

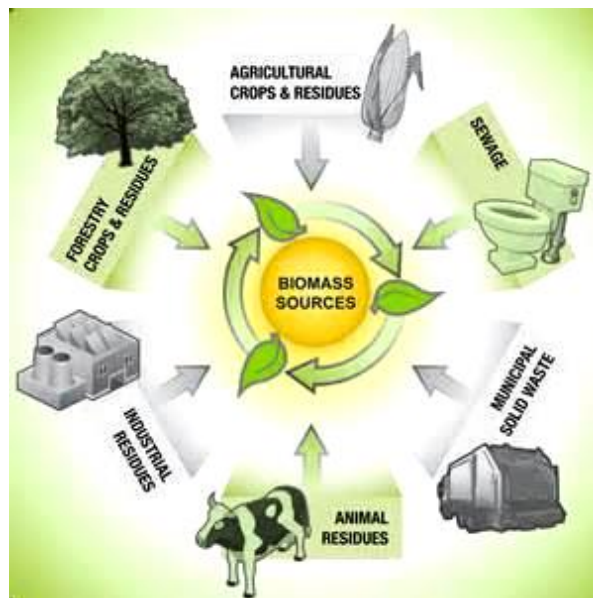


جامعة جنوب الوادي  
South Valley University



# INDUSTRIAL MICROBIOLOGY

FOR 2<sup>nd</sup> YEAR STUDENTS  
FACULTY OF SCIENCE



*BY*

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*2022- 2023*

## GENERAL INTRODUCTION

### Industrial Microbiology/ Biocatalysts

Industrial Microbiology mean use of microorganisms under control conditions for production of useful materials with high economically values. 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: -

- a) Employing microorganisms to produce a desired product.
- b) 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.

The main development for this work started during the twenty century when the bacteria were used for acetone production during the First World War, fungi and actinomycetes were used for antibiotic production during the Second World War.

Now a day there is various commercial products of economic value made by microbes are:

- 1- Medicines i.e. pharmaceuticals, including antibiotics, steroids, vaccines and vitamins.
- 2- Organic acids i.e. citric acid.
- 3- Enzymes i.e. amylase and protease.
- 4- Alcohols i.e. ethanol and methanol.

- 5- Amino acids i.e. glutamic acid, phenylalanine, aspartic acid, lysine and tryptophan.
- 6- Organic solvents.
- 7- Synthetic fuels.

**In addition to these**, quite recently potential of microbes could also be realized in:

- 8- Recovery of metals from ores through bioleaching.
- 9- Recovery of petrol.
- 10- Single cell protein production.

### **The requirements of microbial industries.**

The microbial industries depend on some requirements

#### **Industrial Microbes:**

Selective strains from algae, bacteria, moulds or yeast are using in microbial industries, 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.

The selective strain should keep its activity and purity by reinoculation to the proper medium at intervals. Then should be incubated till it reaches the stationary phase, then stored at low temperature enough to stop its growth. To avoid the possibilities of changes with repeat inoculation and cultivation of selective strain, a copy from this strain should be reserved for long times by using lyophilization. Due to the importance of the selective strains most companies consider it as industrial secrets.

### **Mash:**

The mash is the medium which used for fermentation, this medium should be:

- 1- Suitable for the microbe to produce the requested materials.
- 2- Cheap in price and available locally. For examples **molass** (byproduct for sugarcane manufacturing), **whey** (waste product from milk industries), **corn steep liquor** (waste product from starch industries) and **sulfite-liquor** (waste products from paper industries).

3- Mash is preferable to be selective to be suitable for growth of the requested organism more than competitive organisms. For the growth of yeast and moulds media should be acidic to be more selective.

**Starter:**

Starter is industrial microbe adds to the mash with 1-10% from the volume of the mash. Due to great volume of used mash, so stock culture should be prepared from starter. Starter requires 4-5 stages of multiplication and incubation under proper conditions till the requested quantity. These procedures require high accuracy to produce pure and active starter.

**Cultural conditions:**

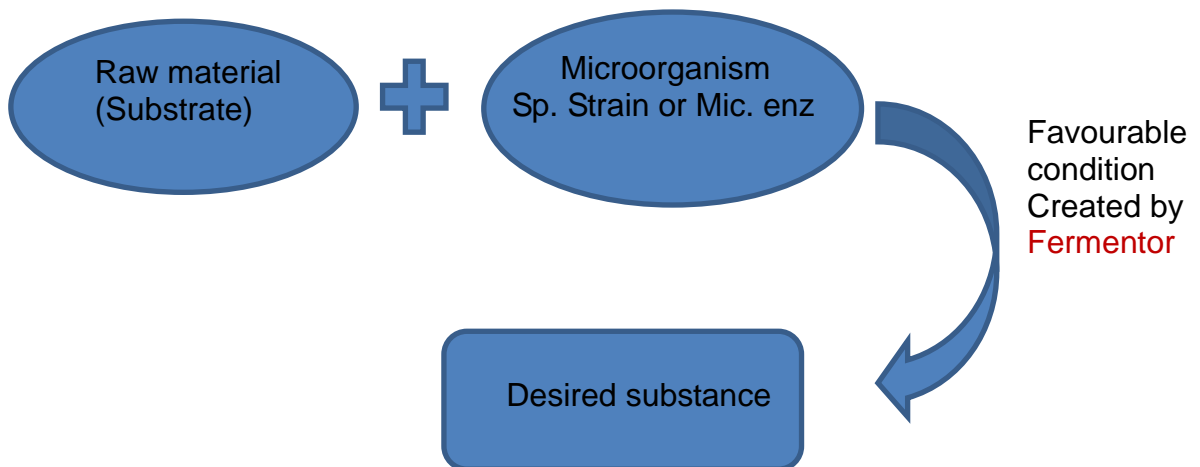
During the production all the suitable nutritional including nutrition elements and environmental conditions including pH, humidity, temperature and aeration (aerobic or anaerobic) should be available.

**Practical considerations:**

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

## Fermentation Processes

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 favorable environment (created in a fermentor) to produce the desired substance.



The cost of fermented product depends on several factors such as:

- 1- Cost of raw materials.
- 2- Cost of additives like enzymes and vitamins.
- 3- Cost of running the fermentation system.
- 4- Maintaining the inoculums.

- 5- Maintaining sterility of the medium.
- 6- Control of temperature, aeration and viscosity of the medium.
- 7- Time take for the fermentation cycle.

Constant check must be maintained on the quality of the inoculums because contamination by unwanted microorganisms, or alteration by mutations, reduces yields and complicates recovery of the products.

### **Mode of operation**

#### **A- Batch reactor**

This is the simplest type of reactor operation. In this mode, the reactor is filled with medium and the fermentation is allowed to proceed. When the fermentation has finished the contents are emptied for downstream processing. The reactor is then cleaned, re-filled, re-inoculated and the fermentation process starts again. All the ingredients required for fermentation prior to inoculation. The fermentation is run until the nutrients are exhausted, then the broth is harvested.

**The advantage:** is the simplicity of the operation. It is useful in the fermentation with high yield-per-unit substrate and with cultures that can tolerate high initial substrate concentrations.

**B- Fed batch reactor (semi continuous)**

Is the most common type of reactor used in industry? In this reactor, fresh media is continuous, or some time periodically added to bioreactor but unlike a continuous reactor, there is no continuous removal. The fermentor is emptied or partially emptied when reactor is full the fermentation is finished. As with the continuous reactors, it is possible to achieve high productivities since the growth rate of the cells can be optimized by controlling the flow rate of the fed entering the reactors.

Both batch and fed batch can be run in repeated mode, with small portion of the previous batch left in the fermentor for inoculum. The medium is then added through a continuous sterilizer. Use of the fermentor is increased by eliminating turnaround time, but the risks of contamination and genetic degradation of the culture are increased. In any case, repeated batch mode cannot be repeated indefinitely, due to maintenance and cleaning needs. Usually, repeated fermentations are run for two or three batches.

**The advantage:** are the ability to add large quantities of nutrients to the fermentor by adding them gradually and the ability to control the rate of nutrient addition.

- 1- This allows for high product concentrations without subjecting the culture to inhibition by high levels of nutrients.



- 2- It also allows for control of culture growth rate, which is required in some fermentations to maximize productivity and yield.

**The disadvantages are:**

- 1- Increased risk of contamination, due to the addition of nutrient through a continuous sterilizer.
- 2- Increased equipment costs for continuous sterilization and flow control equipment for feed streams

Over all, the use of the fermentor time is better in fed batch than straight batch fermentation, reducing fixed cost.

### **C- Continuous reactor**

Fresh media is continuously added, and bioreactor fluid is continuously removed. As a result, cells continuously receive fresh medium. Products, waste products, and the cells are continuously removed for the processing.

**The advantage:**

- 1- The reactor can thus be operated for long periods of the time without having to be shut down.
- 2- Continuous reactors can be many times more productive than batch reactors.

**The disadvantages:** Is increased risk of contamination, especially since it is difficult to keep contamination from growing through the continuous harvest line.

### **The bioreactor/ fermentor**

In the early days, fermentation technology was simple, for example, anaerobic fermentation was carried out in large vats or open wooden vessels to produce alcoholic beverages or solvents. With the expansion of food processing and pharmaceutical industries based on microorganisms, elaborate systems have been evolved. Fermentation vessels were designed to maintain conditions of sterility, aeration, pH, viscosity and temperature at optimum levels required for each system, these fermentation vessels known as bioreactor or fermentor.

**Bioreactor:** is 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- Non- aseptic systems where it is not essential to operate with entirely pure cultures (e. g. brewing, effluent disposal systems).
- 2- Aseptic conditions are essential for successful productivity (e. g. vitamins, polysaccharides). This type is more difficult to operate, maintain and design.

Structure of the bioreactor system has not changed for many years but recently, novel forms have been developed to suit the needs of specific bioprocesses.

**The essential guidelines for optimizing a bioreactor are:**

- 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 evaporation).
- 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.

**Within the bioreactor:**

- 1- Microorganisms are suspended in the aqueous nutrient medium.
- 2- All nutrients including oxygen must be provided to diffuse into each cell.
- 3- Waste products such as heat, CO<sub>2</sub> and waste metabolites removed.

**Fermentation reactions are multiphase consisting from three phases:**

- 1- Liquid phases (Mash or medium used for microorganism growing).
- 2- Solid microphase (Microorganism cells).
- 3- Gas phase (Oxygen and CO<sub>2</sub>).

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.

- 1- 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.

- 2- 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.

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 monitoring and control**

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 of 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<sub>2</sub> analysis.

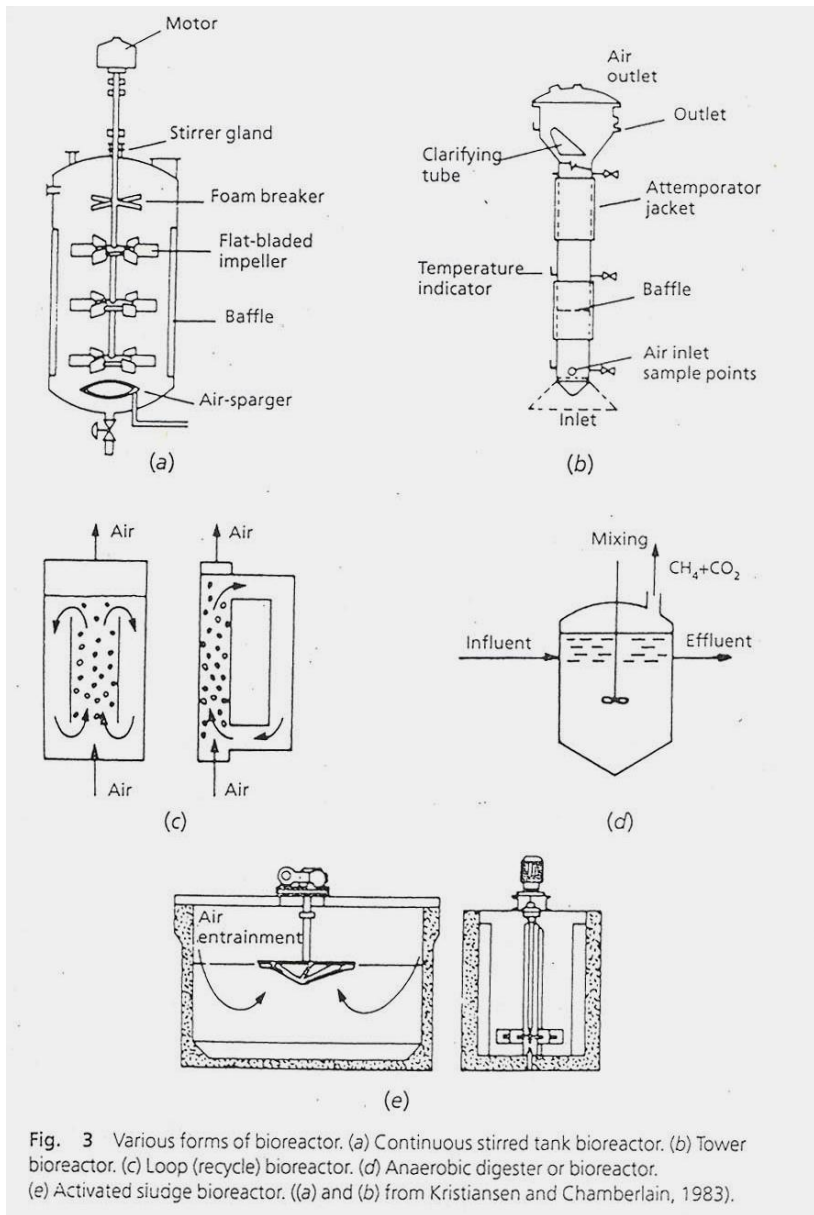


Fig. 3 Various forms of bioreactor. (a) Continuous stirred tank bioreactor. (b) Tower bioreactor. (c) Loop (recycle) bioreactor. (d) Anaerobic digester or bioreactor. (e) Activated sludge bioreactor. ((a) and (b) from Kristiansen and Chamberlain, 1983).

## Scaling- up

Scaling up is the process of expanding a process from small scale to a larger scale. So, here scaling up is defined as the conversion of a laboratory scale plant into a manufacturing unit. This process is the important step for transferring bench- top fermentations to mass production. 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, Erlenmeyer 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, and power). 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**

The extraction and purification of the end-product 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, methods used may include distillation, centrifugation, filtration, ultrafiltration, solvent extraction, adsorption, selective membrane technology, reverse osmosis, molecular sieves, electrophoresis and affinity chromatography. 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.

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

<b>Downstream processing operations</b>	
Methods	Examples
1- Separation	Filtration Centrifugation Flotation Disruption
2- Concentration	Solubilization Extraction Thermal processing Membrane filtration Precipitation
3- Purification	Crystallization Chromatography
4- Modification	
5- Drying	

### **Safety in biotechnology**

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.
- 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.

### **Problems of organism pathogenicity**

Many organisms can infect humans, animals and plants and cause disease. Symptoms result from successful interaction between the pathogenic organism and the host. Many factors are involved in the process, only a few of which are well understood.

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.

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.

A classification of the degree of potential hazard of microorganisms has been drawn up by the European Federation of Biotechnology. **Group E** contains those microorganisms that present risks only to the environment, particularly to animals and plants.

**Classification of microorganisms according to pathogenicity:**

<b>Class</b>	<b>Description</b>
<b>Class A</b>	Microorganisms that have never been identified as causative agent of disease in human and that offer no threat to the environment.
<b>Class B</b>	Microorganisms that may cause human disease and might therefore offer a hazard to laboratory workers. They are unlikely to spread in the environment. Prophylactics are available, and treatment is effective.
<b>Class C</b>	Microorganisms that offer a sever threat to the health of laboratory workers but a comparatively small risk to the population at large. Prophylactics are available, and treatment is effective.
<b>Class D</b>	Microorganisms that cause severe illness in human beings and offer a serious hazard to laboratory workers and to people at large. In general, effective prophylactics are not available and no effective treatment is known.
<b>Class E</b>	This group contains 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 are already in existence in contexts other than biotechnology (e.g. for phytosanitary purposes).

**Problems of biologically active biotechnology products**

Vaccines and antibiotics are obvious examples of biologically active products, and 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, and regulations have been constructed for that management.

**Safety and public acceptance of new biotechnology foods**

The public have a negative attitude to excessive manipulation of foods, in particular, to genetic engineering of foodstuffs. 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.

## DAIRY PRODUCTS

### Dairy Fermentation

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 color. 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. Primary microflora are fermentative lactic acid bacteria which cause the milk to curdle. During dairy product production, milk is first pasteurized to kill bacteria that cause unwanted spoilage of the milk and of the downstream milk 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 includes several different types of bacteria (*Leuconostoc*, *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 products 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. Thus, milk type and bacteria will determine the dairy product produced.

### **Cheese production:**

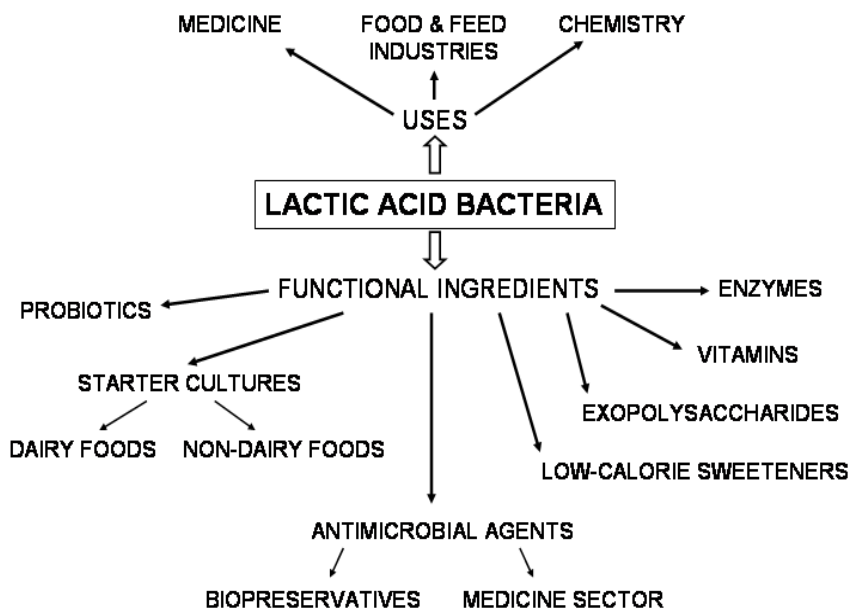
- In the past days, these fermentations were accidentally occurring by the natural presence of lactic acid bacteria. In the present time, an inoculum (of pure cultured bacteria) is added to the milk for obtaining the best final product.
- The early cheese production processes arisen from the use of animal stomachs (sheep) in which the milk is heated and soured by



naturally occurring bacteria and contaminated with the enzyme “*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 flavor in the fermented milk.
- 3- They have beneficial health effects on intestinal microflora.



**The uses of lactic acid bacteria**

Cheese is an important product of fermentative lactic acid bacteria. 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. Consequently, the art of cheese production has spread throughout Europe, each country manufacturing many different types of cheeses. 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.

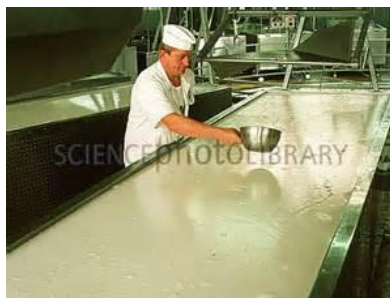
Recent biotechnological cheese productions involve the use of recombinant DNA techniques for chymosin production and commercial use.

In industry, cheese production has three major steps: curd formation, curd treatment and curd ripening.

1- **Curd formation** 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



Adding rennet



Curd collection

2- **Curd treatment** 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.



Draining and compressing curd



Flipping curd

3- **Curd treatment (Secondary microflora)** 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.

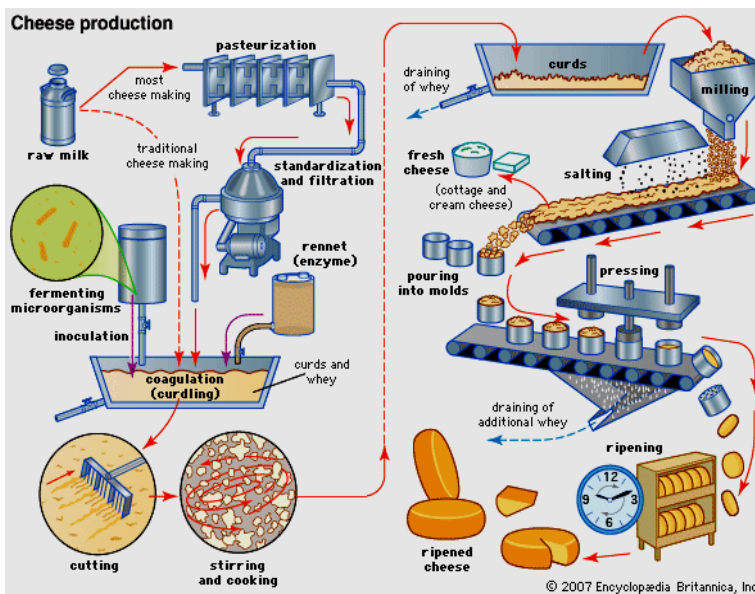


Unripened cheese



Cheese ripening

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



## 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.

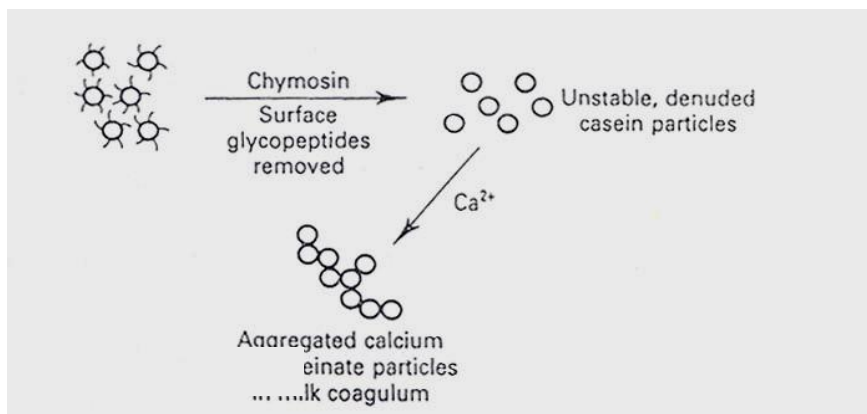
### **Kinds of Cheese**

The numerous cheeses (often named for their place of origin) depend on:-

- 1- The kind and condition of the milk used.
- 2- The processes of making.
- 3- The method and extent of curing.

They may be divided into two major classes, **Hard** cheeses, which improve with age under suitable conditions, and **soft** cheeses, intended for immediate consumption. **Very hard** cheeses include Parmesan and Romano. Among the hard cheeses are Cheddar, Edam, Emmental, Gouda, and Swiss. The semisoft cheeses include brick, Gorgonzola, Limburger, Roquefort, Muenster, and Stilton. Some of the soft cheeses are Brie, Camembert, cottage, and ricotta.

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



### Mode of action of Chymosin (rennet)

Table 1 *types of cheeses*

*Unripened cheeses*

Low fat (cottage cheese)

High fat (cream cheese)

*Ripened cheeses*

Hard cheese (internal ripening)

Ripened by bacteria (Cheddar, and Swiss cheese)

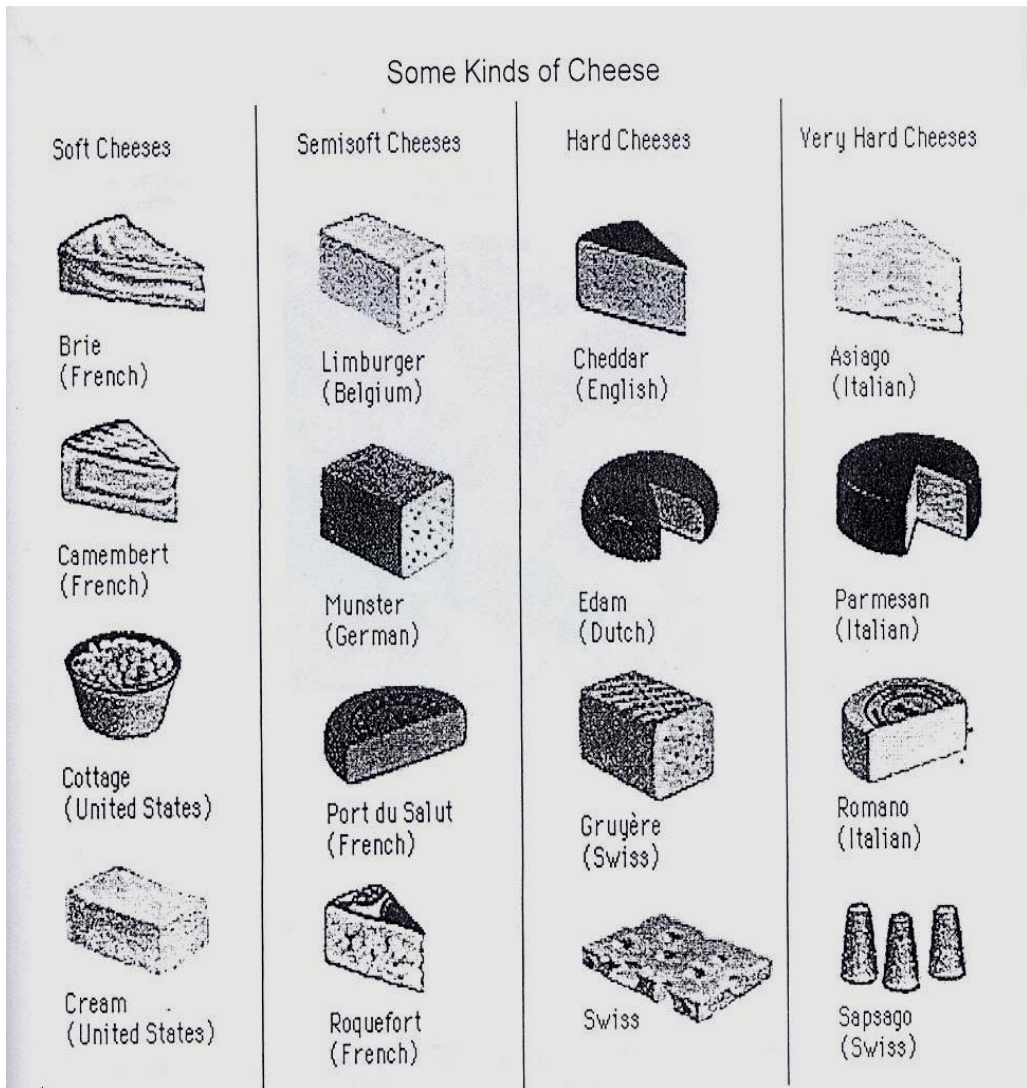
Ripened by mould (Roquefort and other blue cheeses)

Soft cheeses (ripening proceeds from outside)

Ripened by bacteria (Limburger)

Ripened by bacteria and moulds (Camembert)





## 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 cerevisiae*.

### **Bread**

Wheat, and several related grains, make a group of proteins called glutes. 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 flavor 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 flavoring 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<sub>2</sub> production).
  - 2- **Flavor 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.



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 ordinary storage, and its ability to produce desirable flavor.

- 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 yeasts play detrimental roles in industry, particularly as spoilage yeasts in food and beverage production.

Table. Some industrial products of yeast

Commodity	Examples
Beverages	Potable alcoholic beverages: beer, wine, cider, saké, distilled spirits (whisky, rum, gin, vodka, cognac)
Food and animal feed	Baker's yeast, yeast extracts, fodder yeast and livestock growth factor, feed pigments
Chemicals	Fuel ethanol, carbon dioxide, glycerol, citric acid vitamins; yeasts are also used as bioreductive catalysts in organic chemistry
Enzymes	Invertase, inulinase, pectinase, lactase, lipase
Recombinant proteins	Hormones (e.g. insulin), viral vaccines (e.g. hepatitis B vaccine), antibodies (e.g. IgE receptor), growth factors (e.g. tumor necrosis factor), interferons (e.g. leucocyte interferon- $\alpha$ ), blood proteins (e.g. human serum albumin), enzymes (e.g. gastric lipase)

## ETHANOL PRODUCTION

Yeast can convert sugar into ethanol using biotechnological methods, which has various applications including ethanol fuel.

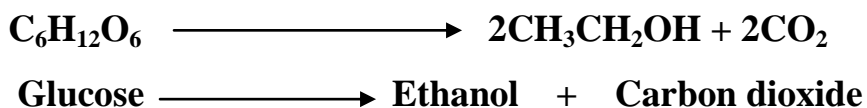
The process: -

- Starts by milling a feedstock, such as sugar cane, sweetcorn, or cheap cereal grains.
- 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.

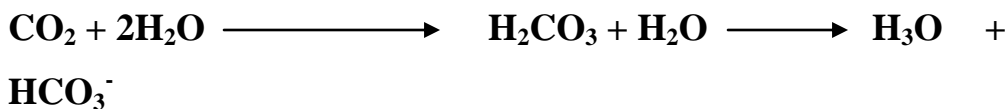
*Saccharomyces* yeasts have been genetically engineered to ferment xylose, one of the major fermentable sugars present in cellulosic biomasses, such as agriculture residues, paper wastes, and wood chips. Such a development means that ethanol can be efficiently produced from more inexpensive feedstocks,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

All living organisms obtain the energy necessary to sustain life, from the oxidation of organic substances by molecular oxygen, in the process of respiration. Under anaerobic conditions (low oxygen concentrations), many organisms, including yeast, obtain

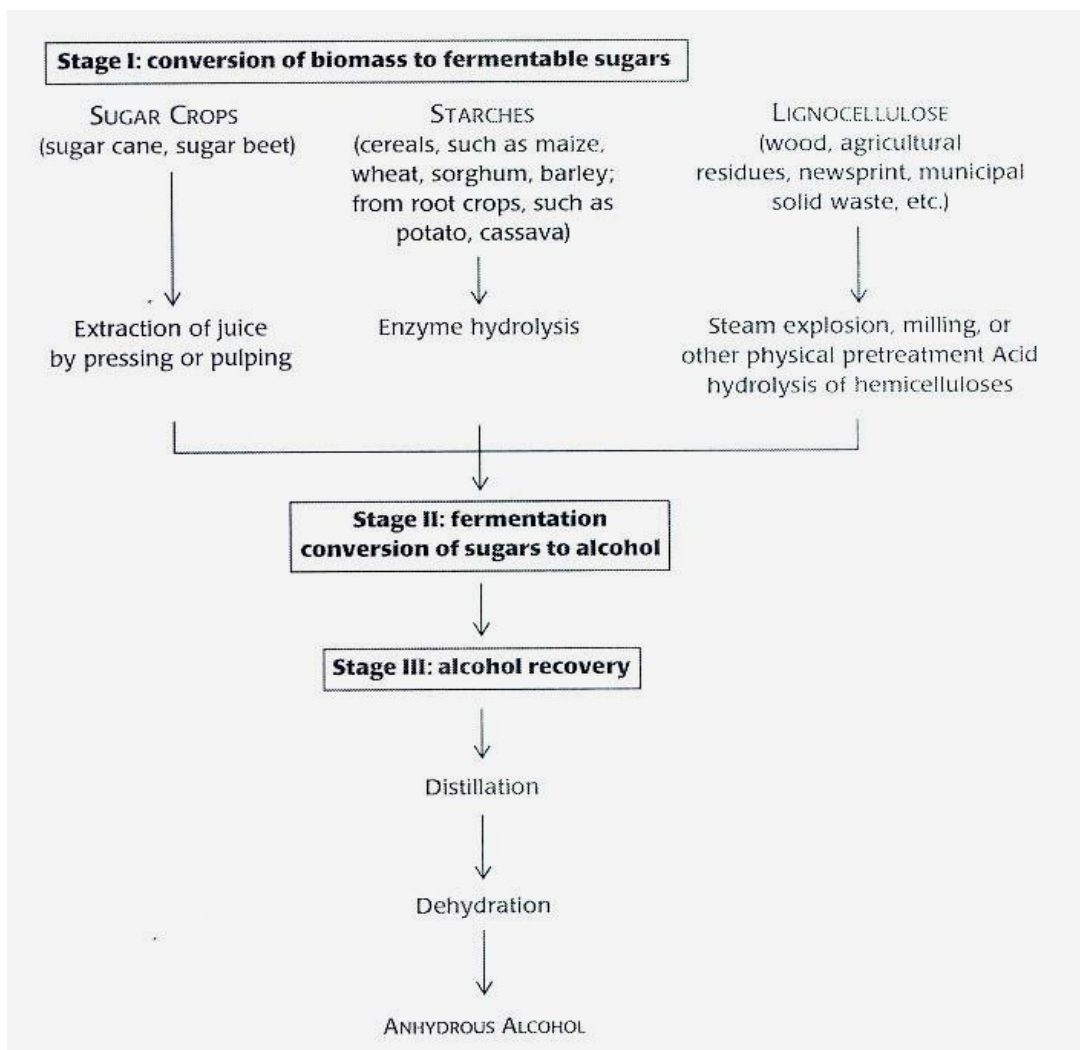
the energy from the process of fermentation. In alcoholic fermentation, characteristic of many yeast species, the fermentation process starts with one molecule of the six-carbon sugar- glucose, and terminates with two molecules of the two-carbon alcohol - ethanol, and two molecules of CO<sub>2</sub>:



The CO<sub>2</sub> released in the process, dissolves in water and form a carbonic acid. This acid dissociates to form hydrogen carbonate and hydronium ions:



In acidic solutions, the dissolution of CO<sub>2</sub> in water decreases then released to the air.



### Stages of alcohol production from different sources

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). Is the energy present in the alternative fuel greater than the nonrenewable



energy used in producing the fuel? 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, with values ranging from 1.10 to 1.34. 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 soluble), corn oil, corn gluten meal, and corn gluten feed. The CO<sub>2</sub> released during the fermentation is captured and sold for carbonating beverages and the manufacture of dry ice.

Yeast or Bacteria	Substrates
<b>Yeast</b>	
<i>Saccharomyces</i> spp.	
<i>S. cerevisiae</i>	Glucose, fructose, galactose, maltose, maltotriose, xylulose
<i>S. carlsbergensis</i>	Glucose, fructose, galactose, maltose, maltotriose, xylulose
<i>S. rouxii</i> (osmophilic)	Glucose, fructose, maltose, sucrose
<i>Kluyveromyces</i> spp.	
<i>K. fragilis</i>	Glucose, galactose, lactose
<i>K. lactis</i>	Glucose, galactose, lactose
<i>Candida</i> spp.	
<i>C. pseudotropicalis</i>	Glucose, galactose, lactose
<i>C. tropicalis</i>	Glucose, xylose, xylulose
<b>Bacteria</b>	
<i>Zymomonas mobilis</i>	Glucose, fructose, sucrose
<i>Clostridium</i> spp.	
<i>C. thermocellum</i> (thermophilic)	Glucose, cellobiose, cellulose
<i>C. thermohydrosulfuricum</i> (thermophilic)	Glucose, xylose, sucrose, cellobiose, starch
<i>Thermoanaerobium brockii</i> (thermophilic)	Glucose, sucrose, maltose, lactose, cellobiose, starch
<i>Thermobacterioides acetoethylicus</i> (thermophilic)	Glucose, sucrose, cellobiose

Some yeast and bacteria that produce good quantities of ethanol and their substrates

## VINEGAR

Vinegar is an alcoholic liquid that has been allowed to sour. It is primarily used to flavor and preserve foods and as an ingredient in salad dressings and marinades. Vinegar is also used as a cleaning agent. The word is from the French *vin* (wine) and *aigre* (sour).

The transformation of wine or fruit juice to vinegar is a chemical process in which ethyl alcohol undergoes partial oxidation that results in the formation of acetaldehyde. In the third stage, the acetaldehyde is converted into acetic acid. The chemical reaction is as follows:



### Raw Materials

- 1) Vinegar is made from a variety of diluted alcohol products, the most common being wine, beer, and rice. Balsamic vinegar is made from the Trebbiano and Lambrusco grapes of Italy's Emilia-Romagna region. Some distilled vinegars are made from wood products such as beech.
- 2) Acetobacters are microscopic bacteria that live on oxygen bubbles. Whereas the fermentation of grapes or hops to make wine or beer occurs in the absence of oxygen, the process of making vinegars relies on its presence. In the natural processes, the acetobacters are allowed to grow over time. In the vinegar factory, this process is induced by feeding acetozym nutrients into the tanks of alcohol.

There are many ways to produce vinegars. These includes the Orleans process, the quick vinegar method and the natural spontaneous fermentation.

### **Natural Fermentation**

It can be made easily by fermenting fresh sap into plastic or earthen jar until it becomes sour. Then pack into plastic bottles and place under the heat of sun for few days. The very common package is a used 1.5 liters Coke bottle. Sugar palm and coconut sap are common examples.



### **The Orleans process**

The Orleans process is one of the oldest and well-known methods to produce vinegar. It is a slow, continuous process, which originated in France. High grade vinegar is used as a starter culture, to which wine is added at weekly intervals. The vinegar is fermented in large (200 litre) capacity barrels. Approximately 65

to 70 litres of high-grade vinegar is added to the barrel along with 15 litres of wine. After one week, a further 10 to 15 litres of wine are added and this is repeated at weekly intervals. After about four weeks, vinegar can be withdrawn from the barrel (10 to 15 litres per week) as more wine is added to replace the vinegar.

One of the problems encountered with this method is that of how to add more liquid to the barrel without disturbing the floating bacterial mat. This can be overcome by using a glass tube which reaches to the bottom of the barrel. Additional liquid is poured in through the tube and therefore does not disturb the bacteria. Wood shavings are sometimes added to the fermenting barrel to help support the bacterial mat.



### **The submerged fermentation method**

1. The submerged fermentation method is commonly used in the production of wine vinegars. Production plants are filled

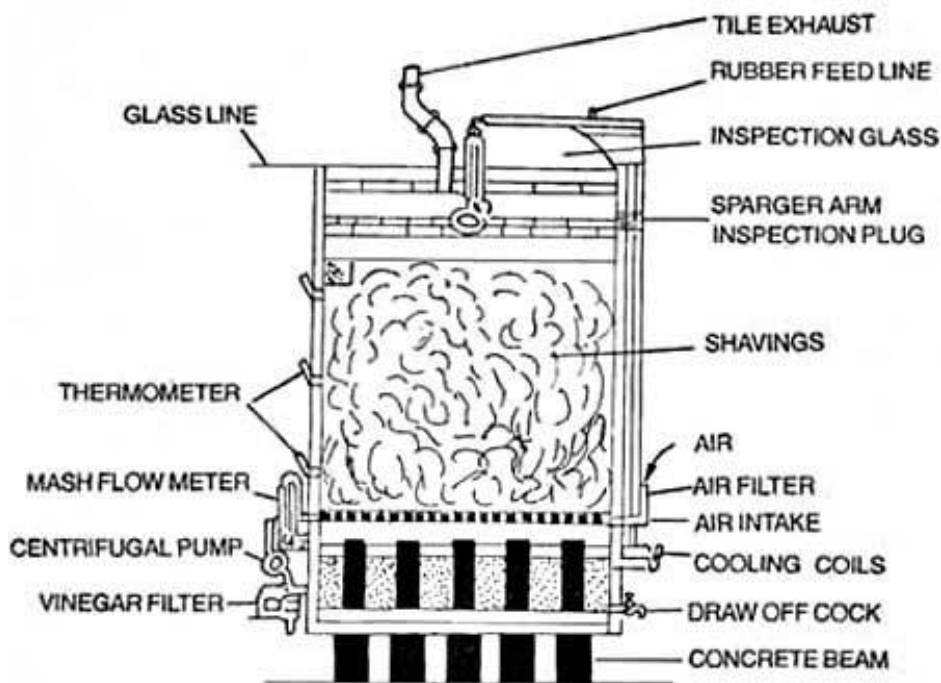
with large stainless-steel tanks called acetators. The acetators are fitted with centrifugal pumps in the bottom that pump air bubbles into the tank in much the same way that an aquarium pump does.

2. As the pump stirs the alcohol, acetozy m nutrients are piped into the tank. The nutrients spur the growth of acetobacters on the oxygen bubbles. A heater in the tank keeps the temperature between 80 and 100°F (26-38°C).
3. Within a matter of hours, the alcohol product has been converted into vinegar. The vinegar is piped from the acetators to a plate-and-frame filtering machine. The stainless-steel plates press the alcohol through paper filters to remove any sediment, usually about 3% of the total product. The sediment is flushed into a drain while the filtered vinegar moves to the dilution station.

### **Quick vinegar method**

Because the prvious process is slow, other methods have been adapted to try and speed up the process. The German method is one such method. It uses a generator, which is an upright tank filled with beechwood shavings and fitted with devices which allow the alcoholic solution to trickle down through the shavings in which the acetic acid bacteria are living. The tank is not allowed to fill as that would exclude oxygen which is necessary

for the fermentation. Near the bottom of the generator are holes which allow air to be drawn in. the air rises through the generator and is used by the acetic acid bacteria to oxidise the alcohol. This oxidization also releases considerable amounts of heat which must be controlled to avoid causing damage to the bacteria.



pencil sketch courtesy of studentguide.in

## 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.



Cultures of some bacteria used as biofertilizers

## CITRIC ACID

Citric acid is 2-hydroxy 1,2,3 propane tricarboxylic acid ( $\text{CH}_2\text{COOH}.\text{COH}.\text{COOH}.\text{CH}_2\text{COOH}$ ), widely used in the food related industries in fruit drinks, jams, preserved fruits. Citric acid is solid at room temperature. It is responsible for the tart taste of various fruits in which it occurs, *i.e.* lemons, limes, figs, oranges, pineapples, pears and goose-berries.

Many microorganisms such as fungi and bacteria can produce citric acid. The various fungi, which have been found to accumulate citric acid in their culture media, include strains of



*Aspergillus niger*, *A. awamori*, *Penicillium restrictum*, *Trichoderma viride*, *Mucor piriformis* and *Yarrowia lipolytica*. But *Aspergillus niger* remained the organism of choice for the production of citric acid.

Molasses is a desirable raw material for citric acid fermentation because of its availability and relatively low price. Over 100000 tons of citric acid are manufactured annually by fermentation processes involving *Aspergillus niger* and molasses as substrate. The fermentation can be as static liquid surface in trays or in deep-tank large scale bioreactors.

Incubation temperature plays an important role in the production of citric acid. Temperature between 25-30 °C was usually employed for culturing of *Aspergillus niger* but temperature above 35 °C was inhibitory to citric acid formation. Citric acid production by *Aspergillus niger* is sensitive to the initial pH of the fermentation medium the maximum production of citric acid was obtained at pH 5.4 in molasses medium.

## 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 tons 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

## PENICILLIN

Refers to a group of  $\beta$ -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.

It is manufactured by growing *Penicillium chrysogenum* on a medium containing a carbon source, growth factors and necessary complement of mineral salts. Although glucose is a useful carbon source for promoting biomass production it is too readily assimilated.

Corn-steep liquor still provides the main source of growth factors for penicillin fermentation. A variety of environmental factors influence penicillin production, optimal pH for production is between 6.8 and 7.7 depending on strain type. The temperature range used for high-yielding strain is 25-27 °C. The fermentor currently used in penicillin production range in volume from 10000 to 20000 liters.

## CEPHALOSPORINS

Some strains of *Cephalosporium (Acremonium)* produce a mixture of antibiotics which are loosely referred to as cephalosporins.

There are three types of cephalosporins namely cephalosporin N, P and C which consider the parent compound for all antibiotics which we now call cephalosporins.

Like penicillin, cephalosporins are produced by batch culture, with glucose as carbon source.

### GRISEOFULVIN

It is the only clinically useful antifungal antibiotics which produced by fungi, being produced by *Penicillium griseofulvin*. It is essentially fungistatic rather than fungicidal, and because of its low toxicity and ability to accumulate in the skin, hair and nails following oral administration, it is used to control superficial dermatophytosis such as ringworm and favus.

Production of griseofulvin is achieved using a medium containing lactose, corn steep liquor, calcium carbonate and potassium dihydrogen phosphate.

### 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.

Interferon was scarce and expensive until 1980 when the interferon gene was inserted **into bacteria using recombinant DNA technology**, allowing mass cultivation and purification from

bacterial cultures. 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. When used in the systemic therapy, IFN- $\alpha$  and IFN- $\gamma$  are mostly administered by an intramuscular injection. 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.

Administered intranasally in very low doses, interferon is extensively used in Eastern Europe and Russia as a method to prevent and treat viral respiratory diseases such as cold and flu. It is claimed that the treatment can lower the risk of infection by as much as 60-70%. Mechanisms of such action of interferon are not well understood; it is thought that doses must be larger by several orders of magnitude to have any effect on the virus. Consequently, most Western scientists are sceptical of these claims.

## 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 microbial products in oil recovery

Microbial product	Role in enhanced oil recovery	Some of the effects
Gases (H <sub>2</sub> , N <sub>2</sub> , CH <sub>4</sub> , CO <sub>2</sub> )	<ul style="list-style-type: none"> <li>• Reduce oil viscosity and improve flow characteristics</li> <li>• Displace immobile</li> <li>• Sweep oil in place</li> </ul>	<ul style="list-style-type: none"> <li>• Improved oil recovery by gases</li> <li>• Miscible CO<sub>2</sub> flooding</li> </ul>
Acids (low molecular weight acids, primarily low molecular weight fatty acids)	<ul style="list-style-type: none"> <li>• Improve effective permeability by dissolving carbonate precipitates from pores throat. Significant improvement of permeability and porosity</li> <li>• CO<sub>2</sub> produced from chemical reactions between acids and carbonate reduce oil viscosity and causes oil droplet to swell</li> </ul>	<ul style="list-style-type: none"> <li>• Enhanced oil flooding</li> </ul>
Solvents (alcohols and ketones that are typical cosurfactants)	<ul style="list-style-type: none"> <li>• Dissolve in oil reduce viscosity</li> <li>• Dissolve and remove heavy, long chain hydrocarbons from pore throat (increase effective permeability)</li> <li>• Involved in stabilizing and lowering interf. tension that promotes emulsification</li> </ul>	<ul style="list-style-type: none"> <li>• Emulsification promotion for increased miscibility</li> </ul>
Biosurfactants	<ul style="list-style-type: none"> <li>• Reduce interfacial tension</li> <li>• Reduce interfacial tension between oil and rock/water surface which causes emulsification, improving pore scale displacement</li> <li>• Alter wettability</li> </ul>	<ul style="list-style-type: none"> <li>• Microbial surfactant</li> <li>• Flooding</li> </ul>
Biopolymers	<ul style="list-style-type: none"> <li>• Improve the viscosity of water in waterflooding and direct reservoir fluids to previously unswept areas of the reservoir</li> <li>• Improve the sweep efficiency of waterflood by plugging high permeability zones or water-invaded zones</li> <li>• Control of water mobility</li> </ul>	<ul style="list-style-type: none"> <li>• Microbial permeability modification (selective plugging)</li> </ul>
Biomass (microbial cells)	<ul style="list-style-type: none"> <li>• Physically displace oil by growing between oil and rock/water surface</li> <li>• Reversing wettability by microbial growth</li> <li>• Can plug high permeability zones</li> <li>• Selective partial degradation of whole crude oil</li> <li>• Act as selective and nonselective plugging agents in wetting, alteration of oil viscosity, oil power point, desulfuration</li> </ul>	<ul style="list-style-type: none"> <li>• Same biopolymers</li> </ul>

<sup>a</sup>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.

## **FOOD WASTE BIODEGRADATION AND 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 to partially decomposed meat scraps.

**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 through 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 to amend soil 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 environment, agriculture and food industry are summarized in the following.

### **Benefits to environment**

- 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.
- Composted manure weights about one-fourth as much as raw manure per ton.

**Benefits to the agriculture**

- 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 using 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.

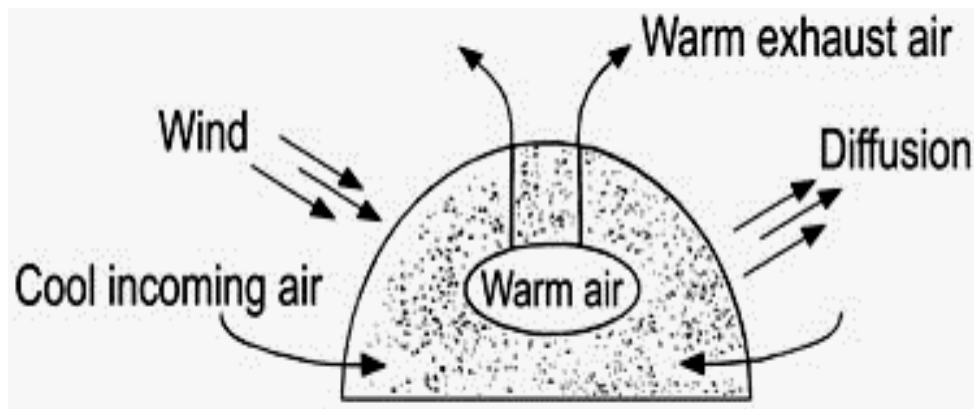
- Minimizes odors from agricultural areas.

### **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 reliable 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.

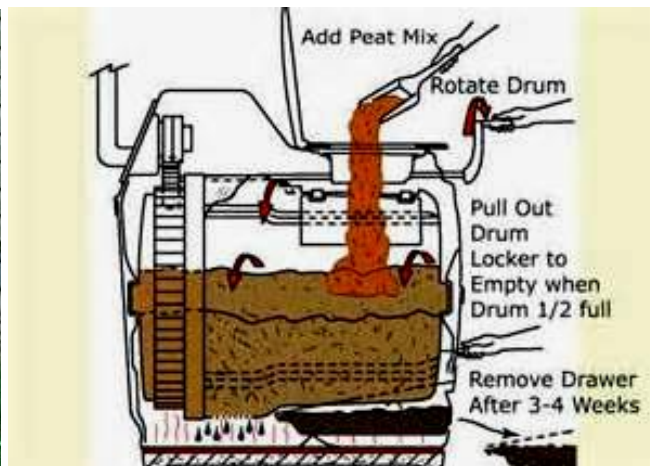


**Windrows in the field**

**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.



**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.

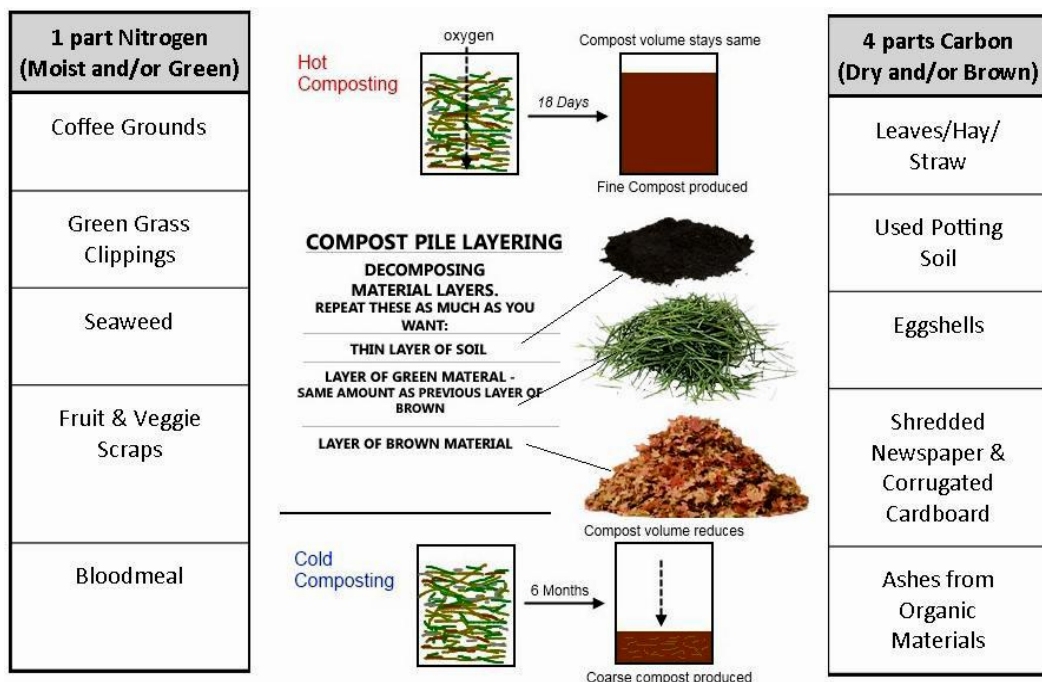


**Vermi-composting** uses worms to consume the food waste and utilizes its castings as high-quality compost. This is usually done in containers, bins, or greenhouses. Typically, 1 pound of worms can eat 4 pounds waste per week. Many schools in the developed countries use this type



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 for good composting:**

**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, and 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. Mesophilic bacteria function between 50 and 113 degrees F to begin the composting process. Thermophilic 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 like humus in appearance, smell, and touch. The finished compost will no longer heat on its 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**

## Microorganisms

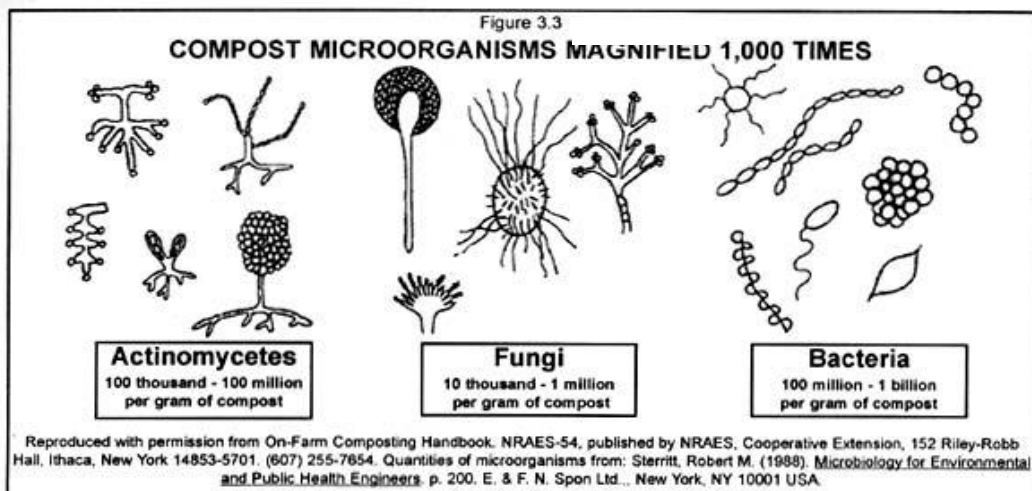


Table 3.6  
MICROORGANISMS IN COMPOST

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

Source: Palmisano, Anna C. and Bariaz, 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 applications 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 as part of an organic matter management system.

Compost can be used to increase pasture quality in intensively managed grazing systems. Compost can also be used as 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.



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## **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  $2 \times 10^{11}$  tonnes of carbon with an energy content of  $2 \times 10^{21}$  joules, representing about 10 times the world's annual energy use and 200 times our food energy consumption. 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 considered as a renewable energy source and 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.



**Biomass from plants**



**Biomass resources**

### **Sources of biomass**

There are three main directions to obtain biomass supply:

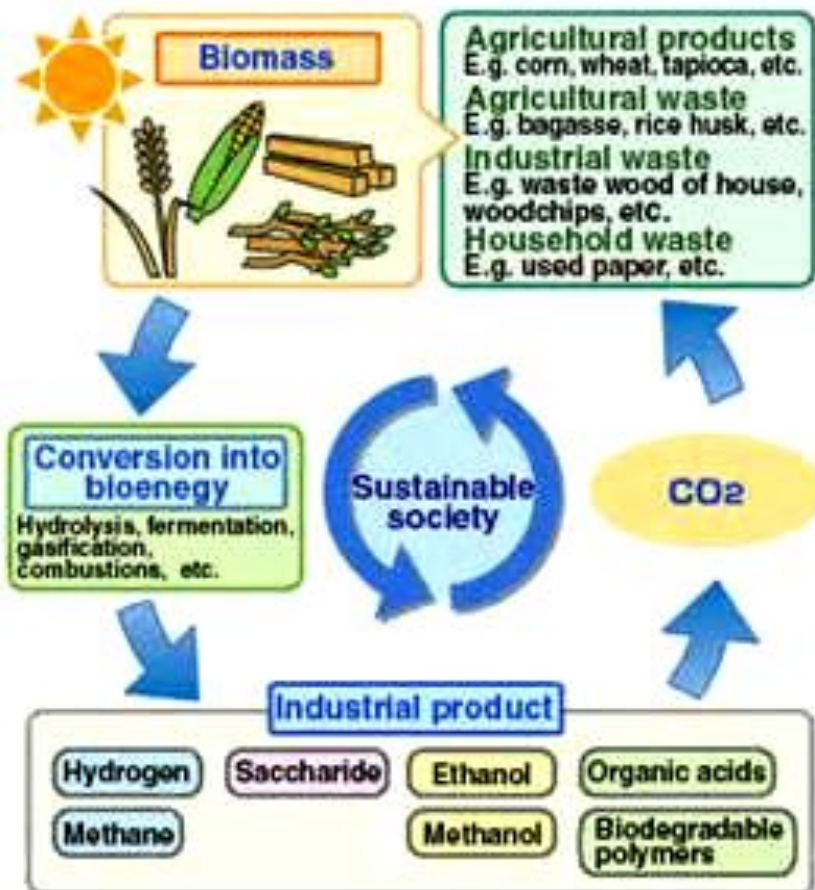
- 1) Cultivation of energy crops.
- 2) Harvesting natural vegetation.
- 3) Utilization of agricultural and other organic wastes.

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 vegetation.

The technical processing of the biomass depends on many factors, including:

- 1) Moisture level.
- 2) Chemical complexity.
  - 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.
  - 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



The present industrial production of ethanol is now depending on non- microbial processes (petrochemical) by the hydration of ethylene. In general, this is carried out in the developed world but, on the other hand, in many developing countries, where raw material is still abundant, the process is depending on microbial fermentation.

The Brazilian program is almost based on **batch fermentation** system. In developed countries, progress in **continuous**

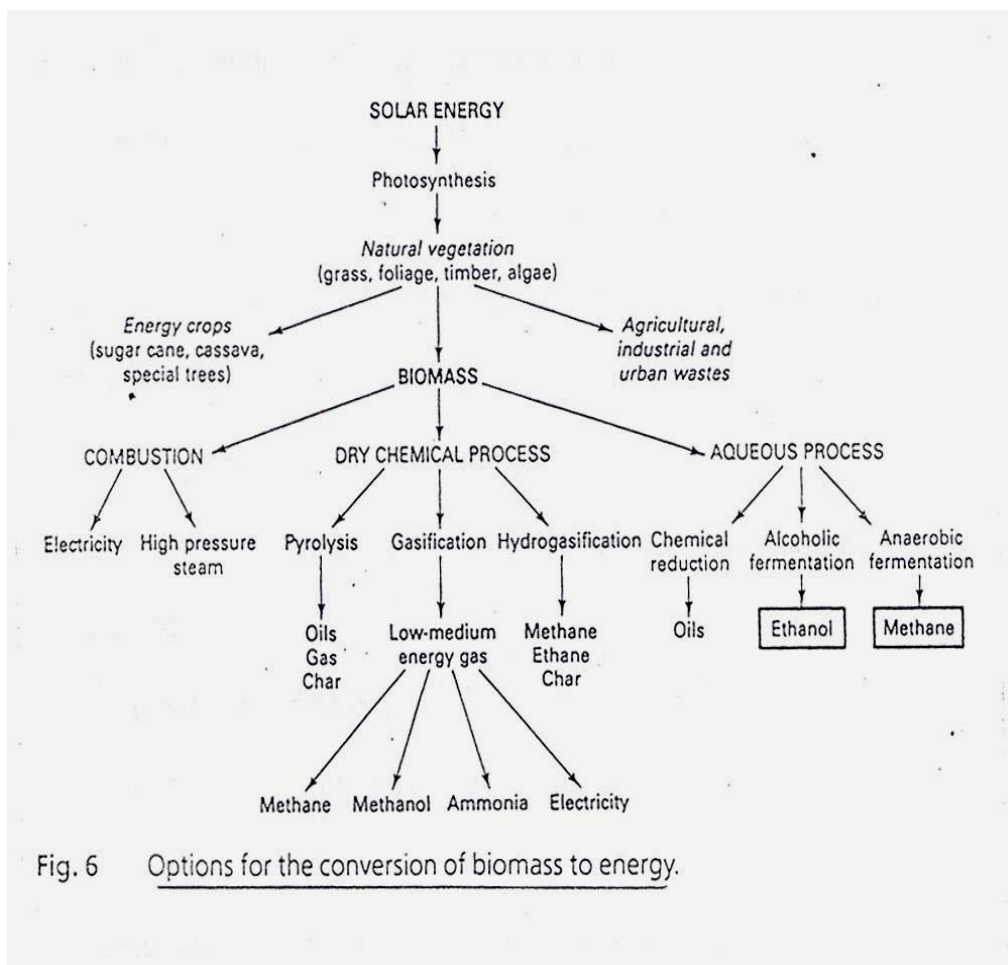
**fermentation** was achieved utilizing various approaches such as retention of the yeast cells in the bioreactor by separation, recycling and continuous evaporation of the fermentation broth.

Table 1.1 *Various raw materials for fuel ethanol production*

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

Note:

<sup>a</sup> Meal from parched corn, often with the hull and germ removed.

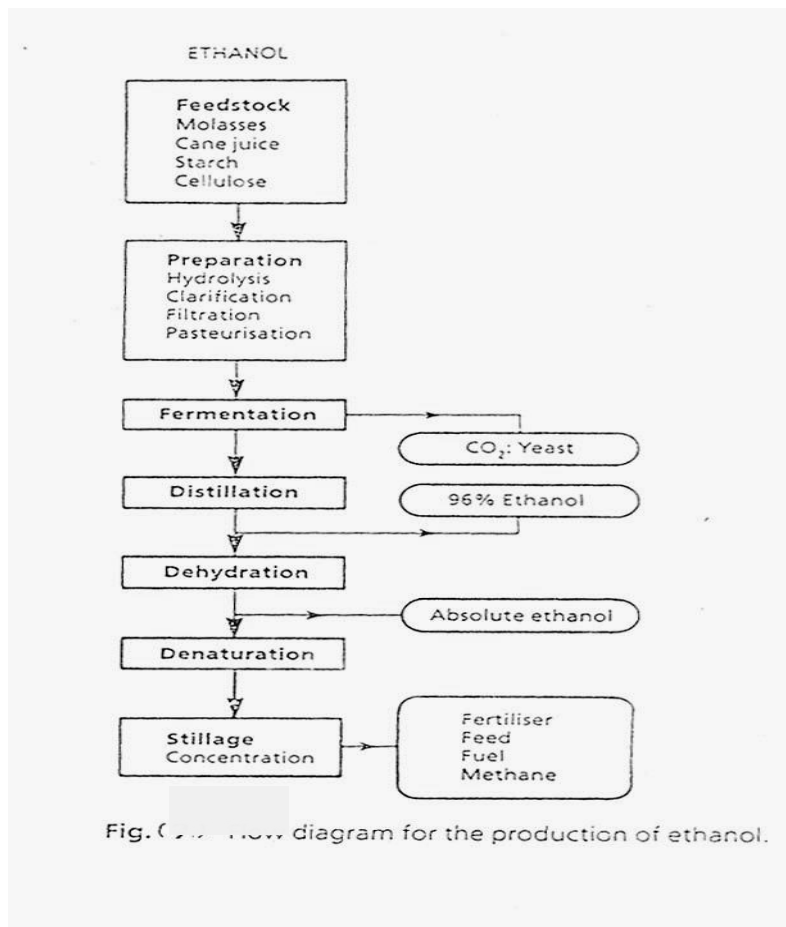


### **Indirect economic advantages of ethanol production from crops in the developing countries:**

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



- b) Mineralization to ash.
- c) Anaerobic fermentation to produce methanol.
- d) Conversion by microorganisms into single cell protein.



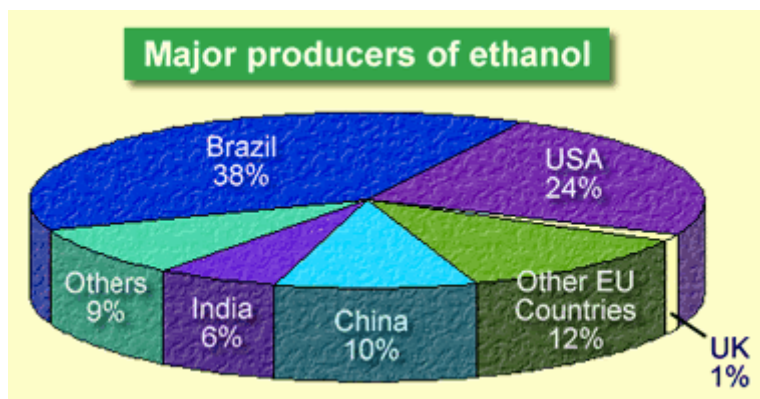
**Biodiesel** is another biofuel made by combining alcohol (usually methanol) with vegetable oil, animal fat, or recycled cooking grease. It can be used as an additive (typically 20%) to reduce vehicle emissions or in its pure form as a renewable alternative fuel for diesel engines.

In general, biofuels can reduce costly petroleum imports, cut greenhouse gas emissions, increase farm income, and boost rural development.

Conventional ethanol is made from sugar cane, corn, and sweet sorghum. Soybean and rapeseed oil are often used to make biodiesel, but coconut, palm, canola and jatropha nut oil are also being used throughout the world.

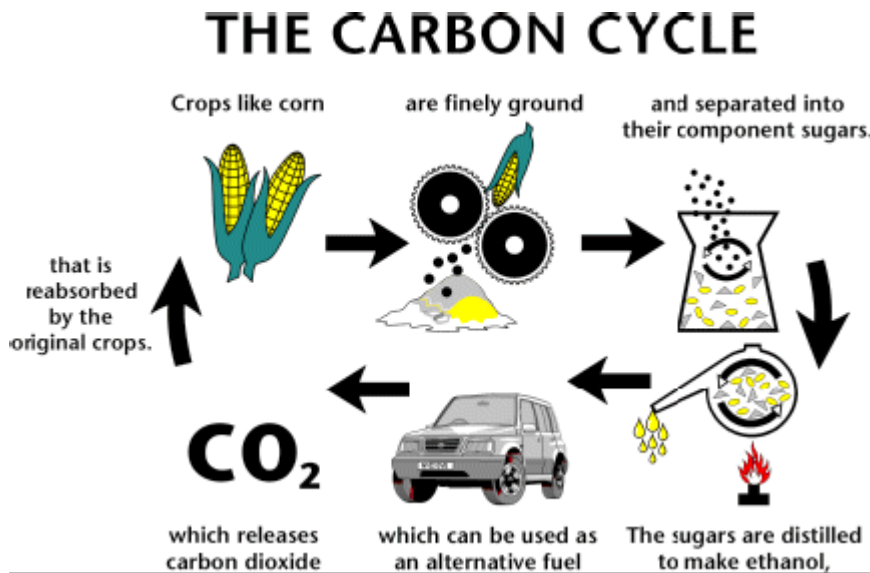
Trees, grass, agricultural residue, and municipal solid waste can also be converted into biofuels. Recent research is making this process less expensive and more energy efficient.

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**

- 1- 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.
- 2- 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.
- 3- 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 (for example E10 fuel). 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. To achieve this, the two most common strategies are:

- ❖ To grow sugar crops (sugar cane, and sugar beet), or starch (corn/maize), and then use yeast fermentation to produce ethanol (ethyl alcohol).
- ❖ To grow plants that (naturally) produce oils, such as algae, or jatropha. When these oils are heated, their viscosity is reduced, and they can be burned directly in a diesel engine.

The oils can also be chemically processed to produce biodiesel.

### **Second generation biofuels**

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. Many second generation biofuels are under development such as biohydrogen, biomethanol, DMF (dimethylfuran), Bio-DME, Fischer-Tropsch diesel, biohydrogen diesel, mixed alcohols and wood diesel.

### **Third generation biofuels**

**Algae fuel**, also called **oilgae** or **third generation biofuel**, is a biofuel from algae. Algae are low-input/ high-yield (30 times more energy per acre than land) and are biodegradable

- 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 15,000 square miles (38,849 square kilometers), which is a few thousand miles larger than Maryland state.

### **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.

## **Methane from biomass**

The uses of methane gas are numerous including:

- 1- Generation of mechanical, electrical and heat energy.
- 2- As a fuel source for domestic and industrial purposes (many countries such as India use methane for these purposes through national gas pipelines).
- 3- Can be converted to methanol and as fuel for internal combustion engines.

Methane gas exists in the atmosphere and is derived mainly from microbial action in natural wetlands, rice paddies and enteric fermentation in animals contributing about 20, 20 and 15%, respectively of the total methane flux.

Domestic cattle are the major contributors, producing about 75% of all animal emissions, whereas humans produce about 0.4%.

After CO<sub>2</sub>, methane is considered the next most important greenhouse gas and is expected to contribute 18% of future warming.

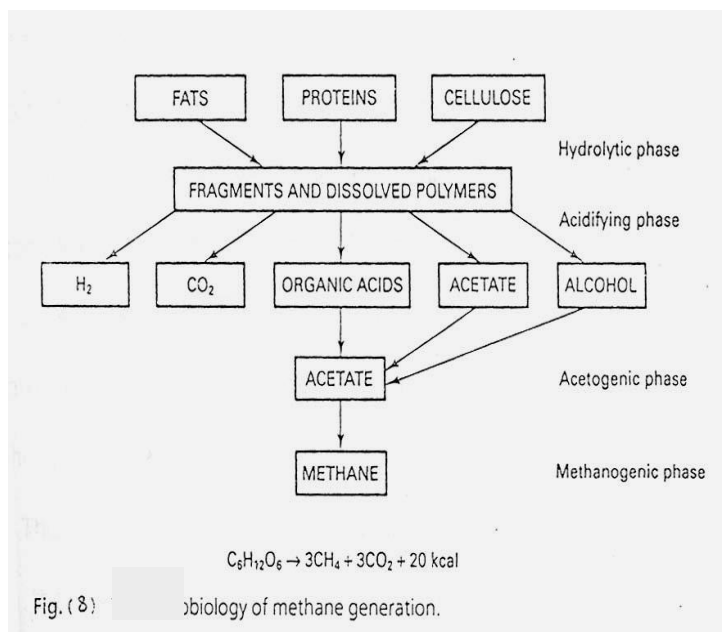
The microbial process is complex, involving mixture of anaerobic microorganisms. The process of anaerobic fermentation proceeds through three main biochemical phases:

- 1- An initial stage requiring the solubilization of complex molecules such as cellulose, fats and proteins that make most of the raw organic matter.

- 2- The resulting soluble, low molecular weight products are converted to organic acids.
- 3- These organic acids (primarily acetic) are specifically decomposed by the methanogenic bacteria to methane and CO<sub>2</sub>.

There are several ways for economical production of methane

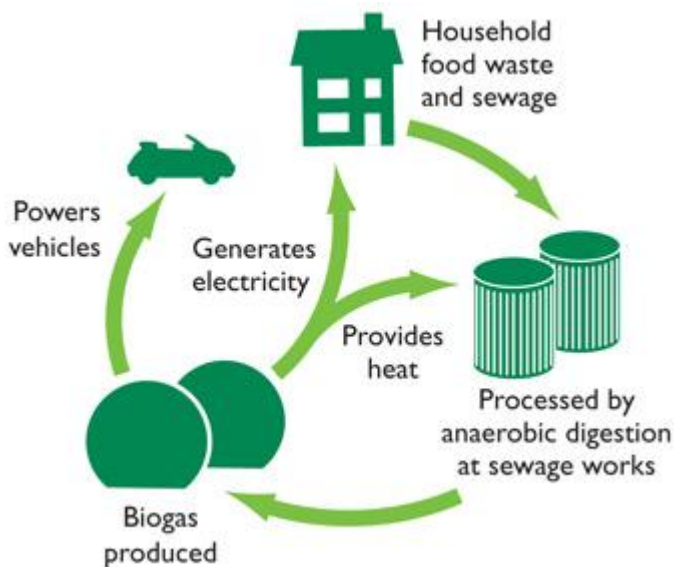
- From sewage.
- From agricultural and urban wastes.
- In biogas reactors.





### A- Production from sewage

The anaerobic digestion of sewage is a long- practiced technique and many municipal systems have devised methods of capturing methane and returning the energy for the needs of the sewage plant. The energy returns are modest and large- scale expansion is not promising.



### B- Production from agricultural and urban wastes

This is a profitable way to generate energy, but the process is complex and subjected to many limitations.

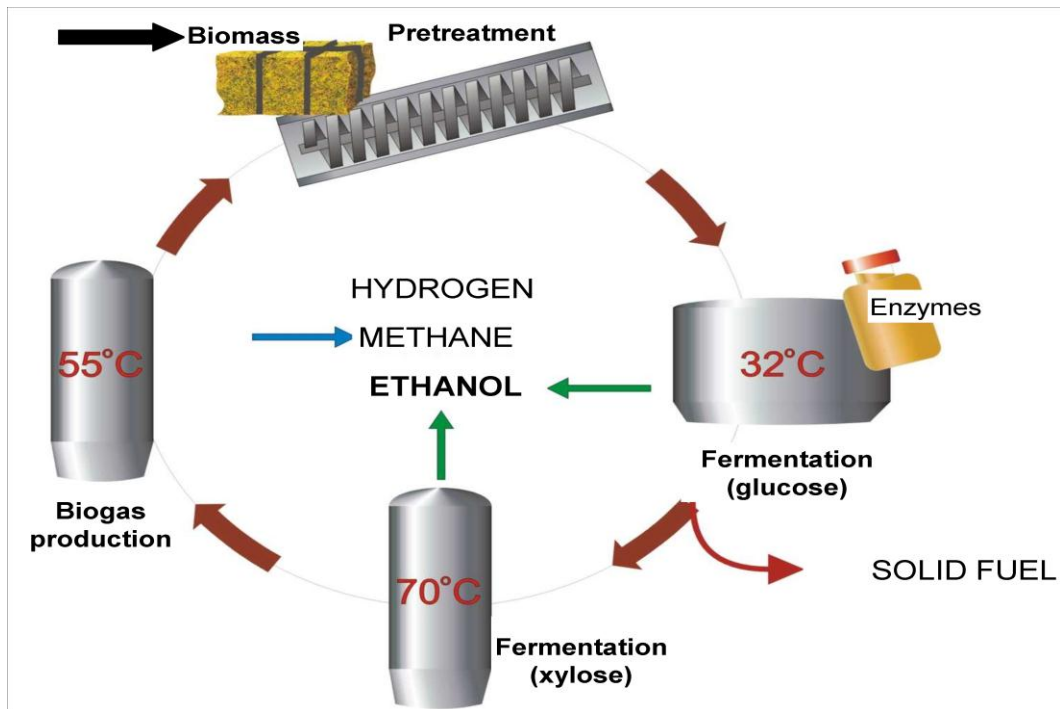
It is possible to convert 30- 50% of urban wastes' energy into methane and with certain vegetable materials and forages, this percentage should be raised to 70%.

**The other advantages of this process may include: -**

- 1- The use of the effluent (rich in ammonia, phosphates and microbial cells) as fertilizers, soil conditioners or animal feed.
- 2- During the process, the mal- odourous and pathogenic wastes may be converted into useful materials.

**Limitations for the process are as follows:**

- 1- The cost of collecting organic matter for methanogenesis is expensive.
- 2- The rate of methane production is low in most processes and much research needs to be carried out on the balance of nutrients for process optimization.
- 3- The presence of lignin in most types of agricultural and urban wastes is the major problem. This is because lignin is difficult to be digested by anaerobic processes. In case of using chemical and physical pre- treatment, this will require more energy and add more cost rendering the process non-economical.



*Economic arguments against large-scale methane production by microbial processes*

- An abundance of methane occurs in nature, particularly in natural gas fields and oil field overlays
- Methane production by gasification of coal is commercially more attractive
- Microbial production of methane is more expensive than natural gas
- Costs of storage, transportation and distribution of gaseous fuels is not yet economically worth while
- Methane cannot be used in automobiles and is difficult and expensive to convert to liquid state

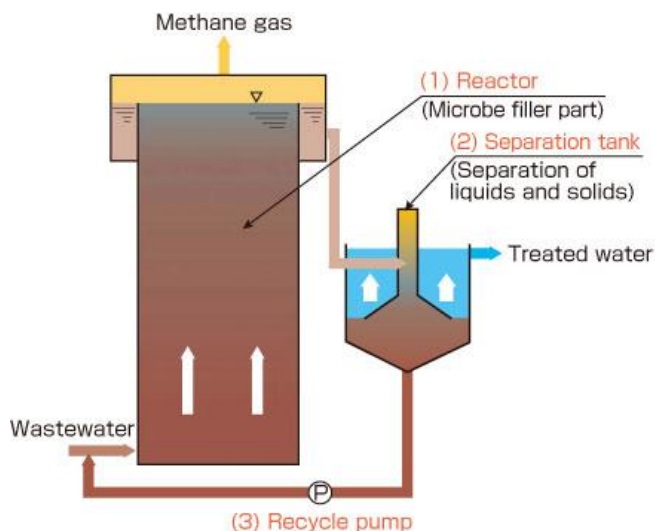
### **C- Biogas reactors**

It is carried out by the fermentation of animal dung and the produced gases are usually referred to as **biogas**. The used system or equipment called biogas plants or bioreactors.

Biogas is a flammable mixture of 50- 80% methane, 15-45% CO<sub>2</sub> and 5% water and some trace gases. The anaerobic microbial process is functioning best at temperature around 30°C. The organisms involved are found naturally in the ruminant manures.

The animal dung is mixed with water and allowed to ferment in near- anaerobic conditions. This is an old practice mainly in India, China and Pakistan. China is the largest user with over 7 million biogas units providing the equivalent energy of 22 million tonnes of coal.

Under ideal conditions, 10 kg of dry organic matter can produce 3m<sup>3</sup> of biogas, that will give 3 hours of cooking, 3 h of lighting or 24 h of refrigeration with suitable equipment.



Biogas combustion is used to derive electricity turbines by steam and, in California, one plant providing up to 20 000 h of electricity by cow manure biomethanation.

The process is still expensive, and a lot of improvements are required. For example, new and cheap construction materials for digesters (bioreactors) and gas storage vessels are required.

Methane is the principal product in the process but there are some other useful by-products such as propanol, butanol and fertilizers. While methane is also of economic value for small-scale production levels, there is considerable doubt about large-scale economic methane production. However, and although of these considerations, biogas production will continue to have high priority in alternative energy research.

## 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
- 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 to convert them into something

harmless. The following general steps are utilized in bioremediation:

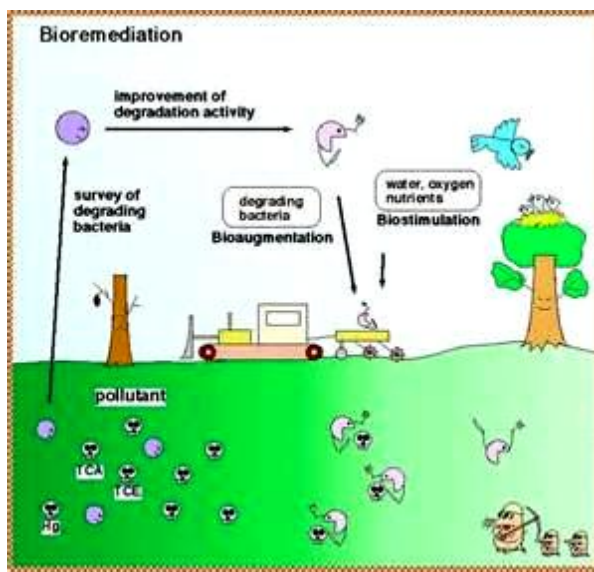
- 1- Define the pollution situation: What pollutants are present, how much of each are there, how dangerous are they, are they spreading and, if so, where and how fast.
- 2- Develop a microbial approach for dealing with the pollutants.
- 3- Isolate or stimulate a microbial population that will, by **natural 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 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.
- 6- 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 limits of the pollution often are ill- defined. For example, leakage from a toxic land fill may have contaminated ground water in an area for years before its discovery and no one knows the extent of the contamination. In some circumstances, pollutants move only inches per year from the source, whereas in other cases

it can travel for miles underground and turn up in well-water at a considerable distance from the pollution source. Defining the extent of underground pollution problems takes years and millions of dollars.

- 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.