A weak solution of water and sugar can be used to determine if yeast is expired. When dissolved in the solution, active yeast will foam and bubble as it ferments the sugar into ethanol and carbon dioxide. Some recipes refer to this as a proof of the viability of yeast before the other ingredients are added.

Some yeasts can find potential application in the field of bioremediation. One such yeast *Yarrowia lipolytica* is known to degrade palm oil mill effluent, TNT (an explosive material), and other hydrocarbons such as alkanes, fatty acids, fats and oils.

PRODUCTION OF ENZYMES

The industrial enzymes find application in many areas. Enzymes leading the market are proteases and amylases.

1- AMYLASES

Higher plants store carbohydrates in the form of starch granules which is composed of 20- 30% amylose (linear polymer of 500- 20,000 α-1,4 linked D- glucose units) and 70- 80% amylopectin (branched polymer formed by joining of linear polymer of 24- 30 α- 1,4 linked D- glucose units by α- 1,6 glycosidic bond). Starch hydrolyzing enzymes are referred to as amylases, and are mainly used in the production of sweeteners for the food industry.

a) α-AMYLASES

α- Amylases are extracellular enzymes which hydrolyze α- 1, 4- glycosidic bonds present in the interior of starch and thus are endo-acting enzymes. α- Amylases are produced by many bacteria and fungi and are classified on the basis of their starch- liquefying and/ or saccharogenic effect, pH optimum, temperature range, and stability. Saccharogenic amylases produce free sugars upon starch hydrolysis, whereas starch- liquefying amylases breakdown the starch polymer but do not produce free sugars. *Bacillus subtilis* Marburg, *B. subtilis* var. *amylosaccharaticus*, and *B. natto* produces saccharogenic α-amylase, whereas *B. amyloliquefaciens* produces liquefying α-amylase. α- Amylases contain a large proportion of tyrosine and tryptophan in the enzyme protein and most of them require calcium as a stabilizer. This enzyme is used in different industries.

Uses of α- amylse in different industries

Industry	Application
Starch Processing	Liquefaction of starch in the production of sugar syrup
Milling	Modification of α-amylase-deficient strains
Baking	Generation of fermentable sugars in flour, and improvement of crust colour
Brewing	Starch hydrolysis during wort preparation from barley
Paper	Liquefaction of starch without sugar production for sizing of paper
Textile	Continuous desizing at high temperatures
Feed	Treatment of barley for poultry and calf
Biological detergents	Starch removal from food stains
Sugar industry	Breakdown of starch from cane juice to improve filterability

Examples of α-amylase producing bacteria

Bacillus subtilis, *B. cereus*, *Lactobacillus*, *Pseudomonas*, *Escherichia*, *Proteus*, and *Serratia* are some α-amylase producing bacteria. However, *Bacillus amyloliquefaciens* and *B. licheniformis* are mainly produced for the industrial production of α-amylase.

Examples of α-amylase producing fungi

Aspergillus, *Penicillium*, *Cephalosporium*, *Mucor*, *Candida*, *Neurospora* and *Rhizopus* are some α-amylase producing molds and *Aspergillus oryzae* is one of the molds used as a source for the industrial production of α-amylase.

b) β- AMYLASES

β- Amylases are the exo- acting enzymes hydrolyzing the α- 1, 4- glycosidic bonds from the non- reducing ends producing maltose and limit dextrin as the major product and are unable to hydrolyze the α- 1, 6 branching present in amylopectin. This enzyme is mainly present in plants but some microbes produce this enzyme including: *Bacillus polymyxa*, *B. cereus*, *B. megatarium*, *Streptomyces* sp., *Pseudomonas* sp. and *Rhizopus japonicus*. β- Amylase has been produced on starch waste by a strain of *B. megaterium* in Submerged fermentation (SmF) and Solid state fermentation (SSF). The starchy wastes from maize, potato, rice, rice husk, tamarind kernel, water chestnut and wheat are used as substrate. This enzyme is mainly used in the production of maltose syrup and other beverages.

c) GLUCOAMYLASES

Glucoamylases hydrolyze starch from the non reducing end producing glucose, maltose, and limit dextrins. *Aspergillus niger*, *A. oryzae*, *Rhizopus niveus*, and *R. javanicus* are examples of the glucoamylases- producing fungi. This enzyme is mainly used for the production of fructose syrup and its production is carried out in submerged fermenter. Starch or dextrin induces the production and, therefore, starch is generally added in the production media. The production of the enzyme is carried at 28- 30 ºC for 3- 5 days depending on the strain.

APPLICATIONS OF AMYLASES

Amylases are used in food, feeding, textile, and pharmaceutical industries.

- In the food sector they are mainly used for liquefaction of starch, manufacture of maltose, high fructose containing syrups, and high

molecular weight branched dextrins. These products are used for various food preparations (cake, candies, etc) adding characteristic sweetness and maintain texture. The use of amylase has replaced the chemical hydrolysis of the starch as the latter used to yield undesirable byproducts and is uneconomical.

- Amylase is also used for the removal of starch sizer from textile (desizing) making the fabric ready for scouring and dyeing.
- Ethanol production from starchy substrates is improved by using amylases or co- culturing the amylolytic strains with ethanol-producing microbes in starch- based media.
- Amylases are also used for processing waste- containing starch generated from food processing plants (reducing the microbial pollution load of effluent).
- Alkaline amylases are used in detergents and for removing the starch stains on cloths and utensils, respectively.
- Amylases are also ingredient of the digestive syrups used for treating digestive disorders.
- Amylase- treated flour is used for preparing animal feed and have improved digestibility.

2- PROTEASES

Proteases catalyze the hydrolysis of peptide bonds of the proteins. The amino acid composition of proteins is very diverse so the proteases responsible for their hydrolysis are also diverse.

Microbes have both intracellular and extracellular proteases. The intracellular proteases are responsible for the maintenance of amino acid pool inside the cell by degrading the unwanted proteins and the extracellular proteases hydrolyze proteins outside the cells into peptides and amino acid required for the cell growth.

Proteases are classified into two major groups: the exopeptidases (peptidases) and the endopeptidases (proteinases). Further the proteases are also classified into alkaline, acid and neutral proteases based on their pH optima of activity.

Both bacterial and fungal proteases are produced commercially and their production conditions are very different from each other. Some organisms are thermophilic especially grown to obtain thermostable proteases for use in detergents.

Industrial uses of proteases

Industry	Protease	Application
Baking	Neutral protease	Dough conditioner
Dairy	Fungal acid proteases	Cheese preparation
Detergent	Alkaline protease from *Bacillus* sp	Laundry detergent for protein stain removal
Food processing	Several proteases	Modification of protein rich material (soy protein or gluten), meat tenderization
Leather	Neutral proteases	Bating of leather, dehairing of hides
Brewery	Neutral proteases	Hydrolyze cereal mash to release peptides and amino acids for utilization by yeast
Photographic	Alkaline protease	Digestion of gelatin for recovery of silver from films
Biopharmaceutical	Several proteases	Digestive syrup, contact lens cleaner, necrotic tissue removal, blood clot digestion
Peptide synthesis	Thermolysin, α-chymosin and several other protease	Reverse reaction to synthesize aspartame and other peptides of pharmaceutical importance

ALKALINE PROTEASES

Strains of *Bacillus*, *Steptomyces*, *Aspergillus* are the major producers of alkaline proteases. Proteases from *Bacillus* (Bacillopeptidases) are mainly used in detergents. Subtilisin carlsberg (protease from *B. licheniformis*) and Subtilisin Novo (protease from *B. amyloliquefaciens*) are the best know proteases used in detergents. These proteases are stable at high temperature, active in alkaline pH (9- 11). Proteases to be used as detergent additive should be stable and active in the presence of surfactants, bleaching agents, fabric softeners and various other formulation of a typical detergent.

In textile industry, proteases may be used to remove the stiff and dull gum layer from the raw silk fiber to achieve improved luster and softness. Protease treatment also modifies the surface of wool fibers to provide unique finishes. The alkaline proteases also have potential application in removal of gelatin from the used photographic films for the recovery of silver from them.

NEUTRAL PROTEASE

Neutral proteases are obtained from plants e.g. papain (from *Carica papaya*), bromelain (from *Ananas comorus*) and ficin (from *Ficus*

spp.), which are cysteine proteases. Neutral proteases are also produced by bacteria (such as *Clostridium histolyticum*, *Streptococcus* spp., *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *B. thuringiensis*, *B. polymyxa*, *Pseudomonas aeruginosa*, *Streptomyces griseus*) and fungi (*Aspergillus oryzae*, *A. sojae*, *Penicillium* spp., *Pericularia oryzae*). The neutral proteases are unstable and require calcium, sodium, and chloride ions for their stability. Not only the pH range for these proteases is small, but they also get inactivated at elevated temperatures. Commercial fungal neutral proteases are used in baking, food processing, protein modification, and in leather, animal feeds and pharmaceutical industries.

ACID PROTEASES

Rennins from calf stomach (used in cheese production as discussed above), pepsin of humans are the well- known examples of acid proteases catalyzing hydrolysis of protein around pH 2- 4. Some of the fungi also produce acid proteases which are rennin- like and used mainly in cheese production. Acid proteases are also used in the preparation of digestive syrup, soy protein digestion during sauce preparation, hydrolyzing the gluten from wheat dough used for preparing biscuits in bakery making them crispy. Silver from the film roll is recovered by digesting the gelatin by acid proteases. *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Serratia*, *Streptococcus*, and *Streptomyces* are some of the bacteria, and *Aspergillus*, *Candida*, *Coriolus*, *Endothia*, *Mucor*, *Penicillium*¸ *Rhizopus*, and *Torulopsis* are some of the fungi producing rennin like proteases.

The rennin produced by *Endothia parasitica* was the first rennin of microbial origin marketed in the year 1967. The fermentation is completed in 48 h at 28ºC, after which the mycelium is removed and enzyme is concentrated and precipitated by evapouration process. The microbial rennins are stable at high temperature, and cause harmful proteolysis. The calf rennin has been successfully cloned into *E. coli* for the production and use of rennin enzyme to avoid the problems encountered with microbial rennin.

PRODUCTION OF AMINO ACIDS

Amino acids are widely used in the food and beverage industries as flavour enhancers, as seasonings, or nutritional additives. World production level is about 600000 tonnes per year, with Japan having the major production proportion. Glutamic acid and lysine are two

amino acids produced by fermentation involving the bacteria *Corynebacterium glutamicum* and *Brevibacterium flavum*, respectively.

Amino acid fermentations -in recent years there has been a rapid development of the production of particular amino acids by fermentation. Microorganisms can synthesize amino acids from inorganic nitrogen compounds. The rate and the amount of synthesis of some amino acids may exceed the cells need for protein synthesis, where upon the amino acids are excreted into the medium.

Some microorganisms are capable of producing sufficient amounts of certain amino acids, to justify their commercial production. The amino acids can be obtained from hydrolyzing protein or from chemical synthesis, but in several instances the microbial process is more economical. Secondly, the microbiological method yields the naturally occurring L-amino acids.

QUESTIONS

1- What are the different industrial uses of yeast?
2- Write on the role of yeast in bread making.
3- What are the most common amino acids produced by microbes? Write in brief on the production.
4- Discuss the main steps of the bread making process.
5- What is the gluten glue?
6- Write on the different kinds of amylases and their uses.
7- Proteases are very important in different industries. Discuss,

PART (7)

II. NON- FOOD PRODUCTS

ETHANOL PRODUCTION

Yeast can convert sugar into ethanol using biotechnological methods, which has various applications including ethanol fuel. The process starts by milling a feedstock, such as sugar cane, sweet corn, or cheap cereal grains, and then adding dilute sulfuric acid, or fungal alpha amylase enzymes, to break down the starches into complex sugars. A gluco amylase is then added to break the complex sugars down into simple sugars. After this, yeasts are added to convert the simple sugars to ethanol, which is then distilled off to obtain ethanol up to 96% in concentration.

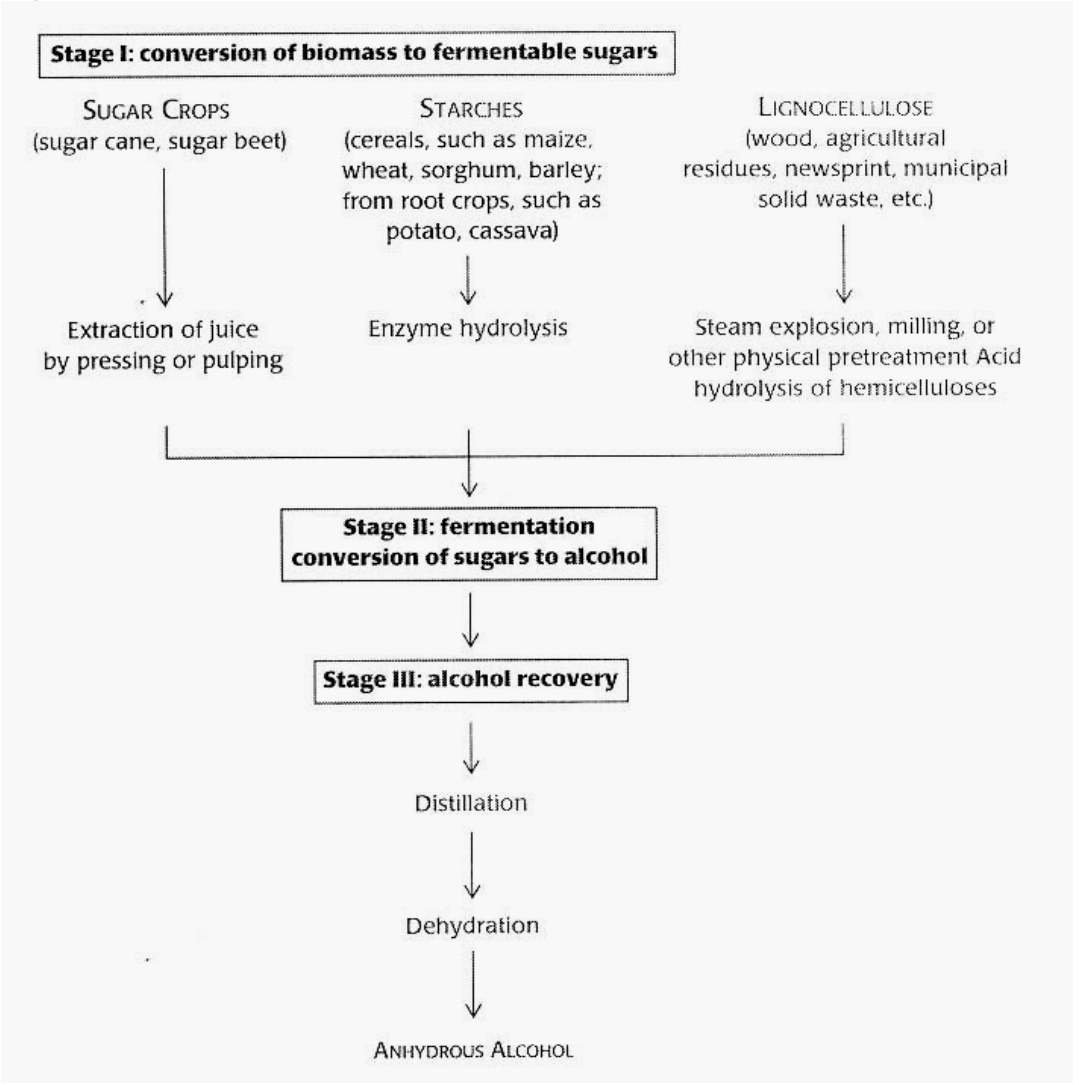
Saccharomyces yeasts have been genetically engineered to ferment xylose, one of the major fermentable sugars present in cellulosic biomasses, such as agriculture residues, paper wastes, and wood chips. Such a development means that ethanol can be efficiently produced from more inexpensive feedstocks, making cellulosic ethanol fuel a more competitively priced alternative to gasoline fuels.

Under anaerobic conditions (low oxygen concentrations), many organisms, including yeast, obtain the energy from the process of fermentation. In alcoholic fermentation, characteristic of many yeast species, the fermentation process starts with one molecule of the six carbon sugar- glucose, and terminates with two molecules of the two carbon alcohol - ethanol, and two molecules of CO_2:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Ethanol +}}{2CH_3CH_2OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

Further distillation procedures are needed to obtain anhydrous ethanol. The most important question that must be addressed by any production process for an alternative fuel is the question of energy balance (the energy output–input ratio). For ethanol, nonrenewable energy is required to grow corn, harvest and transport it, subject it to dry or wet milling, convert the starch in the corn kernels into ethanol, and recover the ethanol by distillation and dehydration. Widely used 2002 estimates of the energy balance for the corn-to-ethanol conversion are modestly positive. The higher value is obtained when credits are assigned to coproducts: stillage (the residue from the fermentation used to produce a high-quality nutritious livestock feed– "dried distillers grains and solubles), corn oil, corn gluten meal, and

corn gluten feed. The CO_2 released during the fermentation is captured and sold for carbonating beverages and the manufacture of dry ice.



Stages of alcohol production from different sources

BIOFERTILIZERS

- Biofertilizers are microbial inoculants containing living cells of either nitrogen fixing or phosphate- solubilizing bacteria. The most important biofertilizer commercially available is the rhizobial inoculant used for legume seed inoculation. Since most legumes are grown without any inorganic nitrogen fertilizers, their growth is dependent on the supply of nitrogen by these bacteria. The technology of producing these inoculants consists of culturing efficient strains of

rhizobia in yeast extract- mannitol medium under controlled conditions in shake flasks or fermentors and mixing the culture broth with sterile powdered foil, charcoal, lignite or peat. The mixture is allowed to cure for a short period after which it is packed and used for seed bacterization. In recent years this technology is also extended to treat cereals with nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum*.

- The blue green bacteria have also been used as biofertilizer in rice cultivation. However, the technology for producing these inoculants on a large scale is different. Efficient strains of blue green bacteria are cultured in open tanks, in water containing adequate amounts of mineral nutrients such as phosphate and molybdate. After adequate amount of growth is obtained, the algal mass is dried and used as inoculant material. Alternatively, this organism can also be cultured directly in the open fields before the rice crop is transplanted.

MICROBIAL RECOVERY OF OIL

- When an oil field is opened up, spontaneous flow and pumping will produce approximately only about one- third of the total petroleum present. Secondary recovery techniques to increase output are involved such as gas pressurizing, water flooding, miscible flooding and thermal methods. Tertiary oil recovery methods include the use of solvents, surfactants, and polymers able to dislodge oil from geological formations to prolong the well life and increase production.
- Microbial- enhanced oil recovery processes involve the use of polymers such as xanthan gum produced by large- scale fermentation of specific bacteria, such as *Xanthomonas compestris*, are useful compounds in oil recovery. Such gums have excellent viscosity and flow characteristics to pass through small pores releasing more trapped oil. Application is usually associated with water- flooding operations.
- A further possible approach is the use of microorganisms in situ for dislodging oil by surfactant production, gas formation or partial microbial digestion to alter oil viscosity. Bio-surfactants may also have a role in releasing oil from tar sands.

The role of microbes and different microbial products in oil recovery is illustrated in the following Table.

The role of microbes and microbial products in oil recovery

Microbial product	Role in enhanced oil recovery	Some of the effects
Gases (H_2, N_2, CH_4, CO_2)	• Reduce oil viscosity and improve flow characteristics • Displace immobile • Sweep oil in place	• Improved oil recovery by gases • Miscible CO_2 flooding
Acids (low molecular weight acids, primarily low molecular weight fatty acids)	• Improve effective permeability by dissolving carbonate precipitates from pores throat. Significant improvement of permeability and porosity • CO_2 produced from chemical reactions between acids and carbonate reduce oil viscosity and causes oil droplet to sweel	• Enhanced oil flooding
Solvents (alcohols and ketones that are typical cosurfactants)	• Dissolve in oil reduce viscosity • Dissolve and remove heavy, long chain hydrocarbons from pore throat (increase effective permeability) • Involved in stabilizing and lowering interf. tension that promotes emulsification • Reduce interfacial tension	• Emulsification promotion for increased miscibility
Biosurfactants	• Reduce interfacial tension between oil and rock/water surface which causes emulsification; improving pore scale displacement • Alter wettability	• Microbial surfactant • Flooding
Biopolymers	• Improve the viscosity of water in waterflooding and direct reservoir fluids to previously unswept areas of the reservoir • Improve the sweep efficiency of waterflood by plugging high permeability zones or water-invaded zones • Control of water mobility	• Microbial permeability modification (selective plugging)
Biomass (microbial cells)	• Physically displace oil by growing between oil and rock/water surface • Reversing wettability by microbial growth • Can plug high permeability zones • Selective partial degradation of whole crude oil • Act as selective and nonselective plugging agents in wetting, alteration of oil viscosity, oil power point, desulfuration	• Same biopolymers

[a]Formation damage; low oil relative permeability; trapped oil due to capillary forces; poor sweep efficiency channeling; unfavorable mobility ratio; low sweep efficiency; water or gas coning.

BIOPLASTICS

Bioplastics, sometimes also called **green plastics**, are plastics that are biodegradable and are made mostly or entirely from renewable resources.

1. Like all plastics, bioplastics are composed of a polymer, combined with plasticizers and additives.

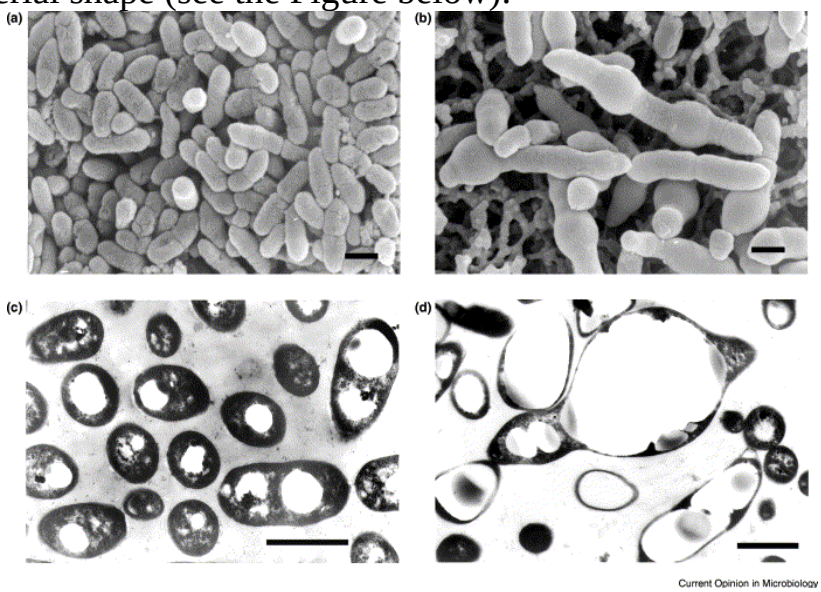
Using fermentation to produce plastics

There are two ways fermentation can be used to create biopolymers and bioplastics:

Bacterial Polyester Fermentation–The bacteria *Ralstonia eutropha* use the sugar of harvested plants, such as corn, to fuel their cellular processes. The by- product of these cellular processes is the polymer. The polymers are then separated from the bacterial cells.

Lactic Acid Fermentation–The final product of fermentation is lactic acid, rather than a polymer. After the lactic acid is produced, it is converted to polylactic acid using traditional polymerization processes.

The number and size of the granules, monomer composition, macromolecular structure and physico-chemical properties vary, depending on the organism. They intracellular light- refracting granules or electron lucent bodies cause a striking alteration of the bacterial shape (see the Figure below).



Scanning **(a,b)** and transmission **(c,d)** electron microphotographs of *P. putida* **(a,c)** and its β-oxidation mutant **(b,d)** cultured in a chemically defined solid medium containing 7-phenylheptanoic acid (5 mM) as a source of aromatic PHAs and 4-hydroxyphenylacetic acid (5 mM) as an energy source. Bar = 1 μm.

Bioplastics can be isolated by centrifugation (cell-free extracts) or by solvent extraction (dried intact bacteria) with chloroform, dichloroethane, propylene carbonate, methylene chloride or dichloroacetic acid.

Currently, the main limitations for the bulk production of bioplastics are its high production and recovery costs. Microbes belonging to more than 90 genera (including aerobes, anaerobes, photosynthetic bacteria, archaebacteria and lower eukaryotes), are able to accumulate and catabolize these polyesters (e. g. *Chromatium vinosum, Thiocystis violacea, Thiocapsa pfennigii*).

The most widely produced microbial bioplastics are PHB, PHA and their derivatives. However, other polyesters can also be produced by microorganisms. Most of them either require similar biosynthetic enzymes or lack current industrial applications.

Macromolecular architecture of the inclusions

PHAs are accumulated intracellularly in granules of different sizes (see the Figure below). They are surrounded by a phospholipid monolayer (PM) containing some other proteins attached to the granule. The function of the PM envelope is believed to avoid the contact of PHAs with water (preventing the transition of the polyester from the amorphous liquid state to a more stable crystalline form), and that it acts as a protective barrier (avoiding cellular damage caused by the interaction of PHAs with internal structures or with cytosolic proteins). Therefore, enzymes specifically involved in the synthesis of the PM, associated with the polyester, must exist.

Applications

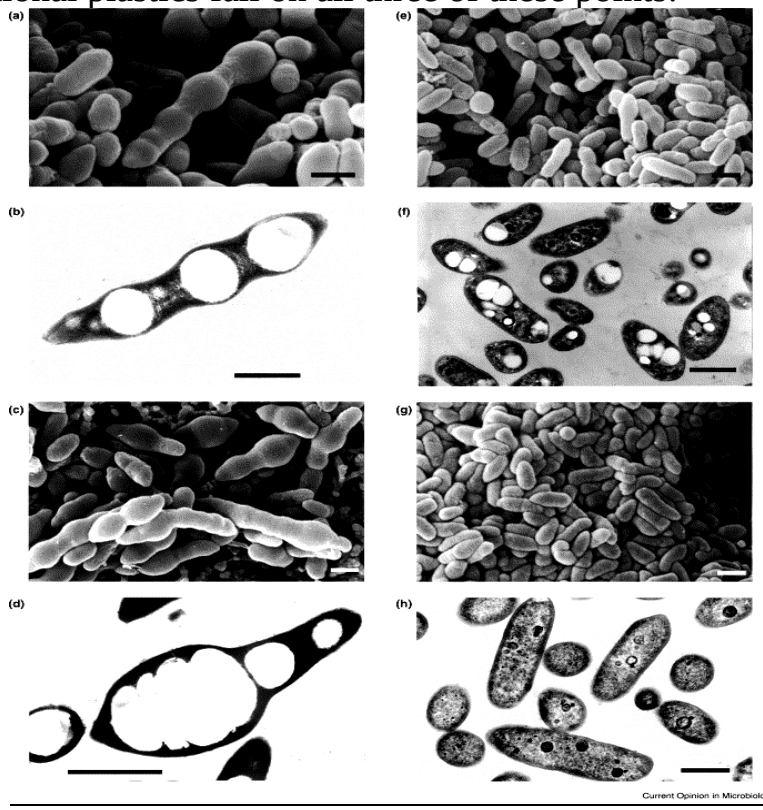
Many different applications have been described for bioplastics since the first industrial production of Biopol1 by ICI Ltd in 1982.

Various products of biopol

There are at least three factors that affect how environment- friendly a material is:

- Renewability: how quickly are the ingredients that go into making the plastic created in the environment?
- Degradability: how quickly can the plastic be re- integrated into the environment after it is no longer being used?
- Production: how much pollution or waste is created during the process of actually making the plastic?

Traditional plastics fail on all three of these points.



Scanning **(a,c,e,g)** and transmission **(b,d,f,h)** electron microphotographs of the *P. putida* mutant **(a–d)** and its double-deleted mutant **(e–h)** cultured in a chemically defined solid medium containing either octanoic acid (5 mM) **(a,b,e,f)** or 7-phenylheptanoic acid (5 mM) and 4-hydroxyphenylacetic acid (5 mM) **(c,d,g,h)**. Bar = 1 μm.

Classification of bioplastics according to their characteristics is presented in the following Table.

Classification of microbial bioplastics according to different criteria.

Biosynthetic origin	Natural bioplastics: those produced by microorganisms from general metabolites (i.e. PHBs and aliphatic PHAs).
	Semisynthetic bioplastics: those that require the addition to the culture broth of some precursors that cannot be synthesised by the microbe (i.e. PHAs containing aromatic monomers)
	Synthetic bioplastics: those polyesters that resemble the natural ones but that can only be obtained by chemical synthesis (i.e. synthetic thermoplastic polymers)
Chemical nature of the monomers	Bioplastic containing aliphatic fatty acid derivatives: saturated or unsaturated (with double or triple bonds) monomers; linear or branched monomers; substituted or not (with functional groups in the monomers).
	Bioplastics containing aromatic fatty acid derivatives
	Bioplastics containing both aliphatic and aromatic fatty acid derivatives
	Bioplastics containing other different compounds (e.g. poly-ɣ-glutamic acid, poly-ε-L-lysine, poly-β-L-malic acid, polyglycolic acid, cianophicin)
Monomer size	Bioplastics containing a short-chain length (sclPHB and derivatives sclPHAs; C3–C5 monomers)
	Bioplastics containing a medium-chain length (mclPHAs; C6–C14)
	Bioplastics containing a long-chain length (lclPHAs; >C14)
Number of monomers in the polyesters	Homopolymeric bioplastic: a single monomer is present in the bioplastic
	Heteropolymeric bioplastic (copolymer): more than one monomer is present in the bioplastic
Type of polyesters accumulated by the microbe	Unique (a single bioplastic)
	More than one (mixed bioplastics)

QUESTIONS

1- How can ethanol be produced by microbial fermentation? What are the main materials used for this process? Give the formula of converting glucose to ethanol?

2- Write in brief on the stages of converting different biomass into fermentable sugars then fermentation to alcohol.

3- What is the meaning of biofertilizer? Give an example including production and benefits?

4- What are the benefits of using microbes in the recovery of the oil?

5- What are bioplastics and why they are called "green plastics"?

6- How fermentation is used to produce bioplastics?

7- Give examples of the biotechnological applications of bioplastics and biopolymers.

8- What are the factors that determine how a material is environment-friendly?

PART (8)

II. NON- FOOD PRODUCTS (Continued…)

SILAGE

Silage is fermented, high- moisture fodder that can be fed to ruminants (animals like cattle and sheep) or used as a biofuel feedstock for anaerobic digesters. It is fermented and stored in a process called ensilage, and usually made from grass crops, including maize or sorghum, using the entire plant, not just the grain. Silage can be made from many other field crops, and other terms (oatlage for oats, haylage for alfalfa) can be used.

It is sometimes a mix of two crops, such as oats and peas. **Haylage** means ensiled forages, made up of grass, alfalfa and alfalfa/ grass mixes. **Balage** is another form of stored forage. In this case hay, alfalfa or grass is cut and baled while still fairly wet. That is, it is too wet to be baled and stored as hay. In this case the dry matter is around 60 to 70%. The bales are wrapped tightly in plastic wrappers. The material then goes through a limited fermentation in which short chain fatty acids are produced which protect and preserve the forage. This method has become popular on smaller farms.

Wrapping with plastic

MAKING SILAGE

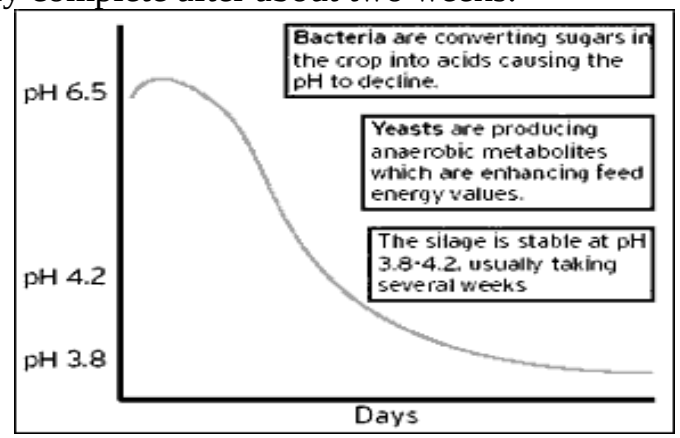
Silage must be made from plant material with a suitable moisture content, about 55% to 70%, depending on the means of storage the degree of compression and the amount of water that will be lost in storage. For corn, harvest begins when the whole- plant moisture is at a suitable level. For pasture- type crops the grass is allowed to wilt for a day or so until the moisture content drops to a suitable level.

The plant material is collected, chopped into pieces about 14 mm long and packed. Silage may also be emptied into a bagger, which puts the silage into a large plastic bag that is laid out on the ground.

Fermentation

Concrete silage silo

Silage undergoes anaerobic fermentation, which starts about 48 hours after the silo is filled. Traditionally, the fermentation is caused by indigenous microorganisms, but today, some silage is inoculated with specific microorganisms to speed fermentation or improve the resulting silage. The process converts sugars to acids and exhausts any oxygen present in the crop material. The fermentation is essentially complete after about two weeks.



Silage inoculants contain one or more strains of lactic acid bacteria, and the most common is *Lactobacillus plantarum*. Other bacteria used include *Lactobacillus buchneri*, and *Pediococcus* species.

Silo effluent

The fermentation process releases liquid. Silo effluent contains nitric acid (HNO_3), which is corrosive. It can also contaminate water courses unless precautions are taken. The high nutrient content can lead to eutrophication (growth of algae blooms).

Storing silage

Silage must be firmly packed to minimize the oxygen content, or it will spoil.

Anaerobic digestion

Anaerobic digesters for silage

Silage is a useful feedstock for anaerobic digestion. If it is not used for feeding animals, silage can be fed into anaerobic digesters to produce biogas.

SAFETY

Silos are hazardous, and people die every year in the process of filling and maintaining them. As well as the risk of injury by machinery or from falls, the fermentation process presents respiratory hazards. Nitrogen dioxide gas is released in the early stages of fermentation, and can kill. Lack of oxygen inside the silo can cause asphyxiation, and molds formed when air is allowed to reach cured silage can cause toxic organic dust syndrome. When filling a silo, fine dust particles in the air can become explosive. The silage itself has no special danger.

Nutrition value

The product retains a much larger proportion of its nutrients than if the crop had been dried and stored as hay. Silage is most often fed to dairy cattle, because they respond well to highly nutritious diets.

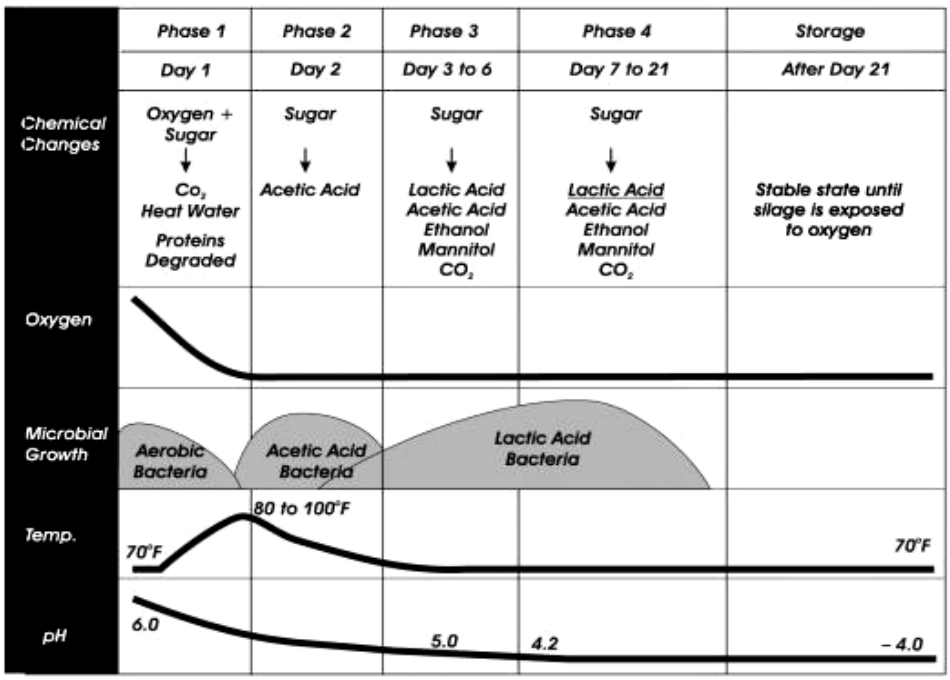
Since silage goes through a fermentation process, energy is used by fermentative bacteria to produce volatile fatty acids (VFA), such as acetate, propionate, lactate, butyrate etc, which preserve the forage. The result is that the silage is lower in energy than the original forage, since the fermentative bacteria use some of the carbohydrates to produce VFA. Thus, the ensiling process preserves forages, but does not improve the quality or the nutrient value. An example of silage nutritional values are in the following Table.

Parameters	Silage	Optimal for silages
Dry matter (%)	43.7	30- 40
Crude protein (% in DM)	13.00	14- 16
Ash (% in DM)	8.90	9 -10
ME (MJ/kg DM)	9.7	9.7- 10.1
pH	4.6	4.9- 5.0
Lactic acid (% in DM)	4.94	
Acetic acid (% in DM)	1.58	2.0- 3.5
Butyric acid (% in DM)	0.00	< 0.3
Propionic acid (% in DM)	0.02	

THE FERMENTATION CONDITIONS

Ideal fermentation is dependent upon decisions and management practices implemented before and during the ensiling process. The primary management factors are:

1. Stage of maturity of the forage at harvest.
2. The type of fermentation that occurs in the silo.
3. Type of storage structure used and methods of harvesting and feeding.



During the ensiling process, some bacteria are able to break down cellulose and hemicellulose to various simple sugars. Other bacteria

break down simple sugars to smaller end products (acetic, lactic and butyric acids). The most desirable end products are acetic and lactic acid. As the bacteria degrade starches and sugars to acidic and lactic acids, dry matter is lost. Attention to details such as speed of harvesting, moisture content, length of chop, silage distribution and compaction can greatly influence the fermentation process and storage losses. Efficient fermentation ensures a more palatable and digestible feed, which encourages optimal dry matter intake that translates into improved animal performance.

Proper packing of the hay and voiding of air (oxygen) provides the environment needed by bacteria to break down fiber components and sugars. The faster the fermentation is completed, the more nutrients will be retained in the silage.

A critical time during the ensiling process occurs after the initial three to five days and requires some 15 to 20 days for completion. The success of the ensiling process is determined during these two weeks. The critical difference between silage and haylage is the effect of moisture content of the forage during this two- week fermentation process. If the forage is too dry, fermentation is restricted and the pH cannot drop sufficiently. If pH of the haylage does not drop sufficiently, spoilage will occur.

Six phases of silage fermentation and storage.

	Phase I	**Phase II**	**Phase III**	**Phase IV**	**Phase V**	**Phase VI**
Age of Silage	0-2 days	2-3 days	3-4 days	4-21 days	21 days-	
Activity	Cell respiration; production of CO_2, heat and water	Production of acetic acid and lactic acid ethanol	Lactic acid formation	Lactic acid formation	Material storage	Aerobic decomposition on re-exposure to oxygen
Temperature Change*	69-90 F	90-84 F	84 F	84 F	84 F	84 F
pH Change	6.5-6.0	6.0-5.0	5.0-4.0	4.0	4.0	4.0-7.0
Produced		Acetic acid and lactic acid bacteria	Lactic acid bacteria	Lactic acid bacteria		Mold and yeast activity

***Temperature dependent on ambient. Ensiling temperature generally is 15 higher than ambient.**

ADDITIONS TO SILAGE

Various additions to silage have been suggested as methods to improve or alter the fermentation process. These materials may be referred to as additives, conditioners and preservatives.

• **Additive**- A material that adds nutrients to silage.

• **Conditioner**- A material that absorbs excess moisture from chopped forage or which increases the moisture content of excessively dry forage.

• **Preservative**- A material that stimulates the fermentation process or a material that inhibits fermentation.

The benefits obtained from silage additives, conditioners and preservatives depend upon their influence on the silage fermentation process. Silage additives, conditioners and preservatives function in the following ways:

• Add dry matter to reduce moisture content
• Add water to increase moisture content
• Alter the rate, amount and kind of acid production
• Acidify the silage
• Inhibit bacterial and mold growth
• Culture silage (inoculants) to stimulate acid production
• Increase nutrient content of the silage

Several chemicals used as silage preservatives also inhibit undesirable bacterial and mold growth. Acids, such as formic and propionic, enhance the preservation of forage. The major benefit of adding weak acids to silage appears to be in reducing spoilage in open storage structures. Formic acid is added to hay crop silages at 0.45 percent of the wet weight or 2.25 percent of the dry matter weight. Propionic acid is added at the rate of 0.5 to 1.0 percent of the wet forage weight.

<u>Increasing the nutrient content of silage</u>

Various materials added to silage will increase nutritive value to the extent that they themselves contain nutrients. Increasing the nutrient content of silage will greatly affect the final cost of the product produced.

Materials such as cereal grains, molasses, dry forages, limestone, urea and anhydrous ammonia are examples of nutrient additions to silage. Commercial products are also available that contain one or more of the above materials.

Non-protein N sources for adding to corn silage and suggested application rates.[1]

NPN Sources	Form	% Nitrogen	Application Rate (lbs/wet ton)
Urea	dry	45	10
Mono-ammonium phosphate	dry	11	20[2]
Pre-mixed ammonia-water	liquid	20-30	17-25
Anhydrous ammonia	gas	81	6-7
Ammonia, cold flow	gas-liquid	81	6-7

[1] Commercial products should be applied at a rate to provide 5 pounds of actual nitrogen/ton of forage. [2] Add 5 lbs. dry urea to provide 5 lbs. of nitrogen.

SILAGE INOCULANTS

A number of commercial products, referred to as fermentation aids and/ or inoculants, are available for adding to silage at the time of ensiling. Since silage is a product resulting from the action of bacterial enzymes on the material stored, attempts have been made to alter or regulate silage fermentation through the addition of materials containing bacteria, yeasts and molds. The primary purpose for adding bacterial inoculants is to increase the number of lactic acid-producing bacteria, thus encouraging more lactic acid production and a well- preserved forage mass.

Research using various bacterial inoculants indicates highly variable results. Products showing consistent, positive results indicate about a 5 percent increase in dry matter preservation. Therefore, cost of the inoculant per ton compared to the dollar value of the dry matter saved will determine the profitability of using a silage inoculant.

The addition of bacterial inoculants to corn silage harvested at the proper growth stage and moisture content has not shown consistently positive results. Results have been more positive when inoculants have been added to alfalfa, a low carbohydrate forage, and sorghum silages.

QUESTIONS

1- What is silage and what it is used for?
2- What are the materials used for making silage?
3- Give a brief idea on how to make silage from grass, corn or other crops?
4- Explain in brief the fermentation of silage and the characteristics of "silo effluent'?
5- Discuss in brief the storage, safety and nutritional value of silage.
6- Write on the fermentation conditions and its phases.
7- Are there any additions to silage? Discuss?
8- Write- in no more than 10 lines- on silage inoculants.

PART (9)

COMPOSTING

• Wastes from food and drinks are becoming an increasing problem, particularly to large production centers, because of the environmental laws that restrict the dumping of the high- biological oxygen demand wastes. In the developed world, efforts are made to use such organic wastes to generate valuable by- products, while achieving active waste removal. There is also a large market for biological waste treatment systems in these countries.

FOOD WASTE COMPOSTING

Composting is the natural process of decomposition and recycling of organic material into a humus rich soil amendment known as compost. For any business or institution producing food waste, this organic material can be easily decomposed into high quality compost.

Food waste composting

Compost constituents

Fruits, vegetables, dairy products, grains, bread, unbleached paper napkins, coffee filters, eggshells, meats and newspaper can be composted. If it can be eaten or grown in a field or garden, it can be composted. Items that cannot be composted include synthetic plastics, grease, glass, metals, foil, silverware, drinking straws, bottles, polystyrene or chemicals. Items such as red meat, bones and small amounts of paper are acceptable, but they take longer to decompose. Add red meat and bones to only a well- controlled compost pile to avoid attracting vermin, pests and insects.

Food waste is unique as a compost agent

Food waste has unique properties as a raw compost agent. Because it has a high moisture content and low physical structure, it is important to mix fresh food waste with a bulking agent that will absorb some of the excess moisture as well as add structure to the mix. Bulking agents

with a high C: N ratio, such as sawdust and yard waste (grass, leaves etc.), are good choices. Food waste is highly susceptible to odour production- mainly ammonia- and large quantities of leachate. The best prevention for odour is a well- aerated pile that remains aerobic and free of standing water. Leachate can be reduced by aeration and sufficient amounts of a high carbon bulking agent. It is normal to have some odour and leachate production. Captured leachate can be reapplied to the compost or used under ornamental plants.

BENEFITS OF COMPOSTING FOOD WASTE

Food waste that is not composted generally goes directly to a landfill. Once in the landfill, organic matter may react with other materials and create toxic leachate. Food waste placed in an airtight landfill stops the earth's natural cycle of decomposition. This cycle plays a crucial role in the health of our environment.

Benefits of compost to the environment and agriculture

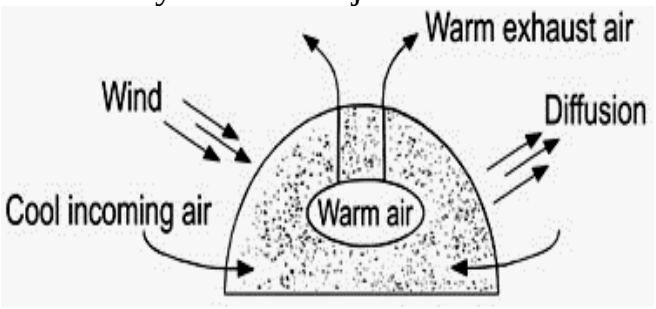
Environment	Agriculture
• Water and soil conservation • Protects groundwater quality. • Minimizes odors from agricultural areas. • Avoids methane production and leachate formation in landfills by diverting organics from landfills into compost. • Prevents erosion and turf loss on roadsides, hillsides, playing fields and golf courses. • Drastically reduces the need for pesticides and fertilizers. • Binds heavy metals and prevents them from migrating to water resources, being absorbed by plants, or being bioavailable to humans. • Off-farm materials can be brought in and added to manure to make compost. • Facilitates reforestation, wetlands restoration, and wildlife habitat revitalization efforts by amending contaminated, compacted and marginal soils. • Long-term stable organic matter source. • Buffers soil pH levels. • Off-farm materials can be brought in and added to manure to make compost.	• Adds organic matter, humus and cation exchange capacity to regenerate poor soils. • Suppresses certain plant diseases and parasites and kills weed seeds. • Increases yield and size in some crops. • Increases length and concentration of roots in some crops. • Increases soil nutrient content and water holding capacity of sandy soils and water infiltration of clay soils. • Reduces fertilizer requirements. • Restores soil structure after natural soil microorganisms have been reduced by the use of chemical fertilizers; compost is a soil inoculant. • Increases earthworm populations in soil. • Provides slow, gradual release of nutrients, reducing loss from contaminated soils. • Reduces water requirements and irrigation. • Provides opportunity for extra income; high quality compost can be sold at a premium price in established markets. • Moves manure to non-traditional markets that do not exist for raw manure. • Brings higher prices for organically grown crops. • Minimizes odors from agricultural areas.

Benefits to the food industry

- Reduces solid waste disposal fees.
- End wasting large quantities of recyclable raw ingredients .
- Educates consumers on the benefits of food waste composting.
- Markets your establishment as environmentally conscious.
- Markets your establishment as one that assists local farmers and the community.
- Helps close the food waste loop by returning it back to agriculture.
- Reduces the need for more landfill space.

COMPOSTING METHODS

Passive composting or **piling** is simply stacking the materials and letting them decompose naturally. This method is simple and low cost but is very slow and may result in objectionable odors.



Aerated composting

In Aerated static piles air is introduced to the stacked pile via perforated pipes and blowers. This method requires no labor to turn compost but is weather sensitive, and can have unreliable pathogen reduction due to imperfect mixing.

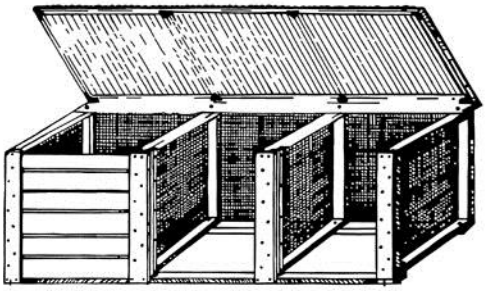
Aerated piles in the field

Windrows are long, narrow piles that are turned when required based on temperature and oxygen requirements. This method produces a uniform product and can be remotely located. However, turning the compost can be labor intensive or require expensive equipment. Windrows are typically used for large volumes which can require a lot

of space. In addition, windrows can have odor problems, and have leachate concerns if exposed to rainfall.

Windrows in the field

In bins using wire mesh or wooden frames allow good air circulation, are inexpensive, and require little labor. Three chamber bins allow for faster compost production utilizing varying stages of decomposition. Bin composting is typically used for small amounts of food waste.



This three-bin system can handle significant quantities of materials. It also allows staged composting, by using one section for storing compostable materials, one section for active composting, and one section for curing or finished compost. Note: You can use discarded wooden pallets instead of new wood to make a three-bin system.

In-vessel systems using perforated barrels, drums, or specially manufactured containers are simple to use, easy to turn, require minimal labor, are not weather sensitive, and can be used in urban and public areas. The initial investment can be high and handling volumes are typically low.

Vermi- composting uses worms to consume the food waste and utilizes its castings as high quality compost. This is usually done in containers, bins, or greenhouses. Typically 1 pound of worms can eat 4 pounds waste per week. Many schools in the developed countries use this type of composting as an environmental education tool. Worm castings bring a premium price but the investment in worm stocking may be high depending on the size of the operation. If too much waste is added anaerobic conditions may occur. In addition, worms cannot process meat products.

IMPORTANT CONSIDERATIONS

 Proper nutrient mix, or carbon to nitrogen ratio **(C: N)** is important for bacteria to process organic material into compost. The optimum ratio to begin composting is **30:1.** If the ratio increases decomposition is slowed, if the ratio decreases foul odors and nitrogen loss can occur. Food waste is typically 15:1, fruit waste 35:1, leaves 60:1, bark 100:1, and sawdust 500:1. For example, a recipe using 1 part leaves and 1 part food waste by volume would achieve close to a 30:1 ratio.

 A moisture content of 60 percent is optimal for microorganisms to breakdown the compost. Moisture contents above 70 percent create anaerobic conditions, slow down the process and can create foul odors. Moisture below 50 percent also slows down the decomposition process. The moisture content of fresh food waste is 80 to 90 percent, sawdust is 25 percent, and yard waste is 70 percent. Compost with a proper moisture content will form a clump and will slightly wet your hand when squeezed. If the clump drips water, it is too wet and may require additional aeration or more bulking agent. If the compost falls through your fingers, it is too dry and may need water additions or more food waste.

 Aeration or oxygen is essential for optimum microorganism populations to effectively breakdown the composting material. This can be done by turning, mixing, the use of blowers, fans, aeration tubes, aeration holes, or raising the compost off the ground.

 Particle size can affect the rate of decomposition of compost. The smaller the particles the more aeration the compost receives and microorganisms can break down smaller pieces faster. This can be accomplished by shredding, chipping, chopping, or cutting composted materials before they enter the compost pile.

 pH levels from **6.0** to **7.8** are considered high quality compost. Proper C: N ratios should create optimum pH levels. Starting with a fairly neutral pH will ensure high levels of microorganisms for efficient decomposition.

 Temperature of the compost is important while biological activity takes place in the decomposition process. Low outside temperature slow down the process, while warmer conditions speed up the process. Mesophillic bacteria function between 50 and 113 degrees F to begin the composting process. Thermophillic bacteria take over and thrive between 113 to 158 degrees F. These high temperatures are what destroy weed seeds and pathogens in the compost. Some composting manures can reach temperatures of 200 degrees F. However, temperatures above 158 degrees F may create conditions suitable for spontaneous combustion.

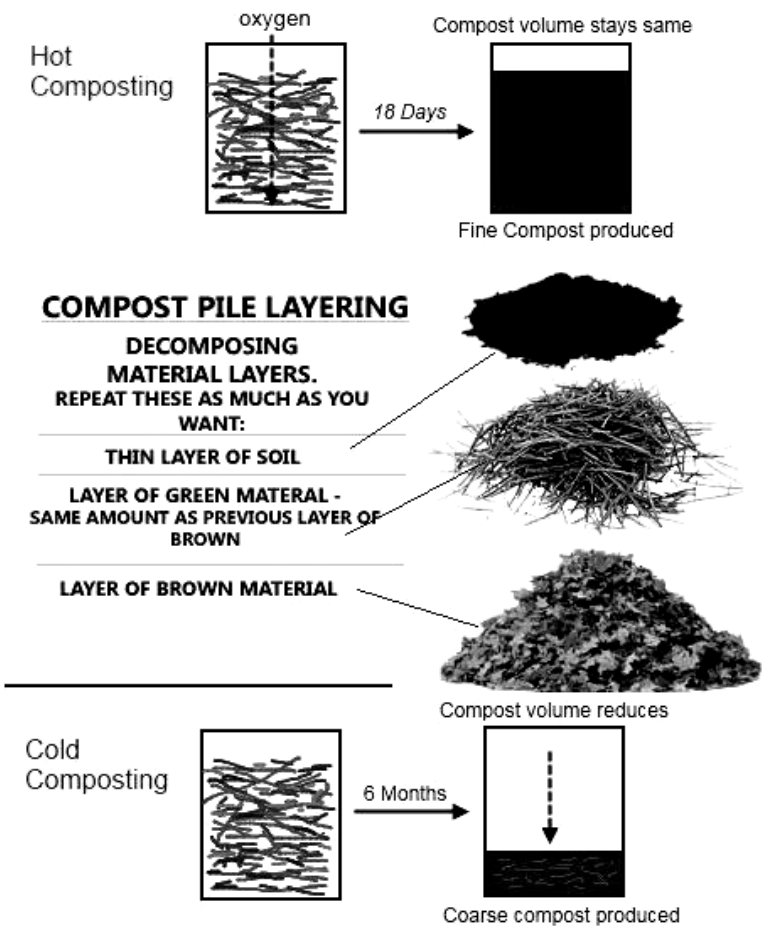
COMPOST RIPENING

Mature or stable compost is similar to humus in appearance, smell, and touch. The finished compost will no longer heat on it's own, thus maintaining the ambient temperature, and there will be no weed seeds or pathogens. The pH will be near 7.0, and the moisture content will be between 35 and 50 percent. The C:N ratio will be 10:1 to 25:1. The organic matter content will be between 40 and 65 percent. It is important to protect the compost from windblown weed seeds until its point of use.

It is very important not to apply unfinished or immature compost, it may have phytotoxins that can kill plants. An inexpensive way to test for mature compost is the watercress test. Watercress seeds will not germinate or grow in immature compost because they are very sensitive to pH and nutrition.

Mature compost

1 part Nitrogen (Moist and/or Green)
Coffee Grounds
Green Grass Clippings
Seaweed
Fruit & Veggie Scraps
Bloodmeal



4 parts Carbon (Dry and/or Brown)
Leaves/Hay/ Straw
Used Potting Soil
Eggshells
Shredded Newspaper & Corrugated Cardboard
Ashes from Organic Materials

Compost microorganisms

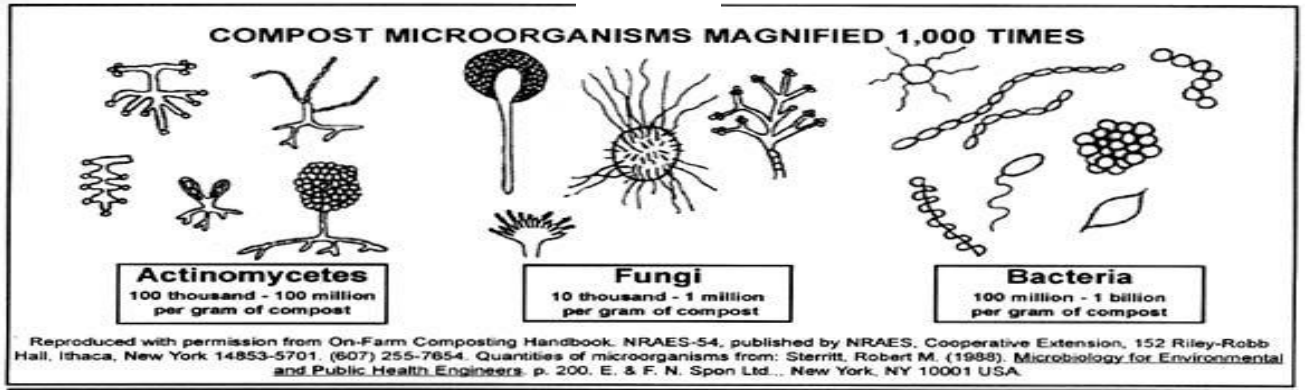


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Table 3.6

MICROORGANISMS IN COMPOST

Actinomycetes	Fungi	Bacteria
Actinobifida chromogena	*Aspergillus fumigatus*	*Alcaligenes faecalis*
Microbispora bispora	*Humicola grisea*	*Bacillus brevis*
Micropolyspora faeni	*H. insolens*	*B. circulans* complex
Nocardia sp.	*H. lanuginosa*	*B. coagulans* type A
Pseudocardia thermophilia	*Malbranchea pulchella*	*B. coagulans* type B
Streptomyces rectus	*Myriococcum themophilum*	*B. licheniformis*
S. thermofuscus	*Paecilomyces variotti*	*B. megaterium*
S. thermoviolaceus	*Papulaspora thermophila*	*B. pumilus*
S. thermovulgaris	*Scytalidium thermophilim*	*B. sphaericus*
S. violaceus-ruber	*Sporotrichum thermophile*	*B. stearothermophilus*
Thermoactinomyces sacchari		*B. subtilis*
T. vulgaris		*Clostridium thermocellum*
Thermomonospora curvata		*Escherichia coli*
T. viridis		*Flavobacterium* sp.
		Pseudomonas sp.
		Serratia sp.
		Thermus sp.

Source: Palmisano, Anna C. and Barlaz, Morton A. (Eds.) (1996). Microbiology of Solid Waste. Pp. 125-127. CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, FL 33431 USA.

USES AND APPLICATION OF COMPOST

Compost has many uses on the farm. It can be used as a soil amendment to improve soil structure, infiltration rate and water holding capacity. It will increase soil microorganism populations, soil organic matter and humus. Compost can also be used as a fertilizer supplement for nitrogen, phosphorous, potassium, and trace elements. Mature compost has no objectionable odour, never "burns" as fertilizers do, can be used to suppress insect pests and soil- borne plant pathogens, and act as a fungicide. In the US, a major California fruit and vegetable grower was able to cut pesticide use by 80 percent after three years of compost applications.

Compost can also be used as a mulch for trees, orchards, landscapes, lawns, gardens, and makes an excellent potting mix. Additional uses for compost include vegetable production, field crops, annual forest plantings, greenhouse crops, mined lands, roads (city, village), and recreation areas (golf courses, trails, athletic fields, and parks).

Application rates will vary depending on crop nutrient needs, field history, and local climate. Applying 300 pounds of compost per 1,000 square feet, at a depth of 6 inches will increase soil organic matter by 0.5 percent. For row crops recommendations range between 3 and 10 tons per acre, while pastures recommendations go up to 4 tons per acre. Some berry and squash crops recommendations are between 25 and 75 tons per acre. While compost is a slow release of nutrients, reports vary in its ability to meet the nutrient needs of crops. You should have your compost analyzed for its nutrient content and adjust your application rates accordingly. Your county extension agent can help you with this.

Questions

1- What are the main uses of food waste in the developed world?
2- How can food waste be used in the developing world to reduce environmental and agricultural pollution? What is the main process for this waste that do so?
3- Can any kind of waste be used for compost making? Explain?
4- How can you judge the ripening of compost?
5- What is the main processes used for compost making?
6- What are the main factors that should be considered for making a good compost?
7- How can compost be applied in the field and what is the rate of applications?
8- How can you make compost in your house fro food waste?
9- How can farmers make compost in the field? Discuss two methods in brief.
10- Mention some examples of actinomycetes, bacteria and fungi that are used as inoculants for compost making.

PART (10)

PHARMACOLOGICAL INDUSTRY

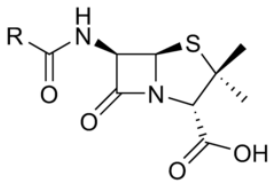
The very useful contribution of microbes to winning a war came through the discovery of penicillin by the English microbiologist Fleming in 1929. The amounts of penicillin were so small in those early days that it was re- isolated from the urine of patients and used again. However, when the second world war came along, the U.S. started the development of penicillin that became, after developing the atomic bomb, the second highest research priority in the war effort.

The revolution in molecular biology offers the possibility of yielding a whole new range of pharmacologically active microbiological produces through the application of genetic engineering technology. It is now possible to move genes from one organism into a plasmid or into the genome of another organism (cloning). In this way a substance that has a specific effect on another gene or gene product can be made in **commercially large quantities**.

For example, although clots are constantly forming in our bodies, they are dissolved before they do serious damage by special "clot-dissolving enzymes". However, their low concentration and difficulty of isolation have, made these clot- busting enzymes too rare and expensive to use widely. Now these enzymes are being made through genetic engineering technology in large enough quantities so as to become a standard treatment for stroke victims. A partial list of therapeutic agents manufactured by molecular biology is given below:

1. **Vitamins (example was given above)**
2. **Amino acids (example was given above)**
3. **Nucleic acids**
4. **Antibiotics**

Example: Penicillin



Penicillin nucleus

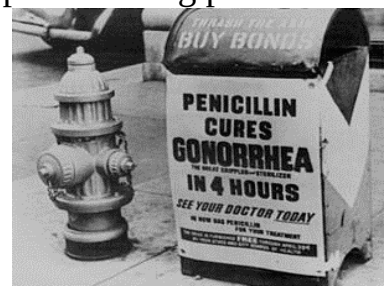
PENICILLIN (sometimes abbreviated **PCN**)

Refers to a group of β-lactam antibiotics used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms.

The discovery of penicillin is usually attributed to Scottish scientist Sir Alexander Fleming in 1928, though others had earlier noted the antibacterial effects of *Penicillium*. Fleming, at his laboratory in St. Mary's Hospital (now one of Imperial College teaching hospitals) in London, noticed a halo of inhibition of bacterial growth around a contaminant blue-green mold on a *Staphylococcus* plate culture. Fleming concluded that the mold was releasing a substance that was inhibiting bacterial growth and lysing the bacteria. He grew a pure culture of the mold and discovered that it was a *Penicillium* mold, now known to be *Penicillium notatum*.

Fleming coined the term "penicillin" to describe the filtrate of a broth culture of the *Penicillium* mold. Even in these early stages, penicillin was found to be most effective against Gram-positive bacteria, and ineffective against Gram-negative organisms and fungi. He noted its laboratory value in the isolation of "*Bacillus influenzae*" (now *Haemophilus influenzae*). After further experiments, Fleming was convinced that penicillin could not last long enough in the human body to kill pathogenic bacteria and stopped studying penicillin after 1931, but restarted some clinical trials in 1934 and continued to try to get someone to purify it until 1940.

On 1942 John Bumstead and Orvan Hess became the first in the world to successfully treat a patient using penicillin.



An advertisement on penicillin during World War II

During World War II, penicillin made a major difference in the number of deaths and amputations caused by infected wounds amongst Allied forces; saving an estimated 12-15% of lives.

Developments from penicillin

The narrow spectrum of activity of the penicillins, along with the poor activity of the orally-active phenoxymethyl penicillin, led to the search for derivatives of penicillin which could treat a wider range of infections.

The first major development was ampicillin, which offered a broader spectrum of activity than either of the original penicillins. Further development yielded beta- lactamase- resistant penicillins including flucloxacillin, dicloxacillin and methicillin. These were significant for their activity against beta- lactamase- producing bacteria species, but are ineffective against the methicillin- resistant *Staphylococcus aureus* strains that subsequently emerged. Other penicillin types were developed later including benzylpenicillin (penicillin G) and Phenoxymethylpenicillin (penicillin V).

Continued from the previous list of products are the following:

5. Alkaloids

6. Steroids

7. Non-Steroid Hormones/cell regulators **(cytokines):**

A. Epidermal growth factor
B. Proinsulin
C. Insulin
D. Human growth hormone
E. Somatostatin
F. Interferons

INTERFERONS (IFNs)

Are natural proteins produced by the cells of the immune system of most vertebrates in response to challenges by foreign agents such as viruses, bacteria, parasites and tumor cells. Interferons belong to the large class of glycoproteins known as cytokines. Interferons assist the immune response by inhibiting viral replication within other cells of the body.

Several different types of interferon are now approved for use in humans, and interferon therapy is used (in combination with chemotherapy and radiation) as a treatment for many cancers.

More than half of hepatitis C patients treated with interferon respond with better blood tests and better liver biopsies. There is some evidence that giving interferon immediately following infection can prevent hepatitis C. However, people infected by hepatitis C often do not display symptoms of HCV until months or years later.

Continued from the previous list of products are the following:

8. Blood coagulating factor XIII

9. Transgenic plants and animals (More effort is currently directed toward making plants not higher yielding but resistant to: herbicides, insects, fungal infections, and viruses)

10. **The restriction enzymes.**
11. **Plasmids**
12. **Vaccines**

VACCINES

NATURE AND IMPORTANCE OF VACCINES

Vaccines are materials that when introduced into the human body help protect the vaccinated person against specified communicable diseases. Communicable diseases are diseases caused by microorganisms, including viruses. Vaccines are preparations of dead or weakened pathogens, or their products, that when introduced into the body, stimulate the production of protective antibodies or T cells without causing the disease. Vaccination is also called **active immunization** because the immune system of the body is stimulated to actively develop its own immunity against the pathogen. **Passive immunity**, in contrast, results from the injection of antibodies formed by another animal (e.g., horse, human) which provide immediate, but temporary, protection for the recipient. The name 'vaccine' comes from the Latin vacca (for cow). This is because the earliest vaccination was done using the cow pox virus (which causes the disease in cow) as a vaccine against small pox in humans. The English physician, Edward Jenner carried out the above vaccination in the late 18th century.

Over the past 200 or so years vaccination has contributed greatly to reducing morbidity and mortality from communicable diseases. The greatest triumph of vaccination is the eradication of smallpox from the earth; no naturally-occurring cases has been reported since 1977. A program to try to eliminate another virus disease, poliomyelitis (polio for short), from the world has been on for some time and the indications are that the number of cases has drastically dropped.

Except for the few cases caused by oral polio vaccine (OPV), the disease has now been eliminated from the Western hemisphere.

VACCINE PRODUCTION METHODS

Traditionally three types of vaccines have been used: attenuated live vaccines, killed vaccines and bacterial toxoids. Recent advances in molecular biology made progresses in vaccine production.

Traditional vaccines

1- Live attenuated organisms

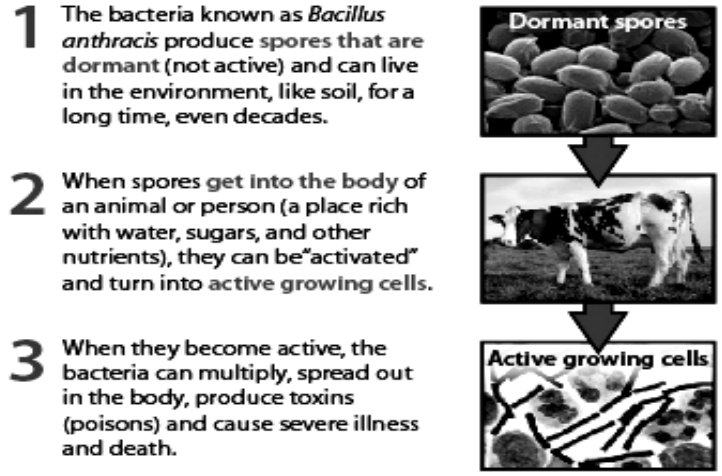
In live attenuated vaccines, the organism has been cultured to reduce its pathogenicity, but still retains some antigens of the virulent form. They consist of the living pathogens with reduced virulence (attenuated) by passing them through hosts different from the usual. Alternatively, non- virulent strains may be used. Live agents may be used for one or more of the following reasons:

(i) When the protection- inducing substance is produced as a diffusible product of metabolizing organisms e.g. *Bacillus anthracis*.

(ii) When it is not feasible to produce sufficient amounts of nonviable agents.

(iii) When immunity is induced by the modification of parasitized cells.

Live vaccines in use include those against polio (Sabin oral polio vaccine - OPV), foot and mouth disease of farm animals, mumps, measles, rubella (German measles), tuberculosis, rabies and yellow fever. For tuberculosis the vaccine is derived the *Bacillus* Calmette-Guérin (BCG) strain of *Mycobacterium tuberculosis*, a weakened version of the bacterium that causes tuberculosis in cows.



Inactivated *vs* attenuated vaccines

	inactivated	attenuated
cost	higher (greater mass required)	lower (agent replicates in the body)
administration	parenteral	oral
adjuvant	needed	not needed
stability	good	poor
reversion	absent	possible
immunity	mucosal immunity absent	mucosal immunity present
	antibody-mediated	antibody-mediate and cytotoxic T cells
	short-lasting	long-lasting

2- Killed vaccines

These consist of suspensions of fully virulent organisms (bacteria or viruses) killed as mildly as possible in order not to destroy the antigenic determinants on the organism. Killing can be achieved by heat, (usually about 60°C for 1 hour) chemicals (phenol, alcohol, formalin) or ultraviolet irradiation. Killed vaccines do not provide prolonged antigenic stimulation as living vaccines and two, three or more injections are required to give adequate protection. Examples of killed vaccines include TAB vaccine against typhoid fever, whooping cough, cholera, and the Salk IPV.

3- Bacterial toxoids

Toxoids are inactivated bacterial exotoxins. The toxins from *Clostridium botulinum*, *Clostridium tetani* and *Corynebacterium diphtheriae* are inactivated by treatment in formalin. Toxoids induce antibody production when injected into the body, although they are themselves harmless. In some diseases, of which diphtheria and tetanus are good examples, the bacterial metabolite, a protein toxin which they liberate, is the cause of the disease and not the bacteria themselves. Exposing the toxin with formaldehyde, denatures the protein can stimulate antibody production.

NEW APPROACHES IN VACCINOLOGY

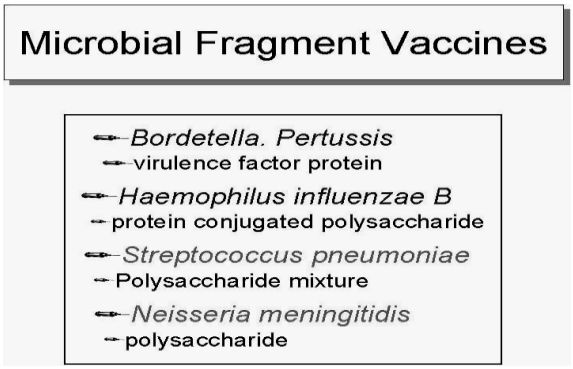
The advent of genomics, proteomics, and biotechnology, as well as the increased understanding of pathogenesis and immune responses to various pathogens have led to the development of safer, more effective and cheaper vaccines. Some of these are described below.

1- Subunit or surface molecule vaccines

Subunit vaccines contain antigens or epitopes that induce protection rather than the whole organism. The materials usually come from the

surface of the organism and hence they are also known as "surface molecule vaccines". The potential advantages of using subunits as vaccines are the increased safety, less antigenic competition, since only a few components are included in the vaccine. One of the disadvantages of subunit vaccines is that they generally require strong adjuvants and these adjuvants often induce tissue reactions. (**Adjuvants are compounds administered with vaccines to increase the immunogenicity of the vaccines**.) Second, the duration of immunity is generally shorter than with live vaccines.

Subunit vaccines are currently available for typhoid and whooping cough. Several vaccines employ purified surface molecules. One of them, the influenza vaccine contains purified hemagglutinins from the viruses currently in circulation around the world.

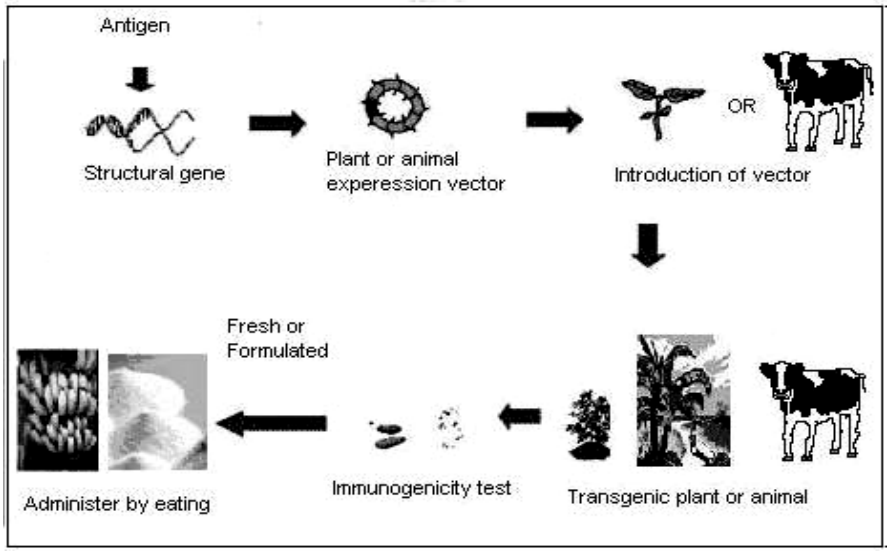


2- Conjugate vaccines

These are similar to subunit vaccines in the sense that only a part of the organism is used in making the vaccine. Some bacteria which are encapsulated cause important childhood diseases such as septicemia, pneumonia and meningitis. The bacteria are *Hemophilus influenzae* type B (HiB), *Neissseria meningitides* and *Streptococuus pneumoniae*. The capsules of these bacteria are made of carbohydrates which the immune system of adults recognize as foreign, but which that of infants do not and hence cannot make antibodies against them. To solve the problem protein from diphtheria or tetanus toxoids is linked or conjugated to the carbohydrate to make a vaccine. This enables a baby's immune system to respond to the combined vaccine and produce antibodies, initiating an immune response against the disease-causing organism. The licensed conjugate vaccines against *Haemophilus influenzae* type b (Hib), previously the major cause of bacterial meningitis in babies and young children, have virtually eliminated the disease in the United States.

Other (experimental) vaccines

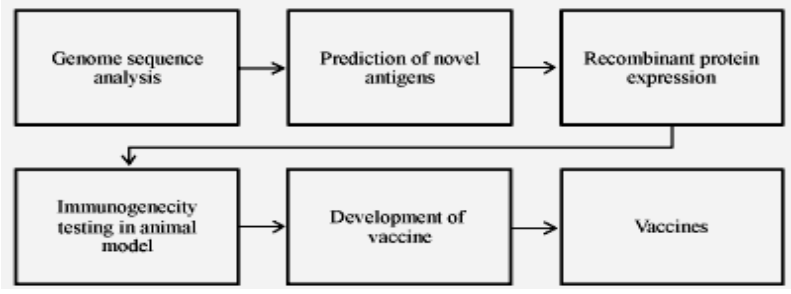
These include polynucleotide (DNA) vaccines and edible vaccines



REVERSE VACCINOLOGY

The name reverse vaccinology means reverse genetics (the process of identifying a protein or enzyme through its gene product). Despite the numerous successful vaccines produced and advances made over the last 200 years of vaccinology, there are still problems with existing approaches. Traditionally, the initial point in preparing a vaccine is to grow the pathogen. However, not all pathogens will successfully grow *in vitro* as is required for conventional methodologies, with Hepatitis B and C viruses being prime examples of such organisms.

Reverse vaccinology utilizes the wealth of information provided by genome sequencing to identify and characterize a whole host of new vaccine targets. Reverse vaccinology is now a standard technology. Vaccines projects are not now undertaken without knowledge of the sequence of the pathogen. Successful examples of genome- based vaccine discovery are pneumococcus, group B streptococcus, *Staphylococcus aureus*, and a variety of viruses.



QUESTIONS

1- What was the great discovery in treating illnesses during the World War II ?
2- What are the main categories of biotechnological and pharmacological products involving microbes?
3- Give an account on penicillin production and benefits?
4- Among the beneficial products are the non- steroid hormones/ cell regulators. Give an example on one of these products?
5- Discuss in brief the nature and importance of vaccines?
6- Compare between the different traditional vaccination methods (live- attenuated, killed and toxoids)?
7- Give short notes on subunit or surface molecule and conjugated vaccines as newer technologies of vaccination.
8- What are the dible vaccines? Give an example.
9- Discuss in brief the reverse vaccination process.

PART (11)

GENERATION OF BIOLOGICAL FUELS (BIOFUELS)

PHOTOSYNTHESIS AS A NEW SOURCE OF ENERGY.

- The world known sources of fossil fuels are coal, natural gas and oil. By applying the current consumption rates, it is assumed that these sources will be depleted within the next 1000, 35 and 15 years, respectively. Approximately 93% of fossil fuel is used for energy production, while only 7% is used by industrial processes such as the production of solvents, plastics and other chemicals.
- In the developed countries, many efforts are increasing now for finding and using alternative sources such as solar, hydro, wind, wave and nuclear power. Biological energy systems are also good alternative in many fields. This depends on biomass production from forests, agricultural and animal residues, and industrial and domestic organic wastes.
- Photosynthetic organisms, both terrestrial and marine, are continuous solar energy converters and are constantly renewable. Plant photosynthesis alone fixes about 2×10^{11} tonnes of carbon representing about 10 times the world's annual energy use. It is well-known also that photosynthesis produced, in the past, all the sources of present carbon fossil fuels. Therefore, the biomass derived from photosynthesis can be converted into storable fuels and chemicals such as alcohols and methane.
- Biomass can be can be converted directly to energy or energy-carrying compounds. These processes can be carried out by direct combustion, anaerobic digestion systems, destructive distillation, gasification, chemical hydrolysis and biochemical hydrolysis.

SOURCES OF BIOMASS

There are three main directions to obtain biomass supply:

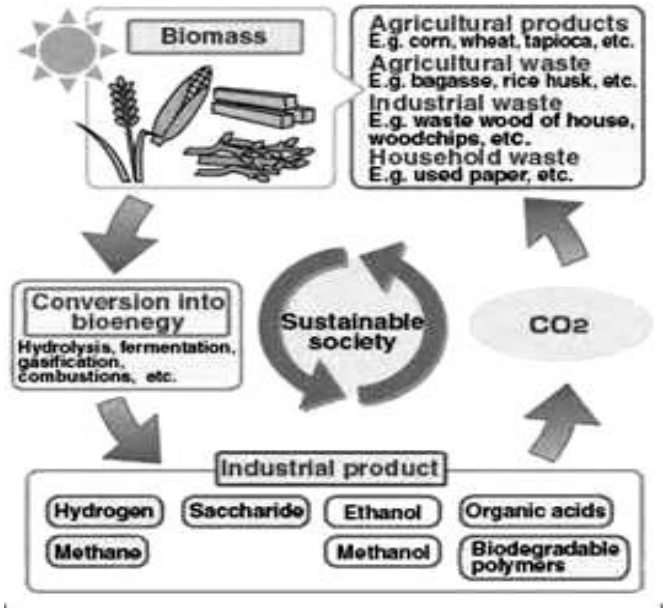
(1) Cultivation of energy crops.
(2) Harvesting natural vegetation.
(3) Utilization of agricultural and other organic wastes.

- Conversion of the resulting biomass into fuel can be achieved by biological or chemical means or by a combination of both. The two main end- products are methane and ethanol, although other products may result depending on the initial biomass and the process utilized.

- For those countries unable to apply these programs, the alternative is using other biomass supplies including the use of organic wastes (agricultural, municipal and industrial) and harvesting natural vegetations.
- The technical processing of the biomass depends on many factors, including moisture level and chemical complexity.

1- Materials with high water content are normally processed through aqueous methods, avoiding the need for further drying. Examples are alcoholic fermentation to ethanol, anaerobic digestion to methane, and chemical reduction to oily hydrocarbons.

2- Materials with low water content such as wood, straw and bagasse can be burnt to give heat or to generate steam for the production of electricity or subjected to thermo- chemical processes to yield gaseous oil, char and eventually methanol and ammonia. And, also it can be treated by alkaline or biological hydrolysis to produce chemical feedstocks for use in further biological energy conversions.



Biomass conversion

- Energy crops that were selected by the U.S. Department of Energy for further development as energy crops were primarily perennials such as switchgrass, willow and poplar. They were selected for their advantageous environmental qualities such as erosion control, soil organic matter build-up and reduced fertilizer and pesticide requirements. There are many other perennial plant species which could be used for energy crops. In addition, some parts of traditional

agricultural crops such as the stems or stalks of alfalfa, corn or sorghum may be used for energy production.

Switchgrass

ETHANOL FROM BIOMASS:

- The following formula represents the fermentation of sugar to alcohol which is an ancient microbial process:

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

- The Brazilian program is almost based on **batch fermentation** system. In developed countries, progress in **continuous fermentation** was achieved utilizing various approaches such as retention of the yeast cells in the bioreactor by separation, recycling and continuous evapouration of the fermentation broth.

Potential raw materials for fuel ethanol production

Starch containing	Cellulosics	Sugar-containing	Other
Cereal grains	Wood	Sucrose and invert sugar	Jerusalem artichoke
		Sweet sorghum	
Corn	Sawdust	Molasses	
Grain sorghum	Waste paper	Sugar beet	Raisins
Wheat	Forest residue	Fodder beet	Bananas
Barley	Agricultural residues	Sugar cane	
Milling products	Municipal solid wastes	Lactose	
		Whey	
Wheat flour	Intensive livestock		
Wheat millfeeds	production wastes	Glucose	
Corn hominy feed[a]		Sulphite wastes	
Starchy roots			
Mandioca			
Potatoes			

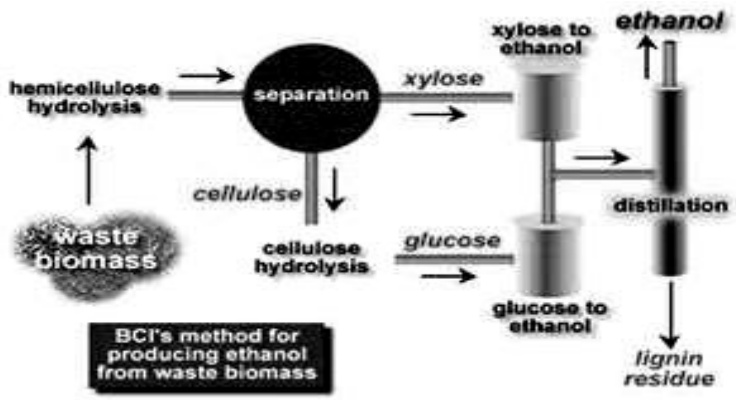
Note:

[a] Meal from parched corn, often with the hull and germ removed.

Indirect economic advantages of ethanol production from crops in the developing countries:

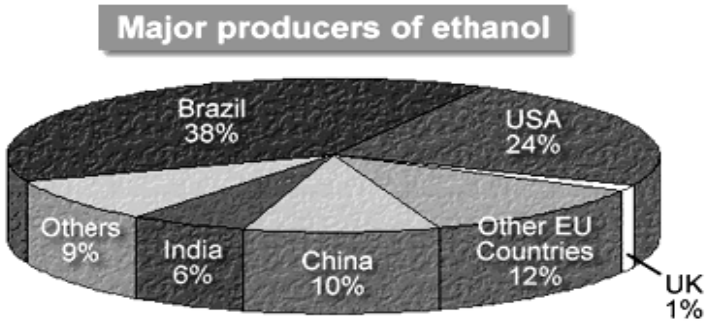
1- Expansion of agriculture.
2- Creating more job opportunities.
3- Applying new technologies for utilizing wastes generated in production of ethanol (stillage) including:

- Evapouration to feed or fertilizers.
- Mineralization to ash.
- Anaerobic fermentation for the production of methanol.
- Conversion by microorganisms into single cell protein.



Biodiesel is another biofuel made by combining alcohol (usually methanol) with vegetable oil, animal fat, or recycled cooking grease. It can be used as an additive (typically 20%) to reduce vehicle emissions or in its pure form as a renewable alternative fuel for diesel engines. In general, biofuels can reduce costly petroleum imports, cut greenhouse gas emissions, increase farm income, and boost rural development.

As the world's top producer, Brazil uses sugar cane to make ethanol. Many other developing countries, produce large amounts of sugar and also have potential to become ethanol producers.



IMPACT ON THE ENVIRONMENT

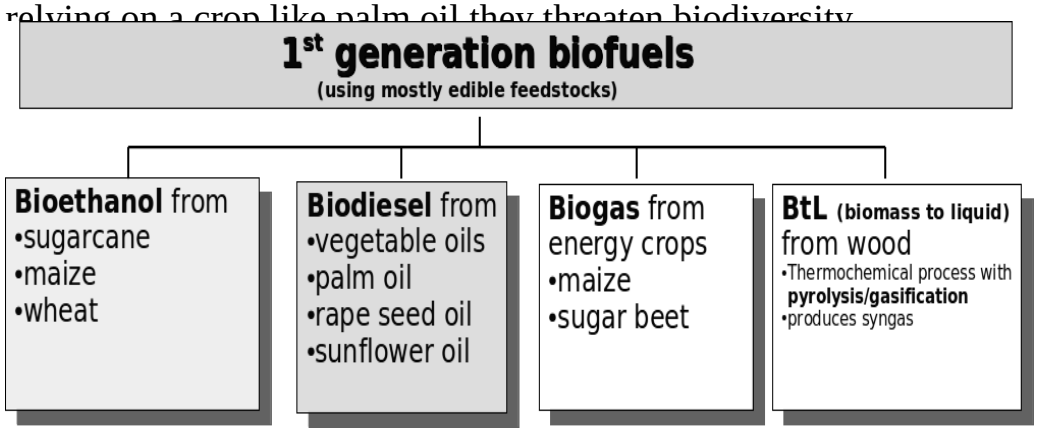
- Plants need carbon dioxide to grow. The carbon dioxide released by the use of biofuels was removed from the atmosphere by plants, and therefore, no new carbon dioxide is emitted. This is not the case for carbon stored in fossil fuels.
- Biofuels are also cleaner burning and reduce emissions of particulate matter, a major component of urban air pollution linked to respiratory and heart disease. Biofuels are biodegradable and nontoxic.
- Perennial cellulosic feedstocks like fast-growing trees and grasses require few inputs, sequester carbon underground in their roots, and do not require annual tilling, thereby reducing soil erosion. These benefits are not present in conventionally produced row crops.

The most common use for biofuels is automotive transport. Increased American and European demand has led to clearing land for palm Oil plantations. In some areas use of pesticides for biofuel crops are disrupting clean water supplies. The greatest technical challenge is to develop ways to convert biomass energy specifically to **liquid** fuels.

GENERATIONS OF BIOFUELS

FIRST GENERATION

First generation biofuels are known for their manifold problems: when made from grains such as corn or canola, they have negative impacts on food prices (this is not the case with sugarcane) and when relying on a crop like palm oil they threaten biodiversity.



SECOND GENERATION

- Second generation biofuel production processes can use a variety of non food crops. These include waste biomass, the stalks of wheat, corn, wood, and special-energy-or biomass

crops. Second generation (2G) biofuels use biomass to liquid technology, including cellulosic biofuels from non food crops.

- Second generation biofuels include biohydrogen, biomethanol, DMF (dimethylfuran), Bio-DME, Fischer-Tropsch diesel, biohydrogen diesel, mixed alcohols and wood diesel.
- Second generation fuels involve a change at the bioconversion step and get rid of the apparent fuel versus food dilemma. Instead of only using easily extractible sugars, starches or oils as in the previous generation, these techniques allow for the use of all forms of lignocellulosic biomass. Grass species, trees, agricultural and industrial residues can all be converted via two main pathways: biochemical and thermochemical.
- Synthetic biofuels and cellulosic ethanol have an excellent carbon balance and may reduce carbon dioxide emissions by up to 90% compared to petroleum based fuels. Moreover, they are ultra-clean and reduce emissions of the other major pollutants (NOx, SOx).

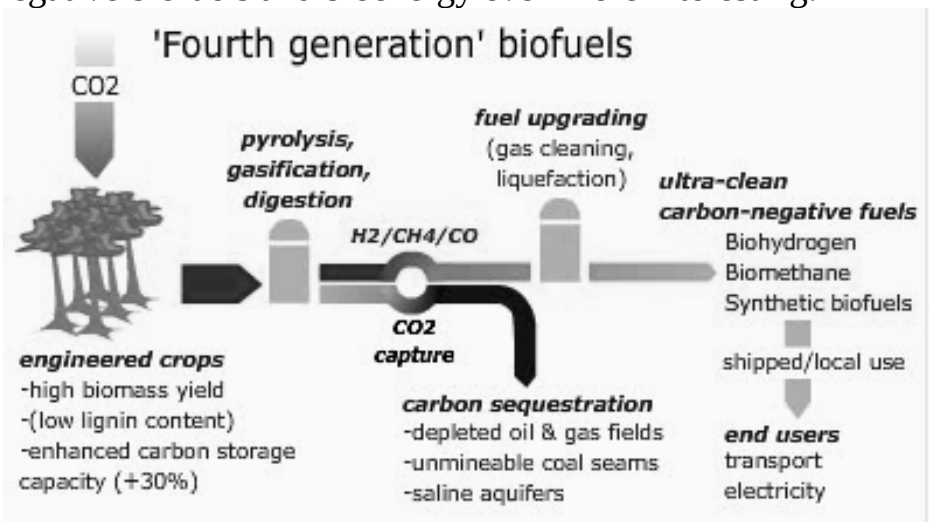
THIRD GENERATION

- Algae fuel, also called oilgae or third generation biofuel, is a biofuel from algae. With the higher prices of oil, there is much interest in alga culture (farming algae).
- One advantage of many biofuels over most other fuel types is that they are biodegradable, and so relatively harmless to the environment if spilled. The United States Department of Energy estimates that if algae fuel replaced all the petroleum fuel in the United States, it would require 38,849 square kilometers, which is a few thousand miles larger than Maryland state.
- This generation takes advantage of new, specially engineered energy crops. Recent advancements in plant biology, the emergence of extremely efficient and fast breeding techniques (molecular breeding), the rapid advancements in the field of genomics, and classic design of transgenic crops promises to result in plants with properties that make them more suitable for conversion into bioproducts.

FOURTH GENERATION

- In fourth generation production systems, biomass crops are seen as efficient 'carbon capturing' machines that take CO2 out of the atmosphere and lock it up in their branches, trunks and leaves.

- The carbon-rich biomass is then converted into fuel and gases by means of second generation techniques (genetically engineered microorganisms).
- Only the utilization of biomass allows for the conception of carbon-negative energy; all other renewables (wind, solar, etc) are all carbon-neutral at best, carbon-positive in practice. Fourth generation biofuels instead take historic CO2 emissions out of the atmosphere. They are tools to clean up our dirty past.
- The fact that fast-growing, high yielding trees are being designed that sequester more carbon dioxide, makes the promise of carbon-negative biofuels and bioenergy even more interesting.



Generations of biofuels

First	Second	Third	Fourth
*Derived from edible plants grown on arable land. * Ethanol and butanol produced via yeast fermentation * Crops include wheat, sugar cane, oily seeds. * Attributed as a potential reason for recent spike in food prices. * Net energy negative.	* produced from non- edible crops grown on non-arable land. * Sources have lignocellulosic content including wood and organic waste. * Net energy positive.	* Produced from algae and other microorganisms. * Resilient organisms that can be grown from sunlight, CO2 and brackish water. * Does not use arable land. * Fastest growing of all biofuel sources. * Potentially carbon neutral.	* Genetic engineering of organisms for efficient production of biofuels. * Include altering lipid characteristics and excretion pathways. * Aim to be carbon negative by creating artificial carbon sinks.

BIOFUELS IN DEVELOPING COUNTRIES

Biofuel industries are becoming established in many developing countries. Many developing countries have extensive biomass resources that are becoming more valuable as demand for biomass and biofuels increases. The approaches to biofuel development in different parts of the world varies. Countries such as India and China are developing both bioethanol and biodiesel programs. India is extending plantations of jatropha, an oil-producing tree that is used in biodiesel production. The Indian sugar ethanol program sets a target of 5% bioethanol incorporation into transport fuel. China is a major bioethanol producer and aims to incorporate 15% bioethanol into transport fuels by 2010.

Amongst rural populations in developing countries, biomass provides the majority of fuel for heat and cooking. Wood, animal dung and crop residues are commonly burned. Figures from the International Energy Agency show that biomass energy provides around 30% of the total primary energy supply in developing countries; over 2 billion people depend on biomass fuels as their primary energy source.

QUESTIONS

1- Why did the need for fuels, other than petroleum and fossil fuel sources, emerged?

2- Describe the main world sources of biomass and its uses in energy production?

3- How can the availability of water limit the programs for biomass production?

4- what are the main problems for technical processing of biomass? Explain?

5- What is the advantageous role of biomass production in reducing global climate changes?

6- Describe one method for producing ethanol from biomass?

7- What are the uses of methane? why it is a very important gas?

8- Describe the process of methane production from biomass? What are the main raw materials? Discuss in details one of these methods explaining the advantages and limitations.

9- What are the indirect advantages of ethanol production from biomass in the developing world?

10- Discuss in brief the generations of biofuels?

11- What is biodiesel? What it is made from?

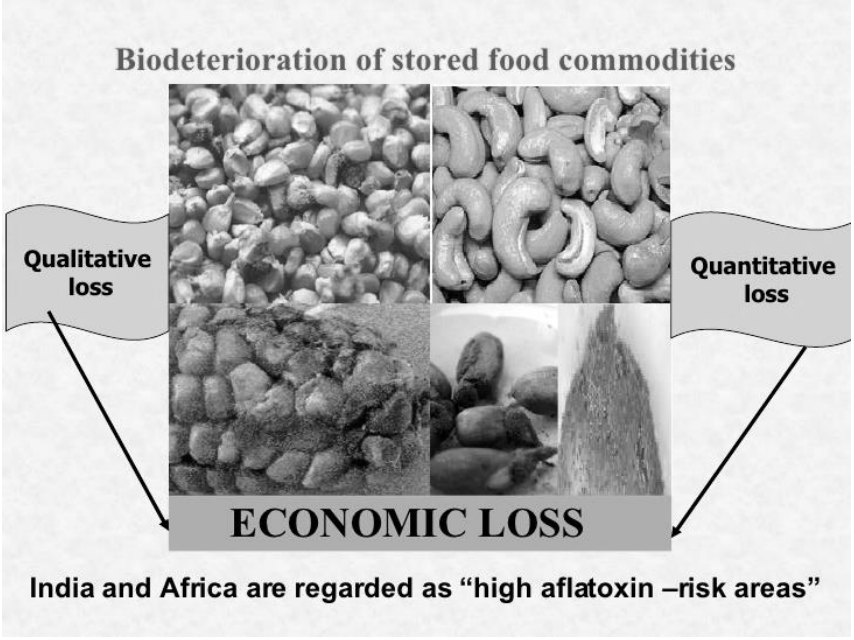
12- Write on the impact of using biofuels on the environment

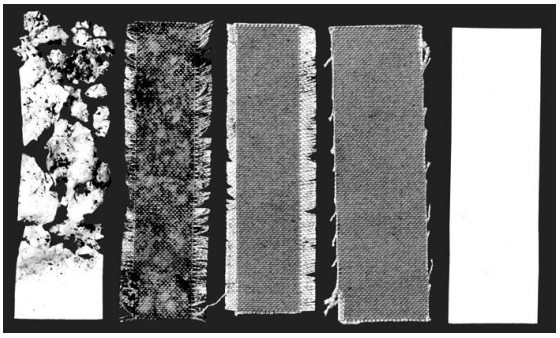
PART (12)

BIODETERIORATION & BIOREMEDIATION

1- BIODETERIORATION

Definition- undesirable change in the properties of materials caused by vital activities of organisms.





Biodeterioration of textile and some resistant textile materials

TYPES OF BIODETERIORATION

1. Physical or mechanical - material is not a food source (e. g. root damage)
2. Fouling or soiling - material is not damaged (e. g. fungus on shower curtain, etc.).
3. Chemical assimilatory - material is used as a carbon and/ or energy source (e. g. food spoilage, degradation of fuels, metals).
4. Chemical dissimilatory - substance not used as carbon and energy source (e. g. acid waste products, tooth decay)

2- BIOREMEDIATION

"Remediate" means to solve a problem, and "bio- remediate" means to use biological organisms to solve an environmental problem such as contaminated soil or groundwater. Bioremediation is defined as "the use of microbes to remove pollutants from the environment".

In a non- polluted environment, bacteria, fungi, protists, and other microorganisms are constantly breaking down organic matter. What would occur if an organic pollutant such as oil contaminated this environment? Some of the microorganisms would die, while others capable of eating the organic pollution would survive. Bioremediation works by providing these pollution- eating organisms with fertilizer, oxygen, and other conditions that encourage their rapid growth. These organisms would then be able to break down the organic pollutant at a correspondingly faster rate.

Our industrial-based civilization has produced and contaminated the earth's surface with a huge number of dangerous pollutants, both natural and made-made. Many of these substances are toxic and/ or carcinogenic or harmful to the environment in other ways. Below is a small list of some prominent industrial wastes polluting our environment. The **bold** ones are carcinogenic/ toxic, the **regular-font** ones are just toxic (to liver, brain, kidneys and other organs and tissues):

1. **Benzene**
2. **Phenol**
3. **Chloroform**
4. **Carbon tetrachloride**
5. Gasoline
6. **Motor oils**
7. **Raw petroleum**
8. **Nitrate**
9. Lead
10. DDT

In many cases the soil and ground water leaching from industrial, and municipal toxic waste dumps, contaminate vast quantities of ground water making it dangerous for any subsequent use. The idea behind bioremediation is to (1) isolate microbes that can **degrade** or eat a particular pollutant and (2) to provide the conditions for doing this most effectively, thereby eliminating that pollutant.

The basic principle of bioremediation is the same as that for sewage treatment. That is, the use of microbial metabolism to "eat up" or metabolize pollutants so as to convert them into something harmless. The following general steps are utilized in bioremediation:

1. Define the pollution situation: What pollutants are present, how much of each are there, how dangerous are they, are they spreading and, if so, where and how fast.
2. Develop a microbial approach for dealing with the pollutants.
3. Isolate or stimulate a microbial population that will, by **natural selection**, "eat" or metabolize the pollutants.
4. Grow the **pollution-fighting-microbes** in large quantities or otherwise provide conditions that will stimulate their growth in the polluted environment.
5. Add the pollution-fighting-microbes to the polluted environment and provide the optimum nutrient and environmental conditions to allow the pollution-fighting-microbes to metabolize the pollutants.

The crucial step in this process is the isolation or enrichment of **suitable microbes** that will effectively metabolize the desired pollutant. This is done using a technique developed by the early microbiologists called the **enrichment culture technique**. The enrichment culture technique works like this:

1- a sample of a pollutant is added to a **basic nutrient medium** in which the pollutant chemical (e.g. gasoline, phenol, turkey feathers etc.) is included as the **major** or only carbon and/or energy source.

2- The medium is inoculated with soil which is likely to contain a diversity of microbes (e.g. rich garden soil, sewage etc.).

3- The culture is incubated, usually under aerobic conditions, at a suitable temperature for a period of time and the concentration of the pollutant **monitored**.

4- If the pollutant disappears, an inoculum is taken from the original flask and added to another and the process is **repeated** until you have a culture in which the **pollutant-digesting organism** predominates.

5- This microbe(s) is isolated and studied to see if you can boost its pollutant- metabolizing abilities even more. Finally it is used as outlined above to treat the polluted material.

Problems with bioremediation

- Often the concentration of a given pollutant is so low that it won't support good growth of microbes, yet the level is high enough to be

dangerous. Under such conditions, additional nutrients, at added cost, have to be supplied.

- It is difficult to get the microbes into the polluted soil in a way that they can effectively remove the pollutant. One procedure involves digging up the contaminated soil, mixing it in large tanks with the microbes and nutrients until the pollutant is degraded and then returning the now **pollutant-free soil** to its original place. Clearly, this is an expensive process when large areas of polluted land are involved.
- Many of the pollutants are recalcitrant or difficult for microbes to readily digest and thus the microbes take a long time to degrade them; further adding to the expense of the process.
- The number of pollutants at a site may be unknown or poorly defined, so what works for one pollutant may not work on another pollutant.

Effective bioremediation

- Bioremediation has proven effective at treating pollution problems like aviation fuel spills in the ground on army bases, in removing oil spills and in digesting a host of other organic pollutants. The process is **costly**, but is proving to be more effective than such procedures as digging up contaminated ground and burying it somewhere else, incinerating the soil or treating the polluted water with expensive and dangerous chemicals to destroy the targeted pollutants.
- Bioremediation of a contaminated site typically works in one of two ways:
  1. In the case described above, ways are found to enhance the growth of whatever pollution- eating microbes might already exist in the contaminated site.
  2. In the second, less common case, specialized microbes are added to degrade the contaminants (i. e. isolation of organisms from the site of contamination and inoculating efficient microorganisms).
- Bioremediation provides a good cleanup strategy for some types of pollution but it will not work for all. For example, bioremediation may not provide a feasible strategy at sites with high concentrations of chemicals that are toxic to most microorganisms. These chemicals include metals such as cadmium or lead, and salts such as sodium chloride.

- Nonetheless, bioremediation provides a technique for cleaning up pollution by enhancing the same biodegradation processes that occur in nature. Depending on the site and its contaminants, bioremediation may be safer and less expensive than alternative solutions such as incineration or landfilling of the contaminated materials. It also has the advantage of treating the contamination in place so that large quantities of soil, sediment or water do not have to be dug up or pumped out of the ground for treatment.

BENEFICIAL BACTERIA AND BIOREMEDIATION

Surprisingly, only about 1% of the bacteria present in nature have been identified, and, consequently, microbiology is the branch with the greatest potential for future discovery.

There are many examples of specific microbes or microbial communities in nature that do remarkably useful things, unattainable by other life forms. For example, the lichen is a microbial community that can grow on the bare surface of a rock. A lichen consists of fungi that break down the rock to extract minerals, algae which absorb light energy to make sugars and other carbon compounds, and cyanobacteria that can fix atmospheric nitrogen (N_2) to make organic nitrogenous compounds such as amino acids and nucleic acid bases. Because of this tripartite partnership, lichens can grow with rock and air as sole sources of food. They proliferate in harsh environments such as dry mountainous areas and the Arctic and Antarctic polar regions of the globe. They often provide crucial sources of food for the animals and plants that live in these environments.

Example (1): <u>EPA bioremediation of soil and groundwater</u>

EPA (Environmental Protection Agency, USA), uses many methods to clean up pollution at different sites. Such methods can be quicker and cheaper than more common methods. Microscopic microbes that live in soil and groundwater like to eat certain harmful chemicals, such as those found in gasoline and oil spills. When microbes

completely digest these chemicals, they change them into water and gases such as carbon dioxide.

In order for microbes to clean up harmful chemicals, the right temperature, nutrients (fertilizers), and amount of oxygen must be present in the soil and groundwater. When conditions are not right, microbes grow too slowly or die or they can create more harmful chemicals. If conditions are not right at a site, EPA works to improve them. One way they improve conditions is to pump air, nutrients, or other substances (such as molasses) underground.

Sometimes microbes are added if enough are not already there. The right conditions for bioremediation cannot always be achieved underground. At some sites, the weather is too cold or the soil is too dense. At such sites, EPA might dig up the soil to clean it above ground where heaters and soil mixing help improve conditions. After the soil is dug up, the proper nutrients are added. Oxygen also may be added by stirring the mixture or by forcing air through it. However, some microbes work better without oxygen.

Sometimes mixing soil can cause harmful chemicals to evaporate before the microbes can eat them. To prevent these chemicals from polluting the air, EPA mixes the soil inside a special tank or building where chemicals that evaporate can be collected and treated. Microbes can help clean polluted groundwater as well as soil. To do this, EPA drills wells and pumps some of the groundwater into tanks and water is mixed with nutrients and air before it is pumped back into the ground. Groundwater can also be mixed underground by pumping nutrients and air into the wells. Once harmful chemicals are cleaned up and microbes have eaten their available "food," the microbes die.

Bioremediation is very safe because it relies on microbes that naturally occur in soil. No dangerous chemicals are used in bioremediation. The nutrients added to make microbes grow are fertilizers commonly used on lawns and gardens. Because bioremediation changes the harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed. To ensure that bioremediation is working, EPA tests samples of soil and groundwater.

Often bioremediation does not require as much equipment or labor as most other methods. Therefore, it is usually cheaper. Bioremediation has successfully cleaned up many polluted sites.

The time it takes to bioremediate a site depends on several factors:

• Types and amounts of harmful chemicals present
• Size and depth of the polluted area
• Type of soil and the conditions present
• Whether cleanup occurs above ground or underground

These factors vary from site to site. It can take months or even several years for microbes to eat enough of the harmful chemicals to clean up

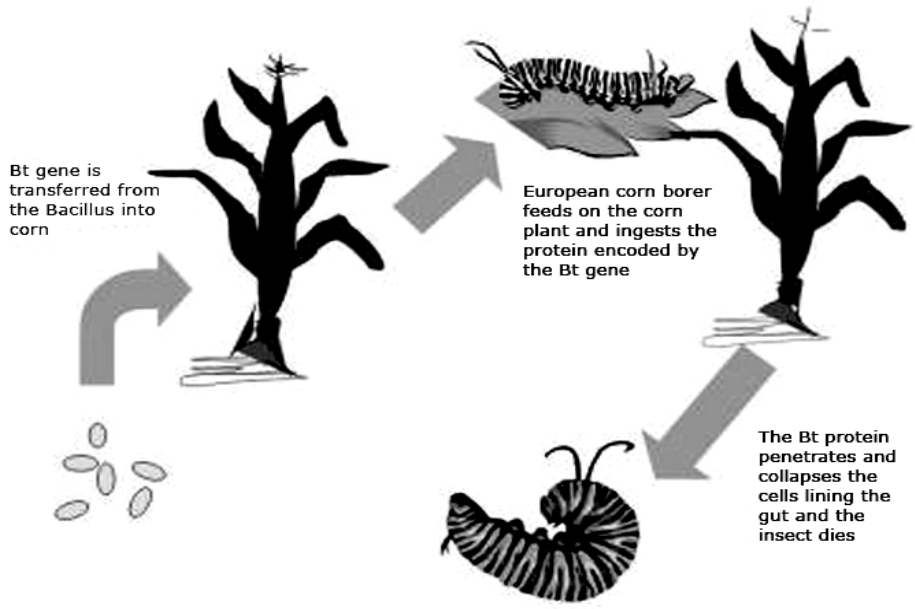
Example (2): <u>Biopesticides (biological control)</u>

They are derived from natural materials such as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. At the end of 2001, there were approximately 195 registered biopesticide active ingredients and 780 products. Biopesticides fall into three major classes:

1- Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest[s]. For example, there are fungi that control certain weeds, and other fungi that kill specific insects.

The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis*, or Bt. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some Bt's control moth larvae found on plants, other Bt's are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.

2- Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest.



3- Biochemical pesticides are naturally occurring substances that control pests by non- toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest.

The advantages of using biopesticides

1. Biopesticides are usually inherently less toxic than conventional pesticides.
2. Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects, and mammals.
3. Biopesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.
4. When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high.

To use biopesticides effectively, however, users need to know a great deal about managing pests.

QUESTIONS

1- Define the biodeterioration?

2- What are the types of biodeterioration?

3- What is bioremediation? What are the main principles of bioremediation concept?

6- What are the steps usually used to remediate pollution biologically?

7- Describe the main problems that occur in bioremediation processes?

8- Give examples on some beneficial bacteria used bioremediation?

9- Define the biopesticides and write on its three major classes.

10- What are the advantages of using biopesticides?

11- Discuss in brief examples of some microorganisms as biopesticide producers.

REFERENCES

1- Biswas, P. K. (2008). Agricultural microbiology, Dominant Publishersand Distributors, New Delhi, 352 pp.

2- Coyne, M. S. (1999). Soil microbiology: an exploratory approach. Delmar Publishers, UK.

3- De Man, J. M. (1999). Principles of Food Chemistry. 3rd ed., Aspen Publishers, Inc., USA.

4- Desrosier, N. W. and Desrosier, J. N. (2004). The technology of food preservation, 4th ED. CBSPublishers and Distributors, India, 558 pp.

5- Edwards, C. (1999). Environmental monitoring of bacteria. Methods in Biotechnology Vol. 12, Humana Press Inc., UK.

6- Mayers D. L. (2009). Antimicrobial drug resistance, Humana Press, Springer Sciences, LLC.

7- Okafur, N. (2007). Modern industrial microbiology and biotechnology. Science Publishers, Infield, USA, 530 pp.

8- Schaechter, M. (2004). The Disk Encyclopedia of Microbiology. Elsevier Academic Press, UK.

9- Sharma, R. (2006). Production, processing and quality of milk products. International Book Distributing Co., India, 275 pp.

10- Shetty, K.; Paliyath, G.; Pometto, A. and Levin, R. E. (2006). Food biotechnology. Taylor and Francis Group, LLC, CRC Press**.**

11- Varma, A. and Oelmuller, R. (2007). Advanced techniques in soil microbiology. Springer- Verlag, Berlin, Heidelberg.

PREVIOUS EXAMINATIONS

South Valley University
Fac. of Science
Dept. of Botany

Third year Exam
Chem/ Botany
Time: 3 hrs

May 2008

(1) Write in brief on each of the following:
The main considerations for safety in biotechnology- cheese production- benefits of compost for agriculture and the environment.
(2) Write in detail on:
The production of ethanol from biomass- tea processing and classification

South Valley University
Fac. of Science
Dept. of Botany

Third year Exam
Chem/ Botany
Time: 3 hrs

Industrial microbial. + Physiol. of fungi

May 2009

***Answer the following questions*:**

Part (1): Industrial microbiology

(1) Write in brief on each of the following:

Food waste- scaling- up in industrial microbiology- using microbes in the recovery of oils- bioplastics- aging of coffee beans- rennet- the role of yeast in bread making- oxidation and drying tea leaves- biofertilizers- bioreactor- European classification of microorganisms according to pathogenicity. (50 marks, 5 each)

(2) Discuss each of the following:

A) The role of pH, aeration, temperature, particle size and the proper nutrient mixture in making a good compost. (20 marks)

B) The main benefits of using lactic acid bacteria in microbiological industries (12.5 marks)

South Valley University
Fac. of Science
Dept. of Botany

Fourth year Exam
Chem/ Microbiol.
Time: 3 hrs

May 2010

(1) Write in brief on each of the following:
The essential guidelines for optimizing the bioreactor- biodeterioration and bioremediation- bread making.
(2) Discuss the main background for each of the following:
Cheese making and lactic acid bacteria- tea processing and classification- food poisoning and preservation methods.

South Valley University
Fac. of Science
Dept. of Botany

Fourth year Exam
Chem/ Microbiol.
Time: 3 hrs

May 2008

(1) Write in brief on each of the following:
The essential guidelines for optimizing the bioreactor- biofertilizers- bread making.
(2) Write in detail on:
Coffee processing- tea processing and classification- The main factors that affect the production of good compost.

South Valley University
Fac. of Science
Dept. of Botany

Fourth year Exam
Chem/ Microbiol.
Time: 3 hrs

Fungi + Industrial Microbiology

May 2009

Part (2): Industrial microbiology

(1) Discuss each of the following:

a) Curd formation, treatment and secondary microflora in cheese making process. (15 marks)

b) Biomass and ethanol production. (17.5 marks)

(2) Write in brief on each of the following:

Classification of tea types according to oxidation- vinegar manufacturing- downstream processes- problems of biologically active biotechnology products- Signs of compost ripening- production of organic acids- the main processes of bread making- types of bioreactors- the main considerations of safety in biotechnology. (50 marks, 5 each)

South Valley University
Fac. of Science
Dept. of Botany

Fourth year Exam
Chem/ Microbiol.
Time: 3 hrs

May 2010

(1) Discuss in brief each of the following:
- The industrial microbes and its characteristics
– the ten main reasons for food poisoning
- the use of bacteria in biomining
- HACCP food safety program.

(2) Write in detail on:
- Scaling- up and downstream processing in industrial microbiology
- Vinegar production
- the main factors that lead to good composting

South Valley University
Fac. of Science
Dept. of Botany

Fourth year Exam
Chem/ Microbiol.
Time: 3 hrs

May 2011

(1) Discuss in brief each of the following:

- Essential guidelines for optimizing the bioreactor
- The main steps of cheese production and the benefits of lactic acid bacteria
- The bread making process
- The used material, the starting and ending C: N ratio and other factors that control good compost making.

(2) Explain in detail on:

- The three main processes for preventing food poisoning
- HACCP food safety program
- The use of bacteria in biomining

South Valley University
Faculty of Science
Microbiol.
Department of Botany

Fourth year Exam
Chem/
Time: 3 hrs

May 2013

Answer the following question:

(1) Discuss in brief each of the following: (8.5 marks each)

- How can you set up a HACCP food safety program.
- The benefits of lactic acid bacteria.
- The characteristics of microbes used in biotechnology
- European classification of hazards in biotechnology
- Why there are so many types of cheeses.

Answer one only of the following:

(2) a) What is the main idea of converting biomass into alcohol. Write on the steps in brief. (20 marks)

b) Explain in brief how can you break the food poisoning chain? (20 marks)

(3) a) How can you solve the problem of organism pathogenicity in biotechnology. (20 marks)

b) write in points on each of the following: personal hygiene in food industry- bread making process- variation of tea products due to differences in fermentation- scaling up and downstream processes. (20 marks)

GOOD LUCK

South Valley University
Faculty of Science
Department of Botany

Fourth year Exam
Chem/ Microbiol.
Time: 3 hrs

Industrial microbiology & Physiology of fungi Exam
May 2020

A) INDUSTRIAL MICROBIOLGY

Part I: Mark on the correct answer as false (F) or True (T).

1) Industrial microbes should be genetically unstable to make genetic manipulation easy.

2) Secondary metabolites are formed during idiophase by few organisms.

3) All growth and production parameters, of the on- going production process, in the bioreactor can be measured online.

4) During the scaling- up process, the chemical factors create more problems than the physical ones.

5) Downstream processes include separation, concentration and purification of the product.

6) Bacteria and harmful organisms cannot grow on food in the refrigerator.

7) Lactic acid bacteria and yeast are involved in the processof coffee and cocoa fermentation.

8) Some acid proteases are used in the bakery products and extracting silver from film rolls.

9) Only some fungi species can produce renin- like proteases for cheese production.

10) Bioethanol can be produced from any biomass materials using microorganisms.

Part II: Choose the correct answer:

1) Industrial microbiology is concerned with:

A. Employing microorganisms in medical treatments.
B. Avoiding the use of microorganisms in industry.
C. Employing microorganisms to produce the desired products.
D. Using microorganisms in small industries.

2) The main areas of food safety in biotechnology include:

A. Aspects related to food workers' safety.
B. Safety from electrical hazards.
C. Pathogenicity, allergy and toxicity of products.

D. Safety from fire and physical injuries.

3) High risk foods are those:

A. Containing large numbers of harmful microorganisms.

B. Ready- to- eat foods without further processing.

C. Cross contaminated foods by workers mistakes.

D. Very hot foods with temperature exceeding 80º C.

4) Cross contamination of food is from:

A. Unused food equipment.

B. Insects and rodents.

C. Dirty kitchen.

D. Other contaminated food.

5) Moving of pathogenic bacteria to food is known as:

A. The route.

B. Cross contamination.

C. Food poisoning.

D. The vehicle.

6) There are three main ways for breaking food poisoning chain including

A. Storage in a refrigerator.

B. Food sterilization and pasteurization.

C. Heating to 60º C.

D. Serving and eating food quickly.

7) Coffee aging is the process of:

A. Leaving coffee beans in dry weather for 6 months.

B. Over fermentation of beans with microorganisms.

C. Exposing unroasted beans to hot air.

D. Storage of beans in moist environment for the required time.

8) Green tea is that:

A. Completely fermented.

B. Half- way fermented.

C. Fermented for only two to three days.

D. Fermented a little and fermentation is stopped by heat.

9) There are thousands of cheese types because:

A. There are many types of milk.

B. Fermentation is carried out by many bacteria and fungi.

C. There are many additives that can be used.

D. Because all of the above reasons.

10) Gluten glue is a type of:

A. Sticky glue produced by bacteria.
B. A type of glue produced from starch.
C. Capsule polysaccharide produced by some encapsulated bacteria.
D. Proteins that form long molecular strings forming dough when kneaded.

جامعة جنوب الوادى
كلية العلوم

الرؤيـــة

كلية العلوم تقدم خدمات تعليمية وبحثية ومجتمعية متميزة

الرســـالة

تلتزم كلية العلوم باعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية وتقديم بحوث علمية متميزة وتطوير مهارات وقدرات الكوادر البشرية بها وتوفير خدمات مجتمعية وبيئية تلبى طموحات كجتمع جنوب الوادى وذلك من خلال مشاركة مجتمعية فاعلة