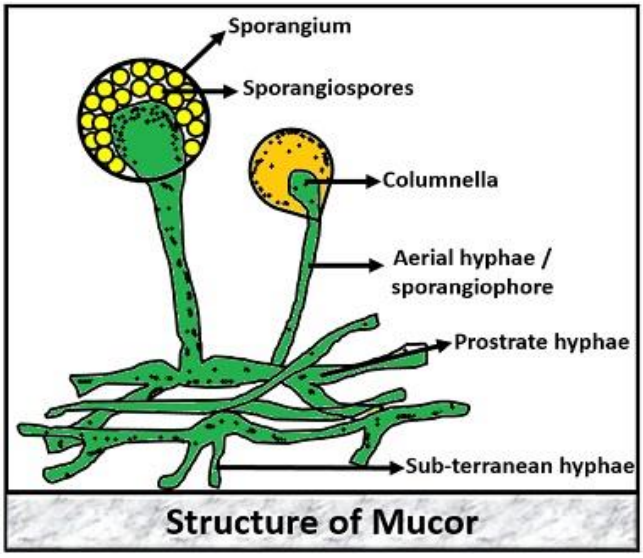
South Valley University

Faculty of Science

Botany & Microbiology Department

Soil Fungi

For third year



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Contents

ACKNOWLEDGEMENT

To my parents

1 Introduction

The word *fungus* comes from the Latin word for mushrooms. Indeed, the familiar mushroom is a reproductive structure used by many types of fungi. However, there are also many fungi species that don't produce mushrooms at all. Being eukaryotes, a typical fungal cell contains a true nucleus and many membrane-bound organelles. The kingdom Fungi includes an enormous variety of living organisms collectively referred to as Eumycota, or true Fungi. While scientists have identified about 100,000 species of fungi, this is only a fraction of the 1.5 million species of fungus likely present on Earth. Edible mushrooms, yeasts, black mold, and the producer of the antibiotic penicillin, *Penicillium notatum*, are all members of the kingdom Fungi, which belongs to the domain Eukarya.

The Fungi: Towards a Definition

It is difficult to define the fungi in simple terms because several unusual organisms as well as the typical fungi are often included in this blanket term. Nevertheless, the typical fungi have a range of features (general characteristics) that separate them from other organisms and which can be outlined here:

1- The fungi are typically filamentous. The individual filaments are called **hyphae** (sing. Hypha) and are surrounded by a wall which often, not always, contains **chitin** as a major component. The hyphae grow only at their tips, so fungi exhibit **apical growth**, and they branch periodically behind the tips, the resulting network of hyphae being termed **the mycelium** (Fig. 1).

2- All fungi are **heterotrophs** (chemo-organotrophs): they require performed organic materials which serve as both the energy source and as carbon

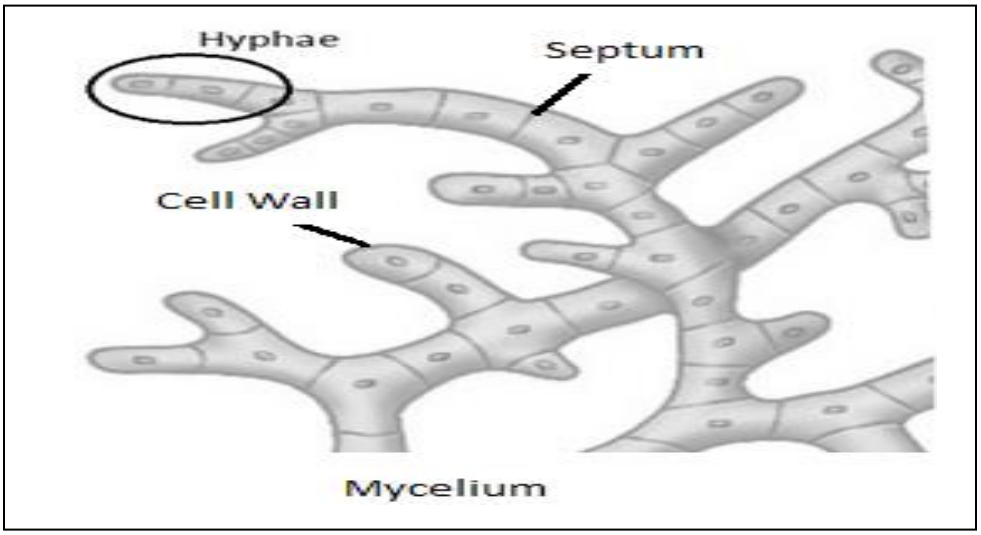

Fig. (1): Hyphae and mycelium

skeletons for cellular synthesis. Because of the rigid cell wall they cannot engulf food, rather they absorb simple soluble nutrients, which may be obtained from complex polymers by releasing extracellular enzymes (**depolymerase**) into the environment.

3- Fungi are **eukaryotic**, they have membrane bound **nuclei**, a range of membrane bound **organelles** and **ribosomes**.
4- Fungi reproduce by both sexual and asexual means, but in either case they usually produce **spores** as the end product. Spores differ greatly in size and shape.

Now we can define the fungi as, *eukaryotic characteristically mycelia, heterotrophs with absorptive nutrition*.

2 Structure and fine structure

General Structure: The hyphae structure

The hypha is essentially a tube, consisting of a rigid wall and containing a moving slug of protoplasm. It is tapered at the tip, the tapered region being termed the extension zone, this represents the region of most active wall growth. The higher fungi have cross walls or septa at intervals, but these are absent in lower fungi except where they occur as complete cross walls to isolate old or reproductive parts from the hyphae (Fig. 2).

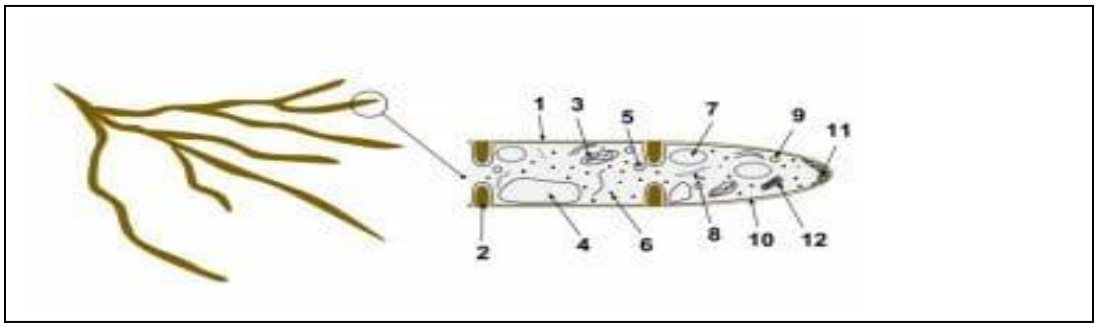

Fig. (2) 1- Hyphal wall 2- Septum 3- Mitochondrion 4- Vacuole 5- Ergosterol crystal 6- Ribosome 7- Nucleus 8- Endoplasmic reticulum 9- Lipid body 10- Plasma membrane 11- Spitzenkörper/growth tip and vesicles 12- Golgi apparatus.

Hyphae Function

Hyphae are associated with multiple different functions, depending on the specific requirements of each fungal species. The following are a list of the most commonly known hyphae functions:

1- Nutrient Absorption from a Host

Some hyphae of parasitic fungi are specialized for nutrient absorption within a specific host. These hyphae have specialized tips called haustoria, which penetrate the cell walls of plants or tissues of other organisms in order to obtain nutrients.

2- Nutrient Absorption from Soil

Some fungal species (e.g., *mycorrihizae*) have developed a symbiotic relationship with vascular plant species. The fungi forms specialized hyphae called arbuscules, which can be found in the roots or phylum of vascular plants, and function to absorb nutrients and water from the soil. In this manner, the hyphae aid the plants by increasing its access to nutrients in the soil while facilitating its own growth.

3- Trapping Structures

In some fungal species, hyphae have evolved into specialized nematode-trapping structures, using nets and ring structures to trap nematode species.

4- Nutrient Transportation

Several fungal species exhibit hyphae composed of chord-like structures, termed mycelial chords, which are used by fungi (e.g., lichens and mushrooms) to transport nutrients across great distances.

Hyphae Classification

In general, hyphae can be classified based on the following traits:

Hyphae Characteristics

Hyphae characteristics are an important method of classifying various fungal species. There are three main hyphae characteristics:

- **Binding**: Binding hyphae have a thick cell wall and are highly branched.
- **Generative**: Generative hyphae have a thin cell wall, a large number of septa, and are typically less differentiated. Generative hyphae may also be contained within other materials (e.g., gelatin or mucilage) and can also develop structures used in reproduction. All fungal species typically contain generative hyphae.
- **Skeletal**: Skeletal hyphae contain a long and thick cell wall with few septa. Skeletal hyphae can also be of a fusiform subtype, with a swollen midsection surrounded by tapered ends.

Hyphae Composition

Fungal species are also further classified based on the hyphal systems they contain. There are four general subtypes:

- **Monomitic**: While virtually all fungal species contain generative hyphae, those with only exhibit this type are referred to as monomitic (e.g., agaric mushrooms).

- **Dimitic**: A species that contains generative hyphae in addition to one other type of hyphae. The most common combination of dimitic fungi is generative and skeletal.
- **Trimitic**: Species which contain all three types of hyphae (generative, binding, and skeletal).
- **Sarcodimitic and sarcotrimitic**: Sarcodimitic hyphae are fusiform skeletal hyphae bound to generative hyphae. Sarcotrimitic species contain fusiform skeletal hyphae, as well as binding and generative hyphae.

General structure: Yeast

In *Saccharomyces cerevisiae* there is a single nucleus, a large central vacuole and the normal range of cytoplasmic organelles (Fig. 3).

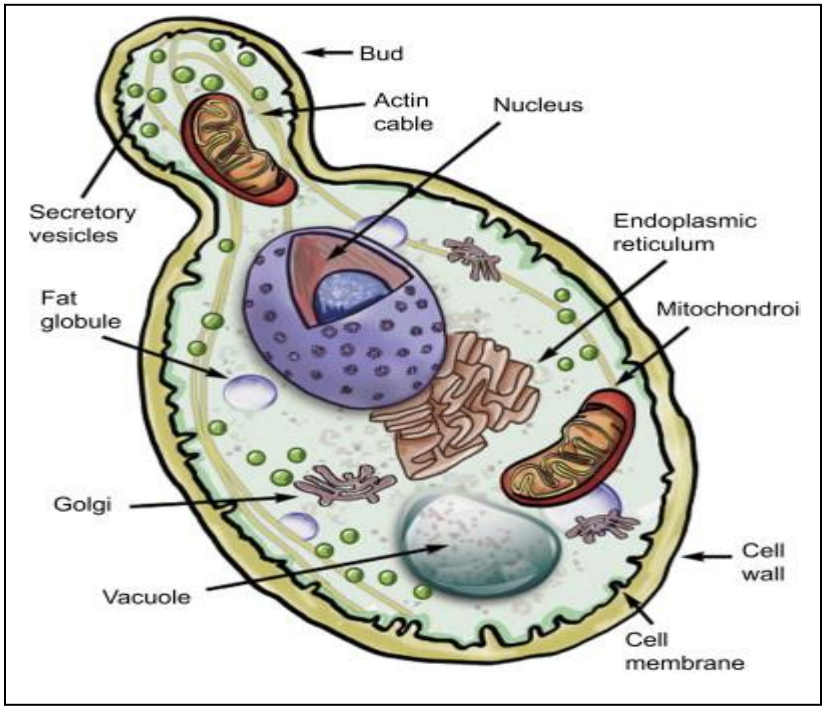

Fig. (3): *Saccharomyces cerevisiae* fine structure.

The cell reproduces by budding, and at maturity the bud separate from parent cell by the formation of the septum. This process leaves a birth scar on the daughter cell and a bud scar on the parent cell.

The bud arises from different point on the parent cell each time so, *S. cerevisiae* is said to exhibit **multipolar** budding. In other yeasts *S.ludwigii* the buds always develop from the same points on the cell, usually at one of the poles termed **bipolar budding**.

Fungal wall

Function of fungal wall:

1- It determines the shape of the cells, because if it is removed by enzymatic treatments the resulting protoplasts are always spherical.
2- Wall acts as interface between the fungus and its environment, it protect the cell from osmotic lysis and perhaps from the metabolites of other organisms.
3- It is a binding site for some enzymes.
4- It can have antigenic properties which mediate the interactions of fungi with other organisms.

Composition:

Gross chemical analysis of fungal walls reveals a predominant of polysaccharides but also significant amounts of protein and lipids. Nevertheless, the wall composition of a fungus should not be fixed because even within a single species the ratio can differ at different stages of the life cycle.

The walls of fungi contain a mixture of

a- Fibrillar components: include chitin and cellulose (Oomycetes). These are straight chain of N-acetylglucosamine and glucose, respectively.

b- Amorphous or matrix: include glucans (polymers or glucose, proteins, polymers of galctosamine and polymers of mannans).

Septa

Septa are found in all filamentous fungi except most members of Oomyctes and Zygomycetes .

Function

1- They acts as structural support of the hyphae.
2- They act as the first line of defense against damage, the septal pores plugged by Woronin bodies as hyphae age or damaged(Fig. 5).
3- Septa have a role in differentiation of fungal groups.

Types

a- Simple septum: found in most of Ascomycotina and Deuteromycotina, in which there is a large central pore 0.05- 0.5 µm diameter.
b- Dolipore septum: in some stages in the life cycles of Basidiomycotina. There is a very narrow central pore bounded by two flanges of amorphous wall material. On either sides of this central pore there are perforated, bracket-shaped membranes, termed parenthosomes, which seem to be a special modifications of endoplasmic reticulum. This type of septa enables cytoplasm to pass from one compartment to another but it usually restricts the passage of nuclei (Fig. 4).

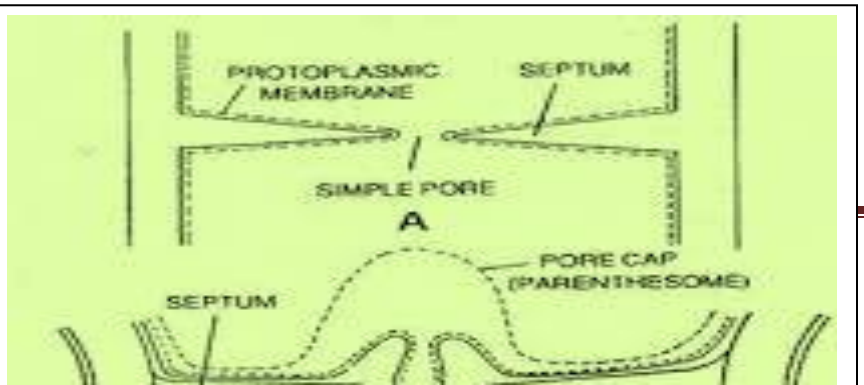

Fig. (4): A, simple pore and B, dolipore

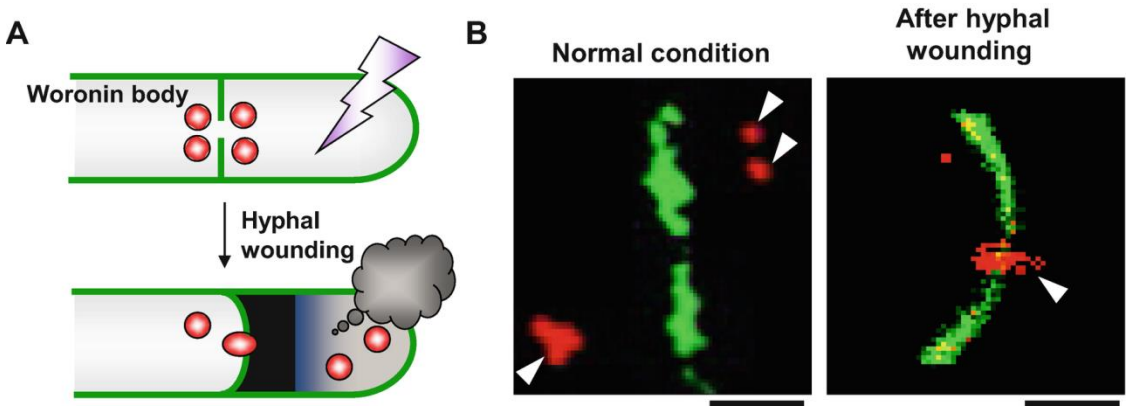

Fig. (5): Function of Woronin body

Membrane and membrane bound organelles

The fungal plasma membrane has a typical tripartite appearance in electron micrographs, (double layer of phospholipids, amounts of protein and sterols).

Permease: govern uptake and release of materials by the cells.

Sterols: help to order the phospholipids and enabling membrane to fuse with another. The main sterol in fungi is ergotsterol.

The endomembrane system

The small membrane-bound vesicles are present in large numbers in the hyphal apex. In higher plants and some algae the vesicles involved in wall growth have been shown to originate from a Golgi body and are thought to move from this to their sites of fusion with the cell membrane, releasing materials into the wall. In most fungi the Golgi body consisting of only a single cistern or a ring-like arrangement of cisternae which are thought to have the same role. The vesicles themselves are thought to be budded off the Golgi cisternae, and their contents are carbohydrate, cellulase, alkaline, phosphatase, glucanase and mannan synthase(i.e enzymes for degradation and synthesis of the wall).

Nuclei

Fungal nuclei are usually small 2-3µm diameter. They are bounded by a double nuclear membrane with conspicuous pores. Most fungi are haploid, although there are some exceptions to these rules. For example *S. cerevisiae* and *Allomyces* can alternate between haploid and diploid generation.

3 Growth

Mechanism of apical growth

We have said that fungal hyphae grow only at their apices. Hyphal apex is surrounded by a wall. Even though it may be thinner and more plastic than the mature hyphal wall. Growth at the apex must involve both a degree of wall lysis and degree of wall synthesis, these processes being so balnced to ensure that the wall becomes neither too weak nor too rigid for further growth.

Spitzenkorper is a body observed in the tips of growing hyphae and disappeared when growth stopped.

The vesicles include some wall precursors, wall synthases and wall lytic enzymes. The functions of the vesicles in apical growth:

1- To transport enzymes that break the bonds between the existing wall components so that the wall stretches as a result of turgor pressure.
2- To transport new wall materials, (precursors) for incorporation into the wall.
3- To increase the surface area of the plasmalemma during growth.

Hypothetical representation of the events in a unit of cell wall growth (Fig. 6):

A- Vesicles containing wall lytic enzymes fuse with plasmalemma.

B- Bonds between existing wall components are broken.

C- Wall stretches as a result of internal pressure vesicles containing wall synthesizing enzymes fuse with plasmalemma.

D- New wall components arise in vesicles or are synthesized from precursors that cross the plasmalemma.

E- A new unit of cell wall has been synthesized.

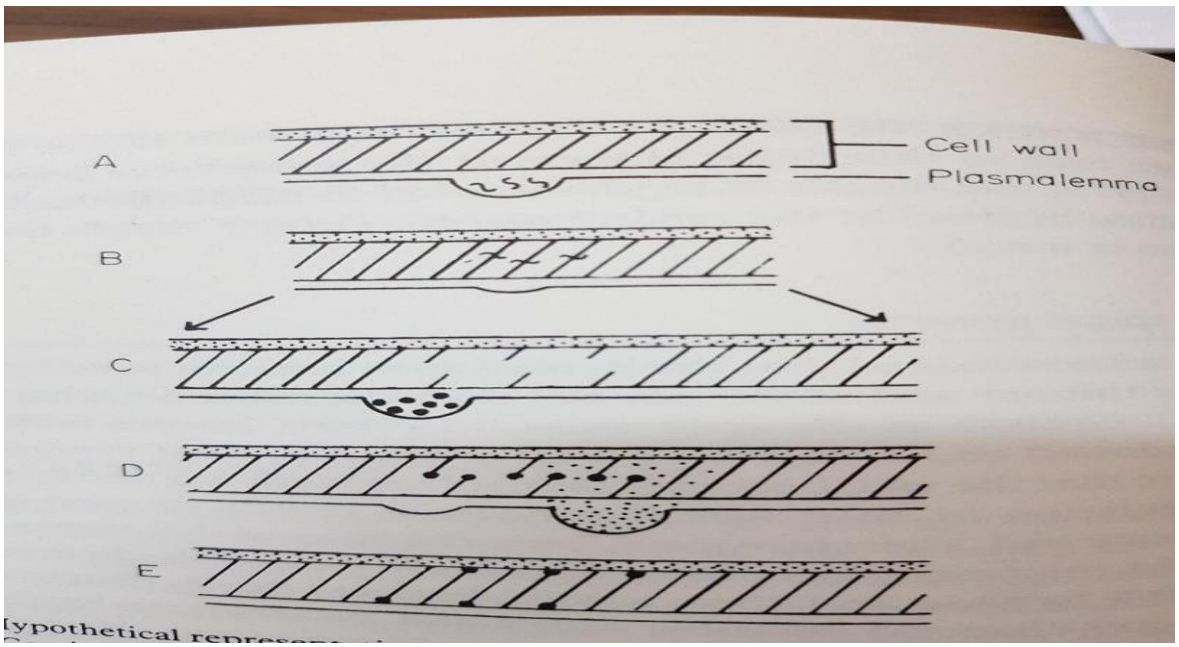

Fig. (6): Hypothetical representation of the events in a unit of cell wall growth.

How does an apex form?

In several fungi such as *Mucor* and *Aspergillus*, spore germination involves an initial phase of swelling as a result of hydration followed by a further phase of swelling that depends on metabolic activity.

Stages in the germination of spores of *Aspergillus niger* (Fig. 7):

a- In normal conditions the spores swells and incorporates a new wall materials uniformly over its surface, later a germ tube emerges and a new wall materials are incorporated at the tip of the germ tube.

b- At 44°C the spore continues to swell and incorporate wall material uniformly, lowering of the temperature to 30°C leads to germ tube outgrowth but the germ tube immediately forms a sporing head.

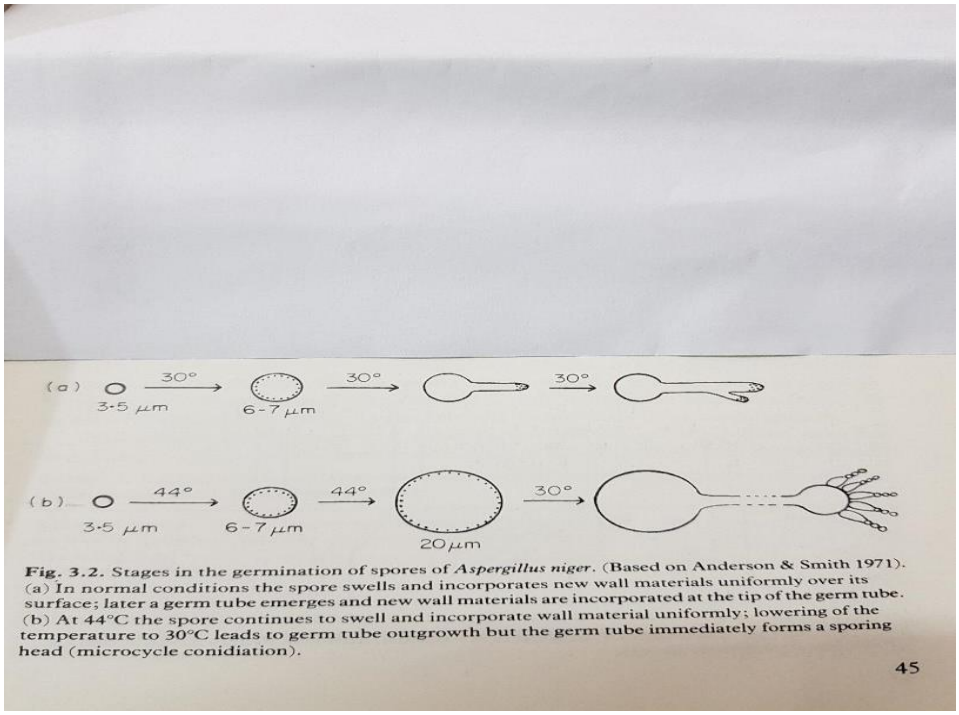

Fig. 3.2. Stages in the germination of spores of *Aspergillus niger*. (Based on Anderson & Smith 1971). (a) In normal conditions the spore swells and incorporates new wall materials uniformly over its surface; later a germ tube emerges and new wall materials are incorporated at the tip of the germ tube. (b) At 44°C the spore continues to swell and incorporate wall material uniformly; lowering of the temperature to 30°C leads to germ tube outgrowth but the germ tube immediately forms a sporing head (microcycle conidiation).

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Fig. (7): Stages in the germination of spores of *Aspergillus niger*.

3 Reproductions

I- Asexual Reproduction

Fungi reproduce asexually by fragmentation, budding, or producing spores. Fragments of hyphae can grow new colonies. Mycelial fragmentation occurs when a fungal mycelium separates into pieces with each component growing into a separate mycelium. Somatic cells in yeast form buds, the nucleus divides mitotically, and the bud ultimately detaches itself from the mother cell.

The most common mode of asexual reproduction is through the formation of asexual spores, which are produced by one parent only (through mitosis) and are genetically identical to that parent. Spores allow fungi to expand their distribution and colonize new environments. They may be released from the parent thallus, either outside or within a special reproductive sac called a sporangium

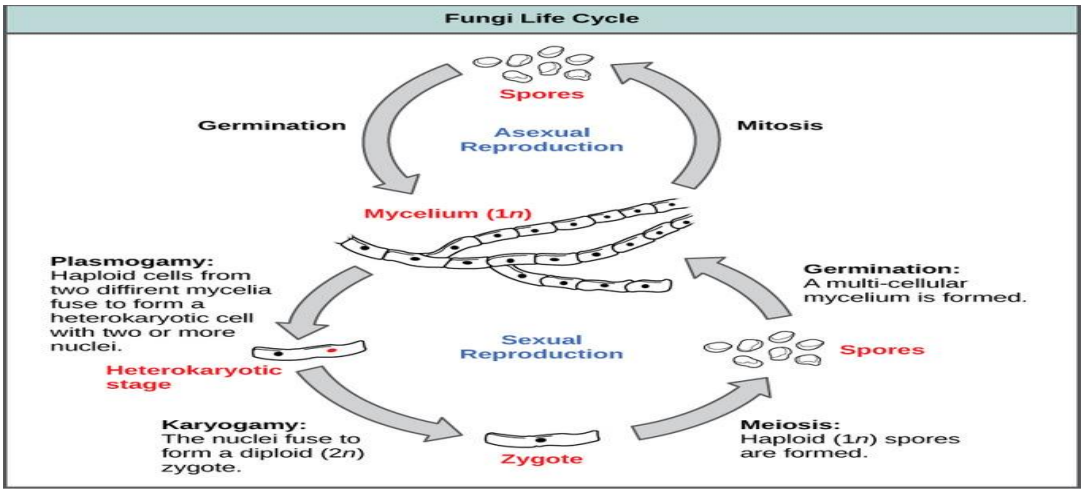


Fig. (8): Fungi Life cycle.

Conidia:

a- Blastic conidia: arise by budding or swelling process and then separated from the supporting structure by a septum.

-Proconidia as this bud enlarges it forms a further bud at its tip and so on until a chain of conidia is formed.

-Phialide another type of plastic conidia formed by a flask shape cell, the phialide. In *Aspergillus* and *Penicillium* after the first conidium has been formed from the phialide all subsequent conidia are extruded from the phialide tip and cut off by a septum. They accumulate one after another in chains (Fig. 9).

b- Thallic conidia: arise by fragmentation or septation of hypha but may be subsequently swell.

In extreme cases of thallic conidia represent little more than hyphal fragments, formed by multible septation of a hypha and separation of the cells by breakdown of the middle region of each septum this type called arthrospore.

The thallic conidia differ from blastic conidia because the former is cut off by a septum at an early stage, before any swelling takes place (Fig. 10).

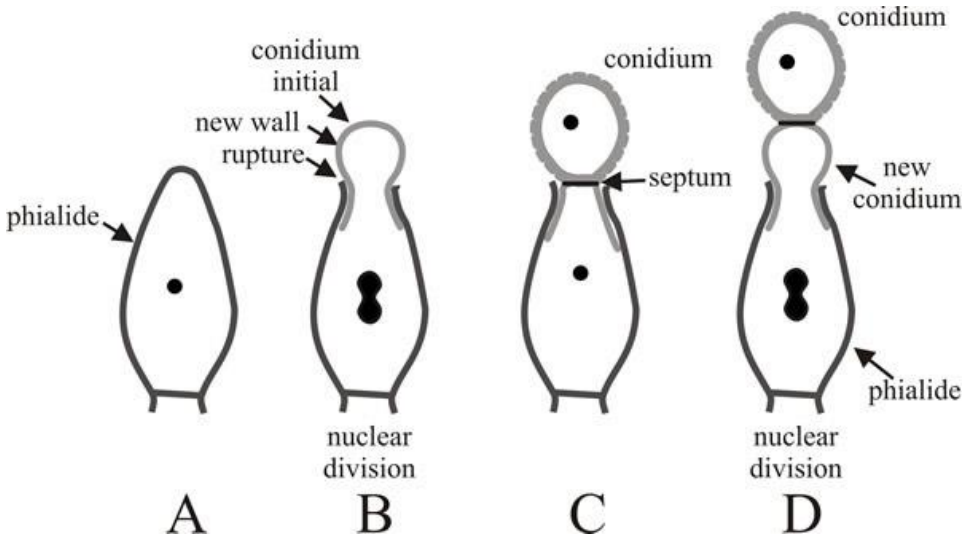


Fig. (9): Phialide

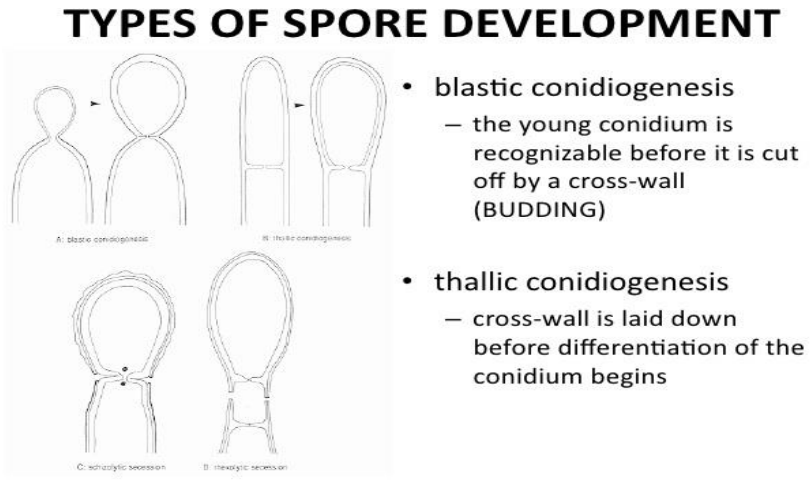


Fig. (10): Types of spores development

Sporangiospores

Sporangiospores develop by cleavage of the cytoplasm around individual nuclei in a multinucleate sporangium (Fig. 11). Cleavage occurs in several ways. In *Saprolegnia* the central vacuole of the sporangium enlarges between the nuclei and its membrane fuses with the plasmalemma, the resulting spores consist of a nucleus, a portion of the cytoplasm and spore membrane derived in part from the vacuole membrane and in part from the sporangium plasmalemma.

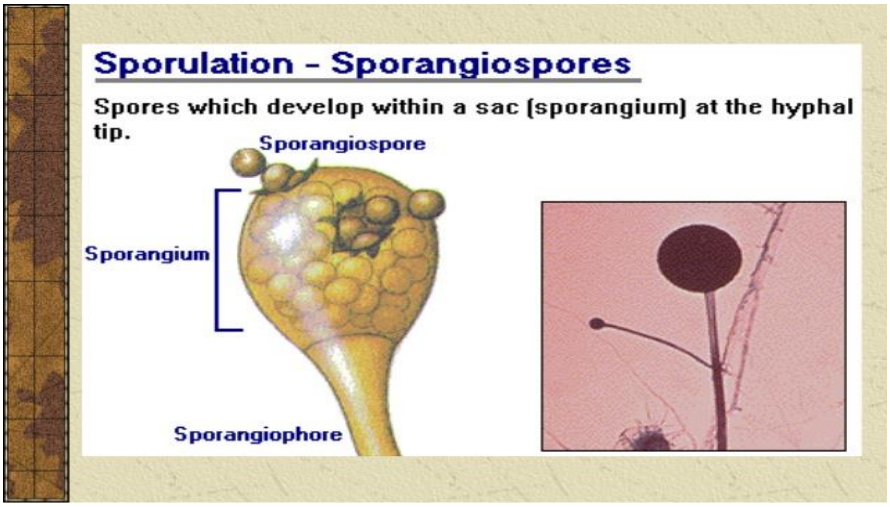


Fig. (11): Development of sporangiospore

II- Sexual Reproduction

Sexual reproduction must be regarded as a whole series of events:

1- Production of sex organs and gametes.
2- Fusion of gametes or sex organs (plasmogamy) followed sooner or later by nuclear fusion (karyogamy).
3- Meiosis in haploid fungi.
4- Development of fruiting bodies to enclose and disperse the spores.
5- Development of sexual spores.

Hormonal systems are known to occur in several fungi (four hormones):

a- Sirenin: is produced by female gametes of *Allomyces* to attract male gametes.
b- Anthreidiol: is produced by female branches of *Achyla* and it initiates the development of antheridia on male hyphal branches.
c- Oogoniol: produced by male branches, which diffuses towards the female hyphae and causes them to develop oognia.

In the Zygomycotina it is common to find different mating types, which are termed + and – because they do not differ in morphology. Species exhibiting this behaviour are termed **hetrothallic** because two different thalli are needed for sexual reproduction, whereas self fertile species are termed **homothallic**.

d- Trisporic acid: the + and – strains of heterothallic species produce different hormone precursors which diffuse in the air towards the opposite mating type and are then converted to active hormone.

Specialized vegetative structure:

Fungi produce a range of structures during penetration of plant surfaces

1- Appressoria: simple swollen cells.
2- Hyphopdia: loped structures.
3- Sclerotia: massive vegetative structures produced by some fungi for dormant survival. They are range from simple of pigmented hyphae as in *Rhizocotina solani* to more complex structures a darkly pigmented ring *(Sclerotinia* spp.)

Fungal growth phases

From the time a spore or a hyphal fragment germinates to form a colony to the time the fungus dies, there are a number of growth phases. Although these phases have been determined under laboratory conditions, it is possible that the same occur in nature. In nature the duration of each phase would be determined by the environmental conditions including other competing micro-organisms.

- **Lag phase**

  Once the growth conditions become favorable for the fungal propagules (i.e., viable spores or mycelial fragments) to germinate, new transport systems must

be induced before growth commences. Thus growth starts slowly and accelerates gradually. This phase is referred to as the lag phase.

- **Exponential or log phase**

  Exponential growth occurs only for a brief period as hyphae branches are initiated, and then the new hypha extends at a linear rate into un-colonized regions of substrate. The biomass of the growing fungus doubles per unit time. As long as the nutrients are in excess growth remains constant during the exponential phase.

- **Stationary phase**

  As soon as the nutrients are depleted or toxic metabolites are produced growth slows down or is completely stopped. The biomass increases gradually or remains constant. During the stationary phase, hyphal growth stops and, in some molds, cell differentiation occurs, resulting in spore formation. During this process nutrients are transferred from the vegetative mycelium to the developing spores. The spores are dispersed by air movement to other areas of the building where they can start new mold growth once the conditions for growth are favorable.

- **The death phase**

  During the death phase, the mycelium eventually dies off. The death phase is usually accompanied by breakdown of the mycelia through self-digestion. Some fungi form spores by fragmentation of the hyphae.

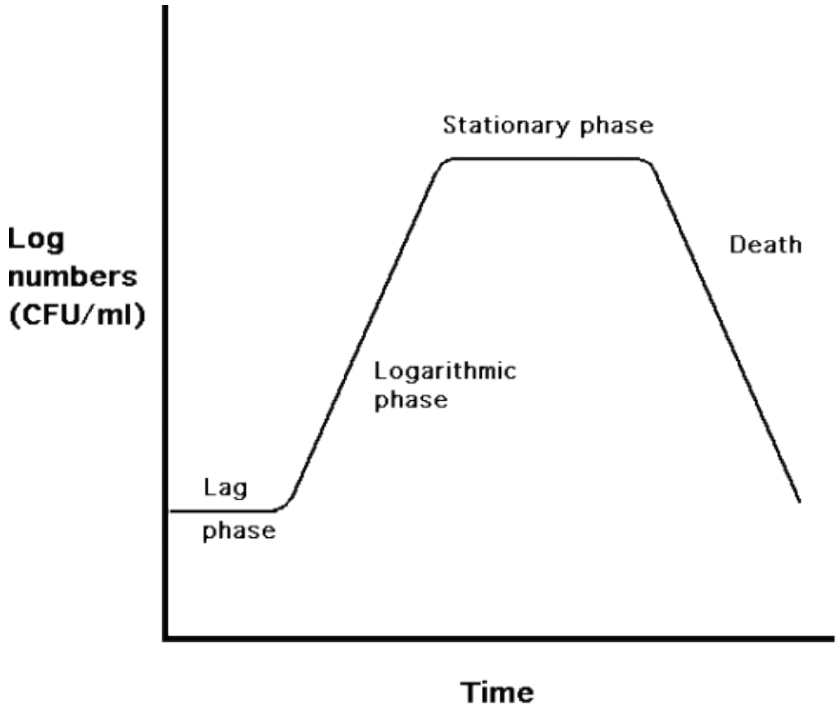


Fig.(12) **Hypothetical bacterial growth curve.**

4 Nutrition

Important classes in which the nutrition of fungi may be classified are as follows:

The fungi are chlorophyll less plants and cannot synthesize their own food unlike green plants from carbon dioxide and water in the presence of sunlight. They are so simple in structure that they cannot obtain inorganic food directly from the soil, and therefore they are always dependent for their food on some dead organic material or living beings.

(a) Saprophytes:

The saprophytic fungi live on dead organic materials produced by the decay of animal and plant tissues. They grow upon dead organic matters such as rotten fruits, rotten vegetables, moist wood, moist leather, jams, jellies, pickles, cheese, rotting leaves, plant debris, manures, horse dung, vinegar, moist bread and many other possible dead organic materials. *Saprolegnia, Mucor, Rhizopus, Penicillium, Morchella, Aspergillus, Agaricus* and many others are good examples of saprophytic fungi.

The saprophytic fungi absorb their food from the substratum by ordinary vegetative hyphae which penetrate the substratum, e.g., *Mucor mucedo*. In other cases of the saprophytic fungi such as *Rhizopus* the rhizoids develop which penetrate the substratum and absorb the food material. In the case of saprophytic fungi the mycelium may be ectophytic or endophytic. In the case of *Rhizopus* the

mycelium is ectophytic whereas the rhizoids remain embedded in the substratum and said to be endophytic.

(b) Parasites:

The parasitic fungi absorb their food material from the living tissues of the hosts on which they parasitize. Such parasitic fungi are quite harmful to their hosts and cause many serious diseases. These fungi cause the great losses to the human beings or indirectly. Many diseases of the important crops are caused by parasitic fungi. The rusts, smuts, mildews and many other plant diseases are important examples of fungal diseases of crops. Their mode of life is parasitic and the relation of host and parasite is called the parasitism.

The parasites which survive on living hosts and only on living hosts are called the **obligate parasites**. Such parasites cannot be grown upon dead organic culture media, e.g., *Puccini, Peronospora, Melampsora*, etc. The parasitic fungi which usually live on living hosts and according to their need they adopt saprophytic mode of life for some time are called the facultative saprophytes, e.g., *Taphrina deformans* and some smuts.

Some parasitic fungi usually pass saprophytic mode of life, but under certain conditions they parasitize some suitable host and are called the facultative parasites, e.g., *Fusarium*, *Pythium*, etc.

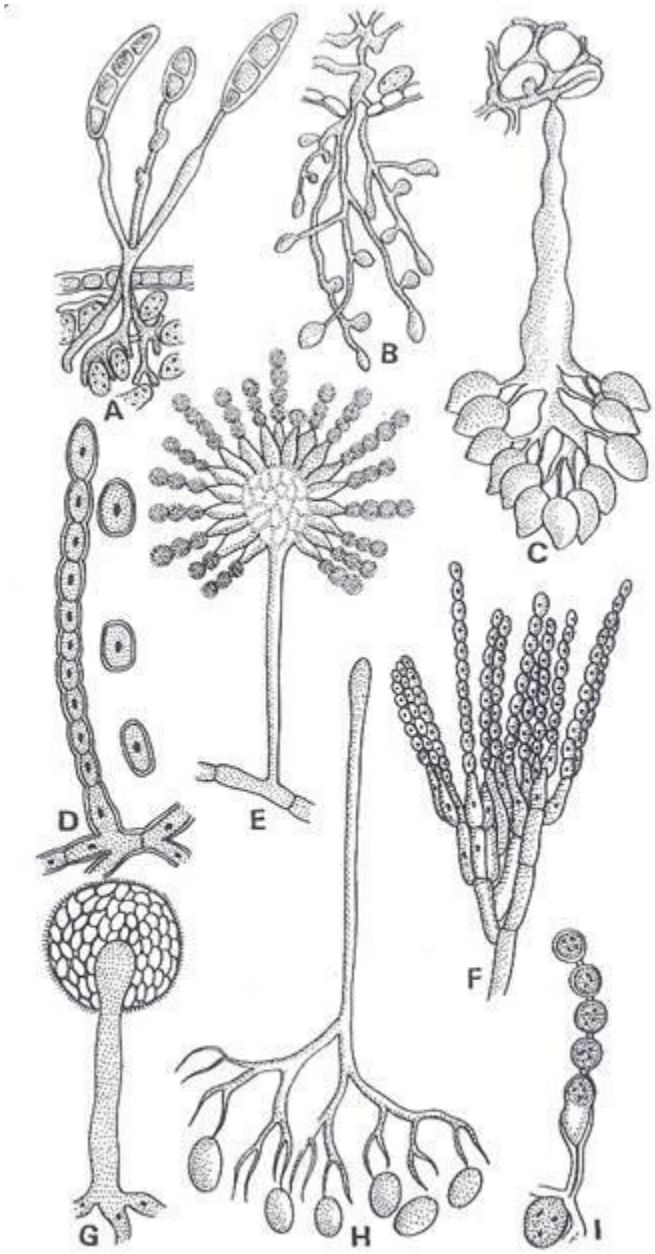


Fig 8.8. *The Fungi*. Various types of sporophores. A, conidiophores and conidia of *Helminthosporium*; B, sporangiophore and sporangia of *Phytophthora*; C, sporophore and sporangia of *Sclerospora*; D, conidiophore and conidia of *Erysiphe*; E, conidiophore and conidia of *Aspergillus*; F, conidiophore and conidia of *Penicillium*; G, sporangiophore and

The parasitic fungi absorb their food from the hosts in different ways. The fungus having the mycelium outside the host is called the **ectoparasite,** e.g., *Erysiphe*, whereas the fungus having the mycelium embedded in the host tissue is called the **endoparasite**. In the former type certain cushion-like appressoria develop on the

surface of the host and from each appressorium a peg-like structure develops which penetrates the host epidermal cell giving rise to a branched or unbranched absorbing organ called the haustorium.

The haustoria may also develop from the mycelium of endoparasites. The haustoria vary in their shapes. They may be small, rounded, and button-like as in *Albugo*, branched and convolute as in *Peronospora* and highly branched as in *Erysiphe*.

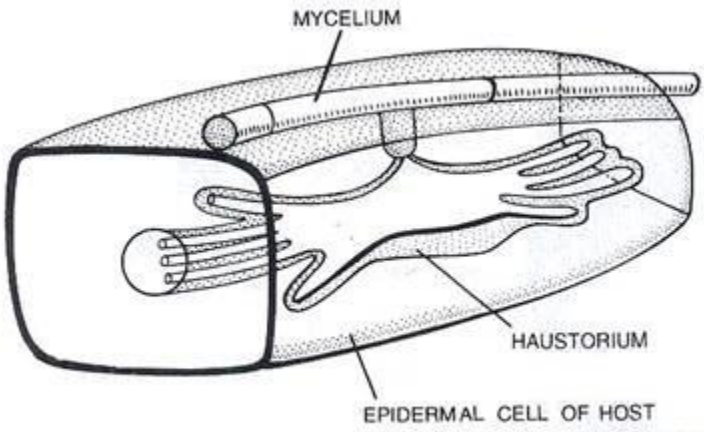


Fig. 8.9. Haustorium. Three-dimensional diagram of an infected epidermal cell with a branched haustorium of *Erysiphe* sp. (powdery mildew).

In the case of rusts and mildews the mycelium remains confined in the pustules and not in the whole body of the plant. This type of fungus is called the **localized** fungus. When the mycelium prevails in the whole of the plant it is said to be **systemic fungus**, e.g., smuts. When the mycelium is confined to the intercellular spaces it is called **intercellular** mycelium and in other cases the mycelium penetrates the host tissue and said to be **intracellular**. Usually the former bears haustoria and the latter does not.

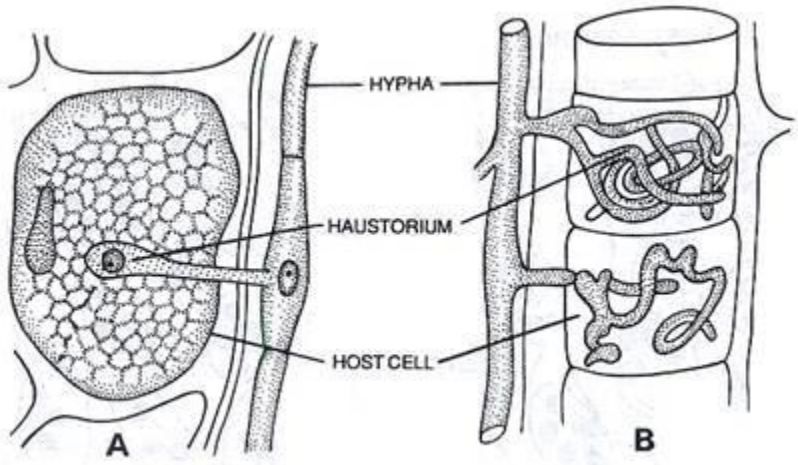


Fig. 8.10. Haustoria. A, elongated capitate haustorium; B, branched or digitate haustorium.

(c) Symbionts:

Some fungi live in close association of other higher plants where they are mutually beneficial to each other. Such relationship is called the 'symbiosis' and the participants are the 'symbionts'. The most striking examples are the lichens and mycorrhiza. The lichens are the resultants of the symbiotic association of algae and fungi.

Here, both live together and are beneficial to each other. The algal partner synthesizes the organic food and the fungal partner is responsible for the absorption of inorganic nutrients and water.

Certain fungi develop in the roots of higher plants and the mycorrhiza are developed. Here the fungi absorb their food from the roots and in response are beneficial to the plants. The mycorrhiza may be external or internal. The **external** mycorrhiza also called the **ectophytic** mycorrhiza are confined to the outer region of the roots whereas the internal mycorrhiza are found deeply in the root cells.

It is to be remembered that in all the cases whether they may be saprophytes, parasites or symbionts, the food is absorbed in the form of solution by cell walls, rhizoids and haustoria.

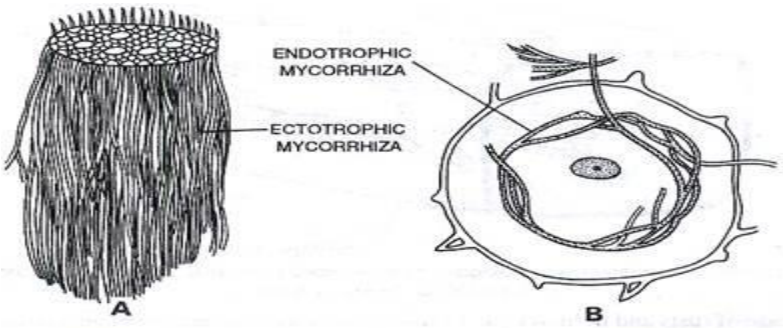


Fig. 8.11. Mycorrhiza. A, ectophytic mycorrhiza, B, endophytic mycorrhiza.

When fungi are cultured in the laboratory on synthetic media, the necessary elements may be supplied in the following way: C is usually supplied in the form of a carbohydrate, such as glucose or maltose sucrose and soluble starch are utilized by many fungi also. N may be supplied in the form of NH_4 salt or as amino acids.

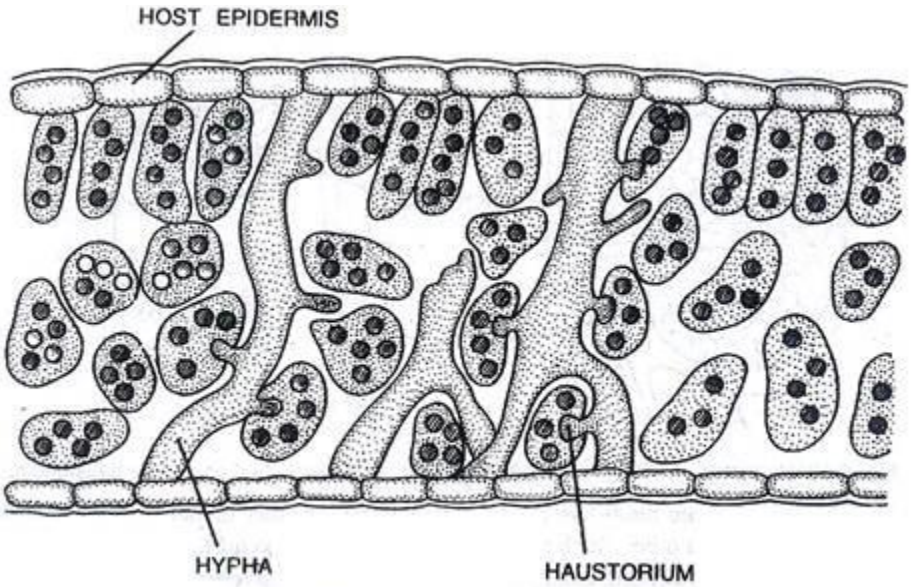


Fig. 8.12. Haustorium. Button-like or globular.

(d) Predacious fungi:

There are many animal trapping fungi which have developed ingenious mechanisms for capturing small animals such as protozoa which they use for food. The most interesting of these mechanisms is that which utilizes a rapidly constricting ring around a nematode which holds it captive while the hyphae sink haustoria into the body of the victim.

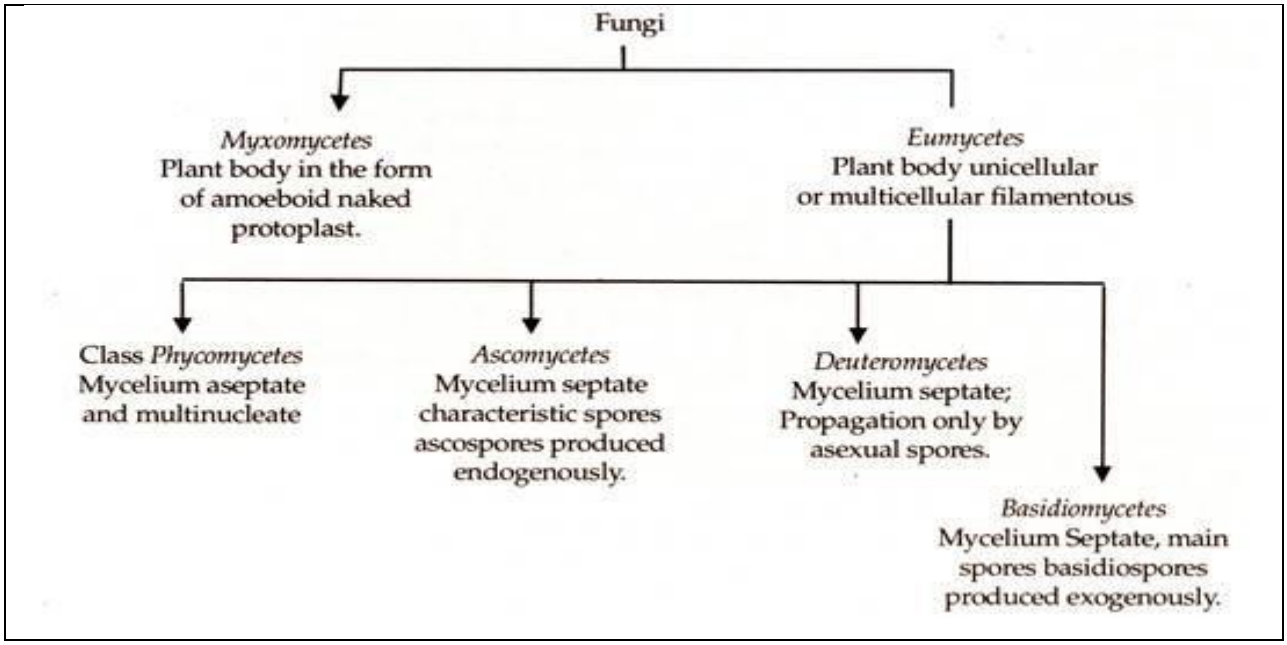
Several species of fungi in the genera *Arthrobotrys*, *Dactylella* and *Dactylaria* employ this method. In the presence of an eelworm population, the hyphae of the

fungi produce loops which are stimulated to swell rapidly and close the opening when an eelworm passing through the loop rules against its inner surface.

5 Classifications

Alexopoulos (1956) places all fungi in the division Mycota. The division Mycota is divided into two subdivisions (1) Myxomycotina (2) Eumycotina (true fungi). Myxomycotina has only one class – Myxomycetes.

Eumycotina has the four classes as shown in the figure.



Subdivision (1) Myxomycotina

Class – Myxomycetes

Occurrence: Myxomycetes are found in cool places, decaying wood and humus rich soil.

Structure: The vegetative stage in Myxomycetes has no cell wall, naked and irregular mass of protoplasm called plasmodium. The plasmodium is amoeboid in shape and multinucleate and moves with the helpof pseudopodia.

Classification: the class Myxomycetes divided into three orders, namely *Plasmodiophorales*, *Stemonitales* and *Acrasiales*.

C: *Myxomycetes*

O: *Plasmodiophorales*

F: *Plasmodiophoraceae*

Ex. *Plasmodiophora brassicae*

Distribution: the fungus causes disease to cruciferous plants mainly to cabbage (club rootdisease).

Occurrence: the fungus is an obligate parasite.

Disease symptoms: irregular growth or hypertrophy in the root.

Structure of the pathogen: naked massof protoplasm called plasmodium.

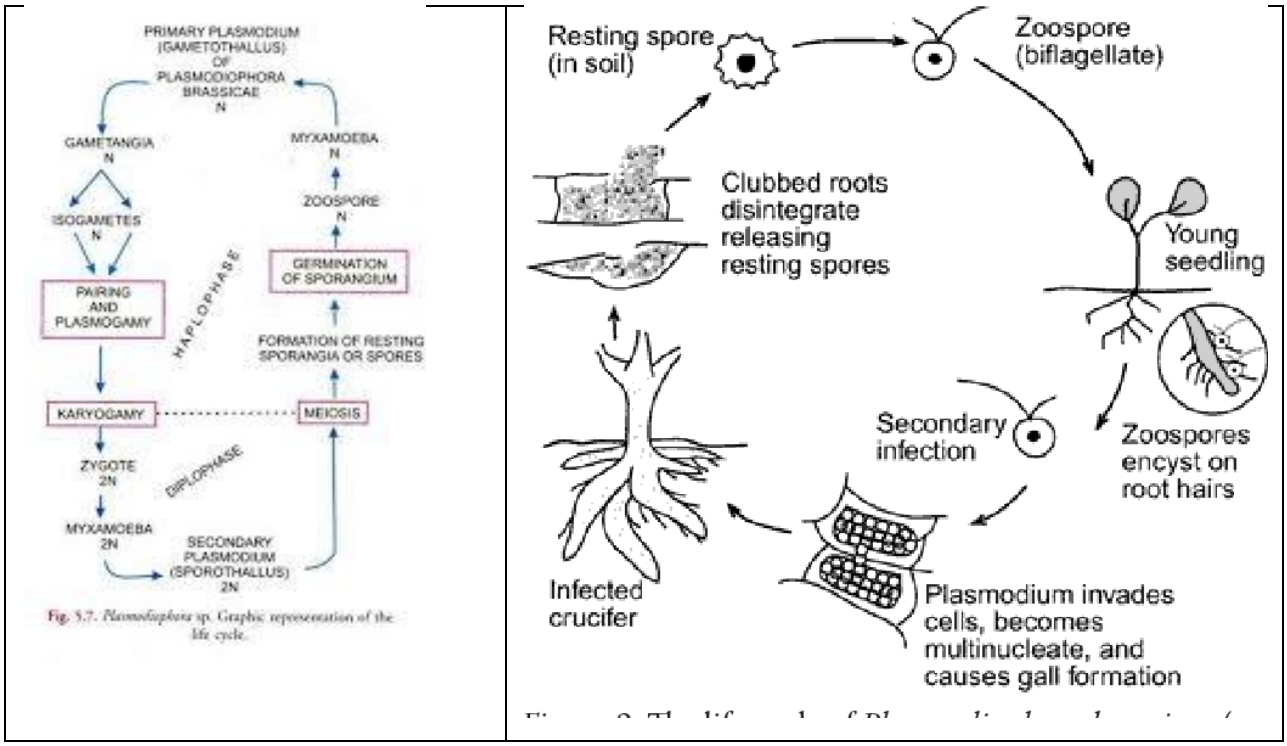
Asexual reproduction:

1- The nuclei in the plasmodium are diploid.They undergo reduction division when the plasmodium are in the cells of root of host plant.

2- Spherical nonmotile spores have a chitinous wall are formed. The death and decay of the root cells of the host sets the spore free.
3- The spores metamorphose themselves into biflagellate zoospores infect the healthy plants forming myxmoeba, which repeatedly divided forming haploid plasmodium.

Sexual reproduction:

4- Each nucleus of the haploid plasmodium gets isolated surround itself by a little cytoplasm and form gametangium.
5- The nucleus of each divides mitotically to form 8-10 biflagellate isogametes.
6- Two isogametes fuse and form a diploid zygote
7- The diploid zygote divides mitotically and develops into diploid pladmodium.



Subdivision (2): Eumycetes

Class 1: Phycomycetes

Occurrence: they are very common in occurrence. The bread mold (*Mucor*), the water mold (*Saprolegnia*), the white rust of mustard all are phycomycetes.

Structure: they are coenocytes, aseptate much branched mycelium (septa appearing to the dead portions or at the time of formation of reproductive structures. Many primitive phycomycetes are aquatic in their distribution even higher forms (except zygomycetes) show dependence on moisture. They may be parasite or saprophyte.

Reproduction:

Asexual reproduction is brought about by

1- Fragmentation 2- Spore formation

Phycomycetes produce both zoospores and conidia. *Saprolegnia* , *Phytophthora*, *Pythium* and others produce zoospores. *Albugo* produces conidia. In Mucorales, asexual reproduction is by aplanospores. Chlamydospores also found in some members.

Sexual reproduction

1-Planogametic copulation. 2- Gametangial copulation. 3- Gametangial contact.

Stages in sexual reproduction

a- Plasmogamy b- Karyogamy c- Meiosis

Classification

The filamentous phycomycetes are divided according their mode of reproduction into 2 orders namely

(1) Oomycetes: reproduction is oogamous.

(2) Zygomycetes: reproduction is isogamous.

The nonfilamentous phycomycetes having rounded lobed mycelia thallus are placed in (3) Archymycetes.

Alexopoulos (1956) divides phycomycetes into 7 orders. Chytridiales, Monoblepharidales, Plasmodiophorales, Saporolegpiales, Peronosporales, Mucorales and Entomophthorales.

The following examples are discussed here;

1- Saprolegniales ex. *Saprolegina.*
2- Mucorales ex. *Mucor*.

C: Phycomycetes

O: Saprolegniales

F: Saprolegniaceae

Ex.: *Saprolegnia* sp.

Occurrence: *Saprolegnia* sp. is commonly called water mold, because of their frequent occurrence in water. Often they grow on dead insects, fishes etc. Most of them are saprophytic the only exception is *S. parasitica* which infects fishes causes salmon disease.

Mycelium: is coenocytic and branched forming white mold. Hyphae are aseptate and septa are formed only when it enters the reproductive phase. Cytoplasm contains several nuclei. Food is stored in the form of globules or glycogen.

Asexual Reproduction: takes place by the formation of pear shaped biflagellate zoospores produced in club shaped zoosporangia. These are usually produced at the terminal regions of somatic hyphae.

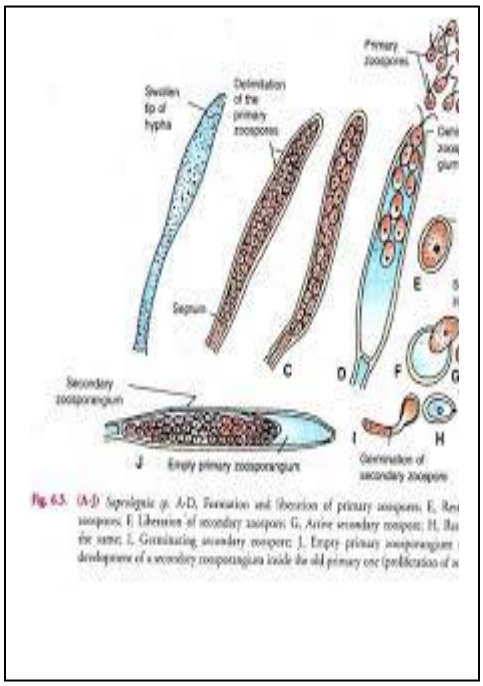
Development of zoosporangium:

1- The apical portions of certain of the hyphae enlarge into swelling.
2- This show dense cytoplasm contents into this migrate many nuclei.
3- Later this is cut off by transverse septum at the base.
4- The young zoosporangium shows multinucleate protoplasm.

Formation of zoospores:

1- The content of the zoosporangium divide into several uninucleate portions by cleavage of the protoplast.

2- Each uninucleate daughter protoplast becomes rounded off and assumes pear shape.
3- Later two flagella develop apically (whiplash type and tinsel type).
4- At maturity the tip of the zoosporangium breaks open and zoospores emerge one after another.

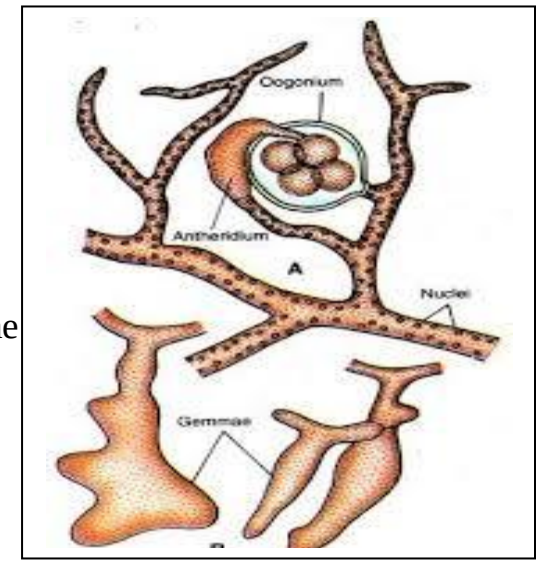


The zoospores are called **primary zoospores**. They swim for some time later they withdraw their flagella become round and enter the resting period. Then the contents of each develop into single kidney shape **secondary zoospore**. These zoospores escape through small pore formed in the cyst. They germinate on a suitable substratum by the formation of germ tube. This tube finally forms the mycelium.

Saprolegnia exhibits two very important phenomena namely:

1- **Diplanestism** : *Saprolegnia* is diplanetic because it produces two types of zoospores, primary and secondary, separated by resting period.
2- **Sporangial proliferation**: after the production and liberation of primary zoospores from the zoosporangium, it becomes empty. Later the basal septum of the emptied zoosporangium enlarges and grows into new secondary zoosporangium inside the old one (produces primary zoospore). This process may be continue.

Sexual reproduction



Sexual reproduction is of typical oogamous type male sex organ is called antheridium and female sex organ is called oogonium. Species of *Saprolegnia* may be homothallic or heterothallic. Sex organs are formed at the tips of the somatic hyphae.

Fertilization:

1- The antheridium becomes closely applied to the oogonium.
2- At the point of contact a fertilization tube is formed.
3- Each branch of fertilization tybe approaches oospheres witin oogonium.
4- On coming into contact with an ooshpere fertilization tube discharge one male nucleus into it.
5- The male nucleus fuses with female nucleus. The fertilized egg secretes a thick wall around it and is now known as oospore.
6- During favorable conditions oospores start germination by a germ tube, it may grow directly into mycelium or into zoosporangium (meiosis occur during germination of zygote).

C: *Phycomycetes*

O: *Mucorales*

F: *Mucoraceae*

Ex. *Mucor*

Occurrence and habitat: *Mucor* lives in a habitat like organic soil, a dead decaying matter of fruits, vegetables and plants, it is essentially saprophyte.

Structure of *Mucor*

Morphological features

Mycelium

The mycelium of *Mucor* is highly branched forms a fine network of hyphae. A mycelium is simply a cluster of hyphae.

Hyphae

These are the thread like and very fine structures that form a "Mycelial network". Hyphae of Mucor is filamentous, aseptate or coenocytic. In *Mucor*, the hyphae categorize into three types:

1. Sub-terranean hyphae
2. Prostrate hyphae
3. Aerial hyphae

Sub-terranean hyphae are the type which is highly branched, more penetrating and is present horizontally to the substratum.

Prostrate hyphae are the type which is also present horizontally between or under the substratum. These two hyphae i.e. sub-terranean and prostrate hyphae help in absorption of water and nutrition.

Aerial hyphae are the type, which originates vertically out from the prostrate hyphae.

Sporangiophore

It is elongated, slightly narrow in shape.

Columella

Sporangiophore swells up to form a dome-like structure called "Columella" which can vary in both shape and size.

Sporangium

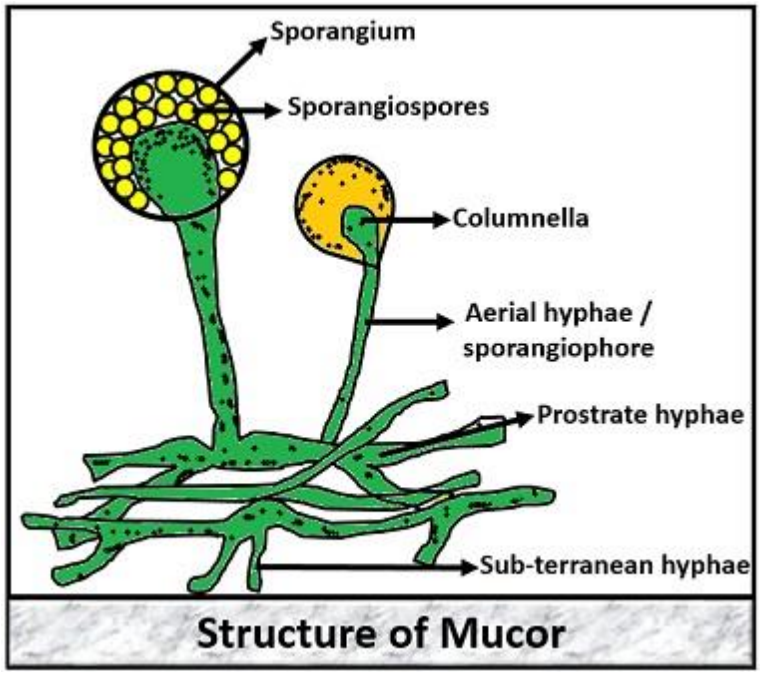
It is the round and thick outer covering which carries numerous spores inside it. It can be globose to spherical.

Spores

These are the reproductive structures forms within the sporangium which are simple, flattened and variable in shape and size.

Nucleus

Multinucleate nuclei present in *Mucor*.



Macroscopic features

- The colony of *Mucor* shows rapid growth.
- The colour of the colony is usually white to grey and turns to brown when the culture becomes old.

Microscopic features

- Hypha: Coenocytic and branched

- Spores: Generally black in colour but can vary with different species. The spores can be motile or non-motile and can exist in variable shapes.

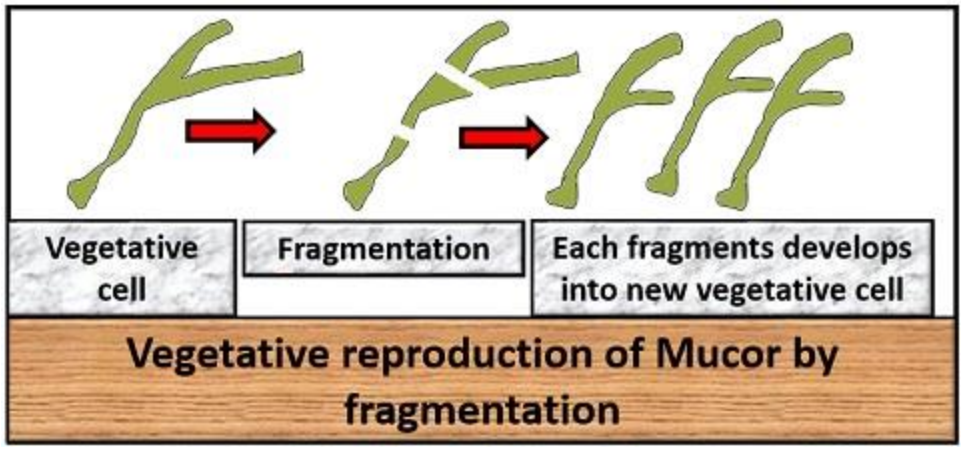
Life cycle of *Mucor*

There are three types of reproduction methods in its life cycle:

1. Vegetative reproduction
2. Asexual reproduction
3. Sexual reproduction

Vegetative Reproduction

It occurs by the fragmentation method, where a vegetative cell breaks into several fragments during some unfavorable conditions. After which, each fragment then develops into a new vegetative body.



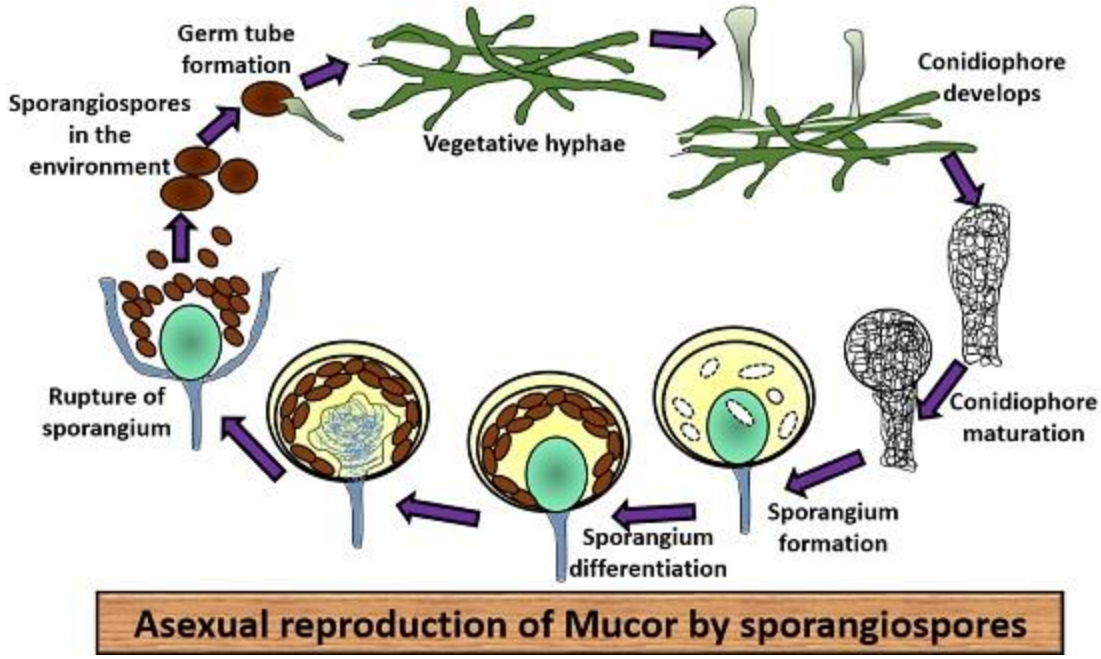
Asexual Reproduction

It occurs through the asexual and non-motile spores like:

- Sporangiospores
- chlamydospores
- Oidiospores

Sporangiospores

These are the spores form within the cell or sporangium and are non-motile. There are following steps involved in asexual reproduction of *Mucor* through sporangiophores:



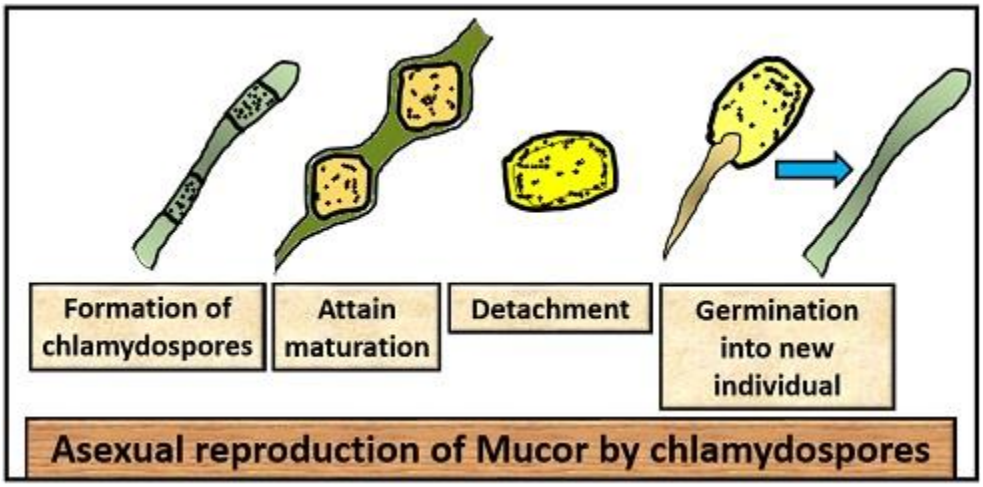
- From the hyphae, first **sporangiophores** arise singly and are erect in position and unbranched.
- Then, maturation of sporangiophore occurs where the cytoplasm and nuclei push upwards by making the aerial hyphae swollen from the apical end.
- After that, it develops a large round **sporangium**.
- During this, maturation phase, sporangium differentiates into:

- **Sporoplasm**: It is thick, dense, multinucleate and present inside the sporangial wall.
- **Columellaplasm**: It is vacuolated and nucleated towards the centre.

- After this, a number of small vacuoles appear between these differentiated portions. The space between the vacuoles forms cleavage furrows (cavity for cleavage).
- Then, to the inner side of cavity septum forms that further divides the **inner columella** and **upper sporoplasm**. This septum then grows to form a dome shape and push itself into the sporangium.
- Cleavage occurs in the sporoplasm between the nucleus and the cytoplasm. This division forms a wall around many thin-walled, multinucleate spores called "**Sporangiospores**".
- The sporangiospores then releases out of the sporangia when columnella swells up which creates pressure on the sporangial wall cause **cell lysis**.
- The spores remain dormant for some time and when they obtain suitable substratum they germinate to a new vegetative body through germ tube.

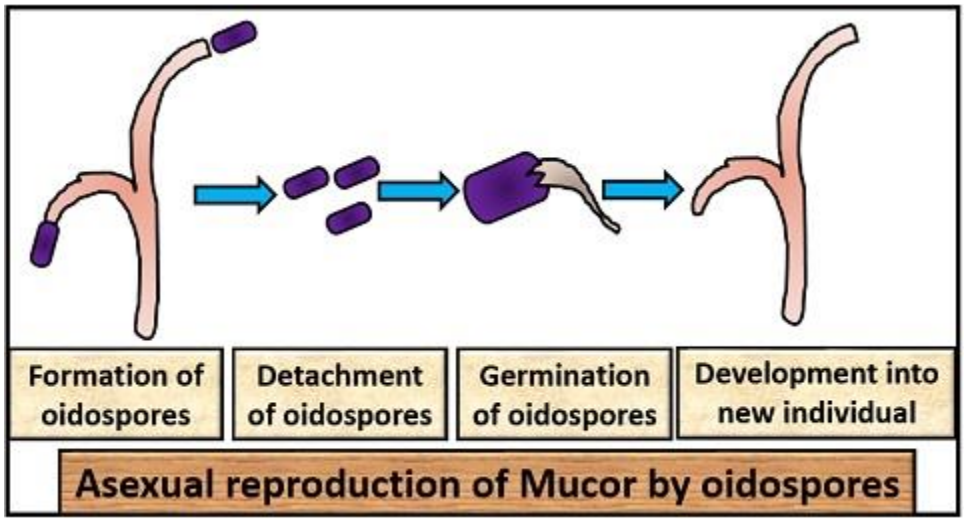
Chlamydospores

These spores are covered by a hard wall, which forms inside the vegetative cell during unfavourable conditions. In unfavourable conditions, mycelium becomes septate by the accumulation of nuclei and cytoplasm in a certain portion surrounded by a thick wall forms Chlamydospores. This spore then detaches from the mycelium and remains dormant. On favourable conditions, they form a germ tube.



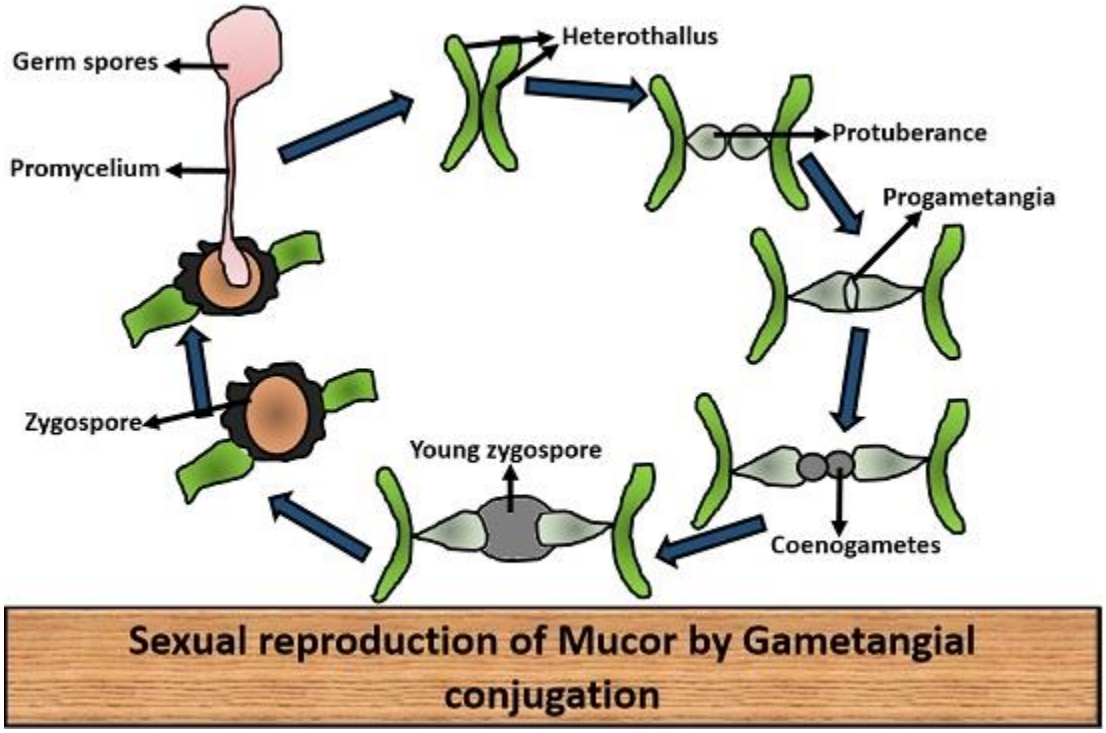
Oidiospores

When a mycelium grows in a substrate (rich in sugar), some small, thin-walled and pearl-like reproductive structure forms. It detaches out of the vegetative cell-like budding in yeast. Then oidospores remain dormant for some time and on favorable conditions it forms a germination tube to form a new vegetative body.



Sexual Reproduction

In *Mucor*, the sexual reproduction occurs by the method refer to "*Gametangial conjugation*" which involves the following steps:



Sexual reproduction of Mucor by Gametangial conjugation

- First, the thallus of two opposite strains i.e. one is (+) and other is (-), comes in contact with each other.
- When they come in contact, there develops a small outgrowth or protuberance from both of the thalli.
- After that, the outgrowth swells to form "**Progametangium**".
- Then septum develops between the progametangium and the fusion of progametangia occurs which results in the formation of gametes refers to "**Coenogametes**".
- Then gametes of both the strains fuse with each other to form "**Zygote**".
- The zygote then enlarges in size and get surrounded by a thick-walled structure called "**Zygospore**".

- Zygospore is dark black in colour which develops and get covered by two layers namely:
  - Outer layer: Also refers to "**Exosporium**"
  - Inner layer: Also refers to "**Endosporium**"

The zygospore remains dormant for some time and on favorable conditions, promycelium develops out from the zygospore, forming a new vegetative body.

Through these three reproductive methods, a *Mucor* completes its reproductive phase and can cause some serial infections or diseases that can affect the ecological system and human health.

Class 2: Ascomycetes

Occurrence :Ascomycetes are found in variety of habitat. The members may be parasitic or saprophytic.

Structure : except for members such as yeast the plant body of ascomycetes is

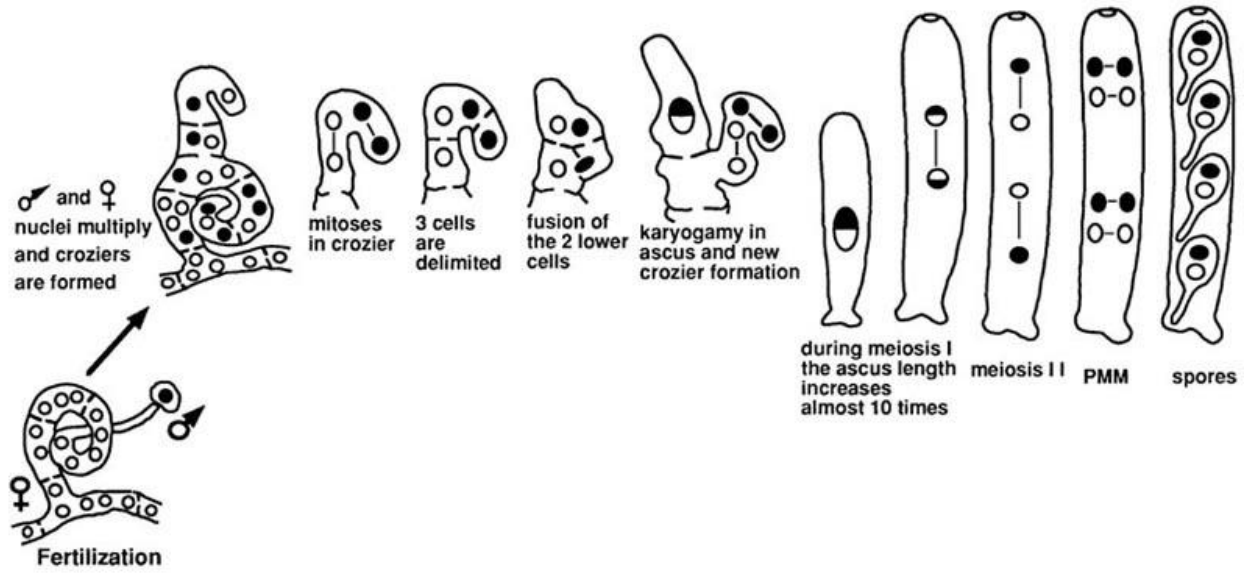
- A septate much branched mycelium.
- Cell wall contains a large portion of chitin.
- The cells of the hyphae may be uninucleate, binucleate or multinucleate.

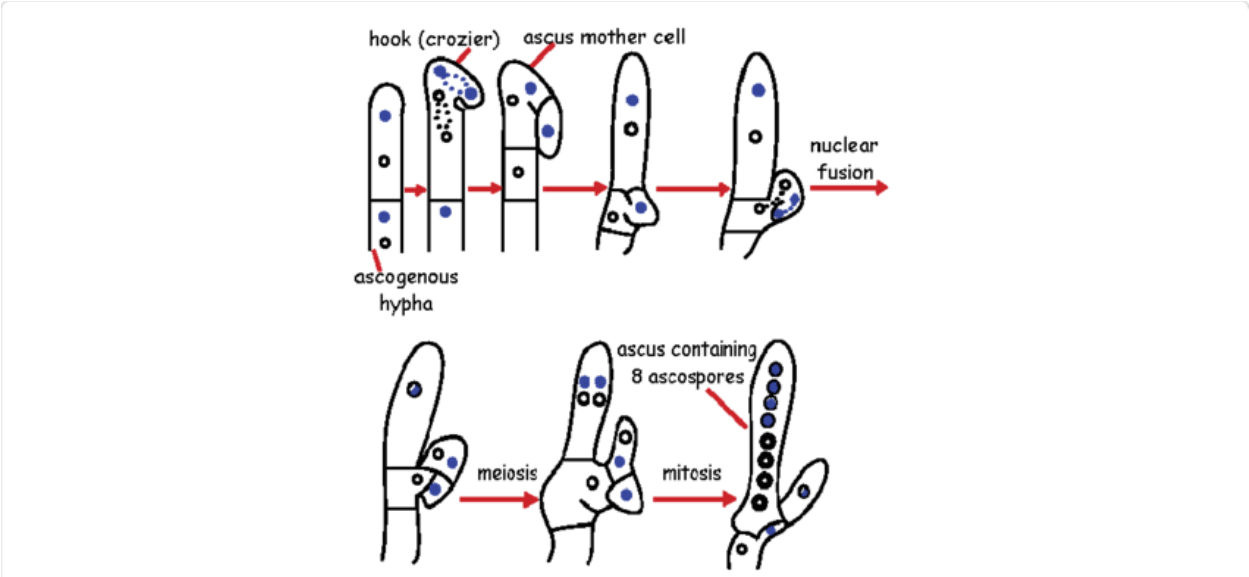
The cross septum dividing the cell is porous permitting the streaming of cytoplasm from one cell to another.

Asexual reproduction; fragmentation, fission, budding, chlamydospore formation and conidial formation.

Sexual reproduction:The male reproductive structure is a club shaped antheridium and the female reproductive structure is flask shaped ascogonium , it has a swollen base and terminal elongated portion called trichogyne. Both the

antheridium and ascogonium are multinucleate. Stages in sexual reproduction are plasmogamy, dikaryotic stage, karyogamy and reduction division.





Ascospore formation

The process of ascus formation:

1- The tip of antheridium comes into contact with the tip of trichogyne. The separating walls dissolve and plasmogamy takes place.

2- The male and female nuclei are irregularly distributed. Later they pair in ascogenous hyphae, one pair into one ascogenous hyphae.

3- Nuclei start dividing and produce many nuclei, attraction between nuclei of different genotype.

4- The ascogenous hyphae now gets divided into basal cell, ascus mother cell and apical cell (croizer).

5- Karyogamy takes place in ascus mother cell forming a diploid nucleus.

6- The diploid nucleus undergoes meiosis followed by mitosis to produce eight haploid nuclei. Each one of the nuclei forms ascospore.

7- The ascus breaks releasing the ascospores which germinate forming monokaryotic mycelium.

Structure and development of fructifications:

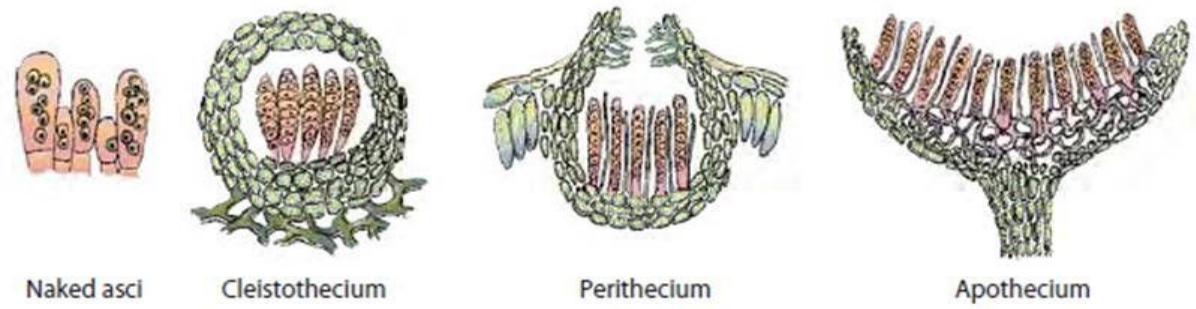
In great majority of ascomycetes the asci are enclosed in compact protective structure called the fruiting body or frutification or Ascocarp. Ascocarps vary in structure and composition, they may be classified into four categories:

(1) **Cliestothecium** is completely closed structure, may be globose or ovoid.

(2) **Perithecium** is flask shaped structures possessing small opening called ostiole .

(3) **Apothecium** is an open type of ascocarp and cup like with a board opening.

(4) **Ascostromata**: asci are formed directly in a cavity in the stroma so there is no wall surrounding the central region of ascocarp.



Classification

Many modern mycologists classify Ascomycetes as follow:

Class: Ascomycetes

Sub-class 1. **Hemiascomycetes** (no ascocarp)

Sub-class 2. **Euascomycetes** (ascocarp present)

Based on the nature of ascocarp the sub class Euascomycetes is divided into three series namely

Plectomycetes (cliestithecium)

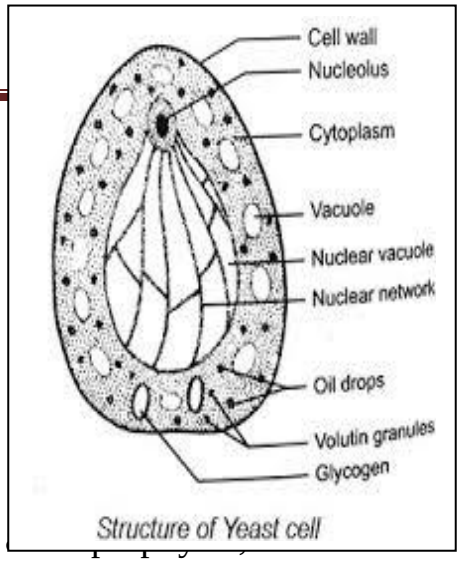
Pyrenomycetes (perithecium)

Discomycetes (apothecium)

The following examples will discussed here:

Hemiascomycetes: *Saccharomyces* (yeast)

Plectomycetes : *Penicillium*



Structure of Yeast cell

C: Ascomycetes

S.C.: Hemiascomycetes

O: Endomycetales

F: Saccharomycetaceae

Ex. *Saccharomyces*

Occurrence: yeasts are widely distributed in nature. They [illegible] commonly found on sugar substrata like fruits.

Cell structure:

Antony Von Leeuwenhoek (1680) was the first to describe the yeast cells. Its unicellular and non-mycelial.

Generally, the shape of cells may vary from circular, spherical, oval, elliptical, elongated, rectangular, dumb-bell shaped to triangular. The cells are minute and range from 2 to 8 µ in diameter and 3 to 15 µ in length. Individually, the cells are hyaline (colorless) but its colonies appear white, cream-coloured or light brown. Each cell consists of a tiny mass of protoplast surrounded by a definite cell wall.

The Cell Wall:

The cell wall is double layered, thin, delicate and flexible. It is composed of two complex polysaccharides, mannan (30%) and glucan (30-40%) with smaller quantities of protein (6-8%), lipid (8.5 – 10.5%) and chitin (2%). Cellulose is absent.

The Protoplast:

Inner to cell wall is a cytoplasmic membrane or plasma membrane. It surrounds the cytoplasm and a nucleus. Under light microscope, a large hyaline structure, occupying a large portion of the cell and a deeply staining body associated on one side of it is seen.

Electron microscopic studies of ultra-thin sections of *S. cerevisiae* and of *S. octosporus* show that the nucleus is surrounded by a nuclear membrane and is distinct from the vacuole.

The nuclear membrane has pores. The cytoplasm in addition to the various cell organelle (mitochondria, endoplasmic reticulum, ribosomes etc.) contains glycogen, proteins, oil and refractile volutin granules (an inorganic metaphosphate polymer) as reserve food materials.

Asexual Reproduction:

Yeasts reproduce asexually either by fission or by budding. Depending on this character they are grouped as fission yeasts, Schizosaccharomyces and budding yeasts, Zygosaccharomyces.

Fission:

1- Yeasts the parent cell elongates (Fig. 217A & B), the nucleus divides into two daughter nuclei

2- Gradually a transverse partition wall dividing the mother cell into two daughter cells (Fig. 217 C & D).

3- The two daughter cells so formed may remain together for some time and begin to divide again or they may separate soon and then divide.

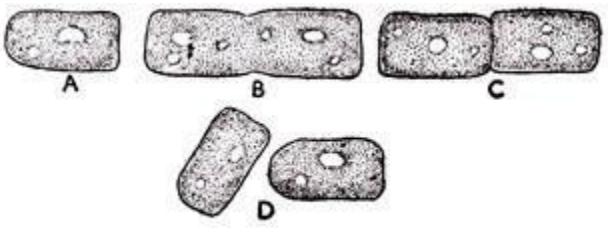


Fig. 217. *Schizosaccharomyces octosporus*. Stages in cell multiplication by fission.

Budding:

Budding yeasts are rather common than the fission yeasts.

1- A small portion of the cell wall, usually near the end, softens.

2- The nucleus of the mother, cell divides mitotically. One of the two daughter nuclei migrates into the enlarging bud (Fig. 218G & D).

3- The bud grows until it attains the size of the mother cell. The daughter cell then becomes separated from the mother cell and the process may be repeated indefinitely (Fig. 218E).

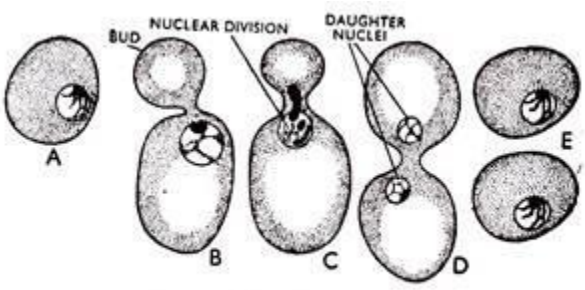


Fig. 218. *Saccharomyces cerevisiae*. Stages in cell multiplication by budding.

Eventually the bud separates from the parent cell leaving a bud scar and the process may be repeated giving rise to chains or groups of yeast cells.

In this way a large number of buds are developed without being detached from one another resulting in the formation of branched or unbranched chains of cells constituting the pseudomycelium. The cells in chains for pseudomycelium are loosely joined together. Sooner or later, however, the chains break into their constituent cells.

Sexual Reproduction:

It takes place by the union of two cells more often similar in size but sometimes they may be dissimilar in appearance, and by the development of short protuberances which unite to form a conjugation tube. This is followed by the dissolution of intervening walls and nuclear fusion which takes place in the conjugation tube.

The subsequent stages' are extremely variable and are discussed separately. The copulating pair of cells may be vegetative cells or ascospores. Often copulation occurs between a mother cell and its bud. This is known as pedogamy and is observed in *Zygosaccharomyces chevalieri*. Yeasts may be homothallic or heterothallic.

Sexual reproduction of yeasts was first clearly recognized by Guillermond (1901-1902). He demonstrated copulation of yeast nuclei and the subsequent stages leading to the ascospore formation. The number and shape of ascospores are variable (Fig. 219). In 1940 Guillermond showed that three life cycle patterns are distinguishable among yeasts.

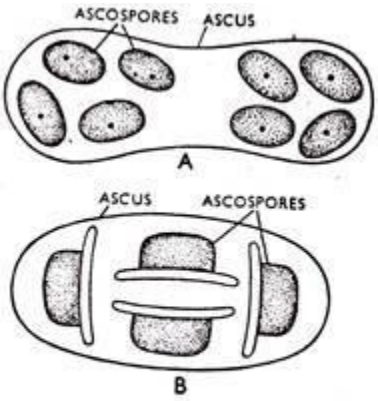


Fig. 219. Various types of yeast ascospores. A. *Schizosaccharomyces octosporus*. B. *Hansenula* sp.

They are:

I. Haplobiontic Life Cycle:

This is exhibited by *Schizosaccharomyces octosporus* which is homothallic. Here the haploid stage (haplophase) is very elaborate. Whereas, the diploid stage (diplophase) is very short being confined to the zygote cell only. Meiosis of the diploid zygotic nucleus takes place immediately after karyogamy. The somatic cells are haploid and elongated. They divide by fission forming daughter cells. Life cycle is presented in Figure 220.

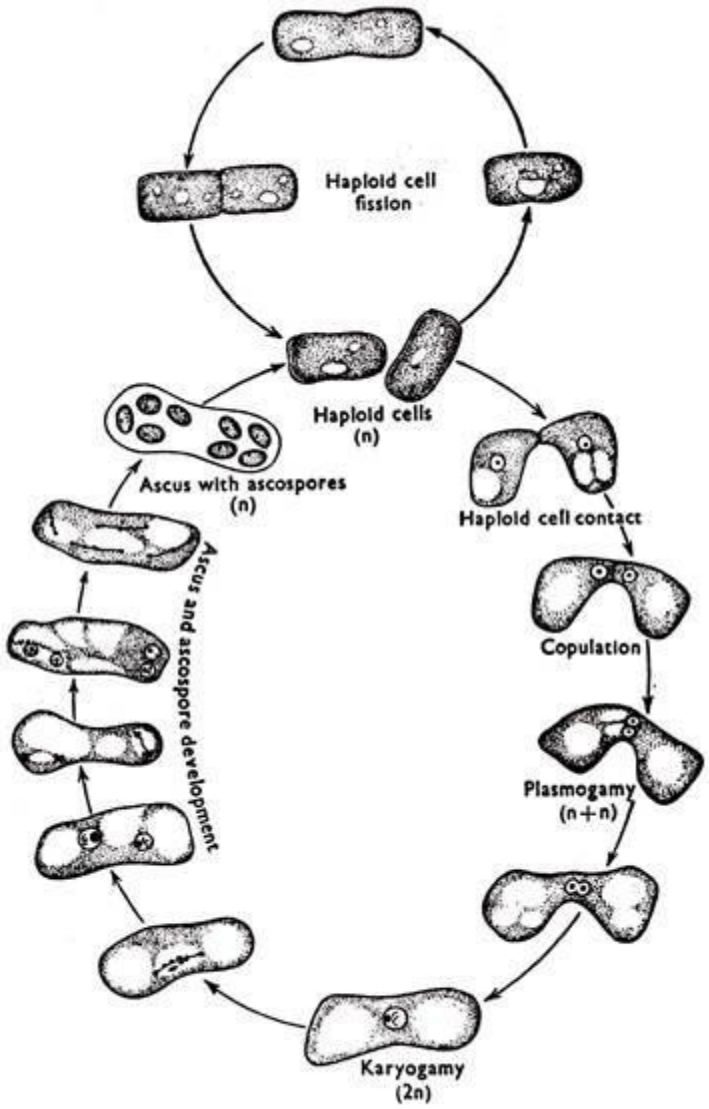


Fig. 220. Haplobiontic life cycle of *Schizosaccharomyces octosporus*.

II. Diplobiontic Life Cycle:

This is exemplified by *Saccharomycodes ludwigii*. Here the diploid somatic stage is long and the haploid stage is very short. The diploid somatic cells produce buds which eventually enlarge to function as asci. The diploid nucleus divides meiotically forming four haploid nuclei around which four ascospores are developed. Life cycle is presented in Figure 221.

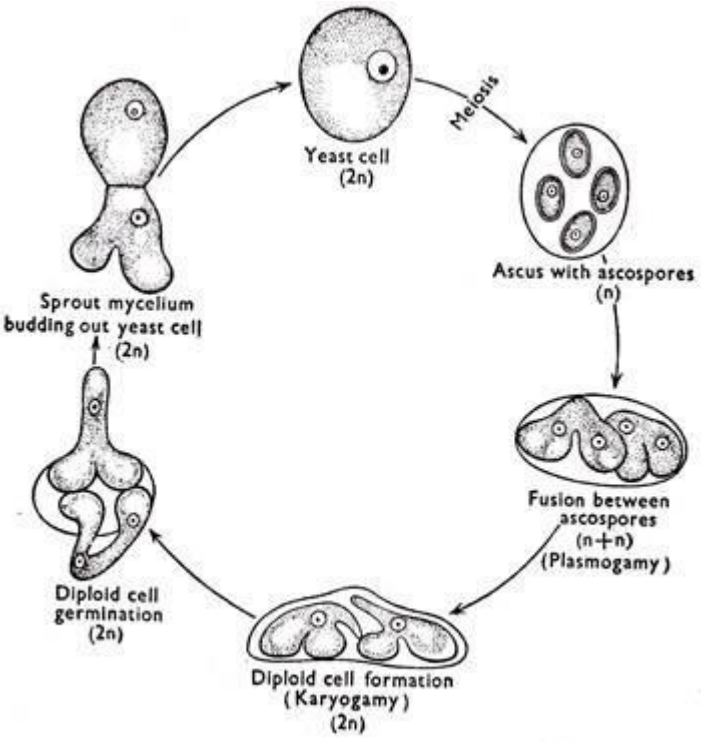


Fig. 221. Diplobiontic life cycle of *Saccharamycodes ludwigii*.

III. Haplo-Diplobiontic Life Cycle:

This is exhibited by *Saccharomyces cetevisiae*. In this type of life cycle both haploid and diploid phases are equally well represented constituting somewhat an alternation of generations. Two haploid cells copulate forming a diploid cell. The diploid cell multiplies by budding producing large number of diploid cells.

Eventually, each diploid cell behaves as an ascus bearing four ascospores and meiosis takes place during the development of ascospores. Life cycle is presented in Figure 222.

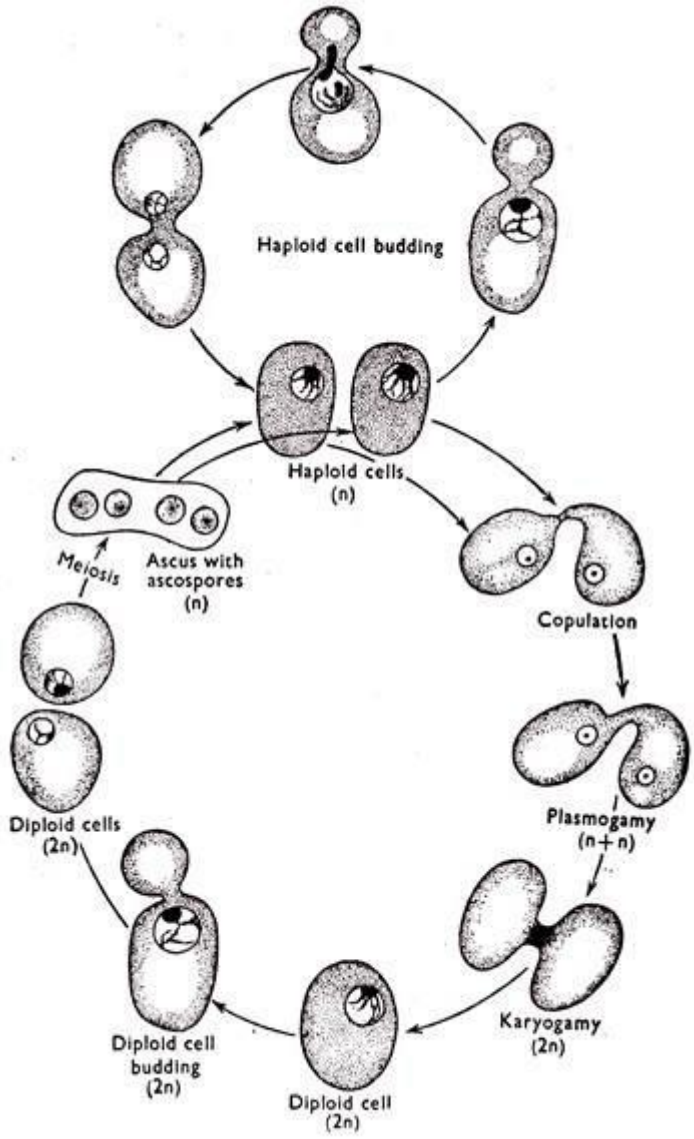


Fig. 222. Haplo-diplobiontic life cycle of *Saccharomyces cerevisiae*.

C: Ascomycetes

S.C.: Euascomycetes

S: Plectomycetes

O:Aspergillales

F: Aspergillalaceae

Ex. *Penicillium*

Occurrence :*Penicillium* is commonly called green or blue mold and is a saprophytic fungus, which grows on rotten fruits, rotten vegetables, meat etc.

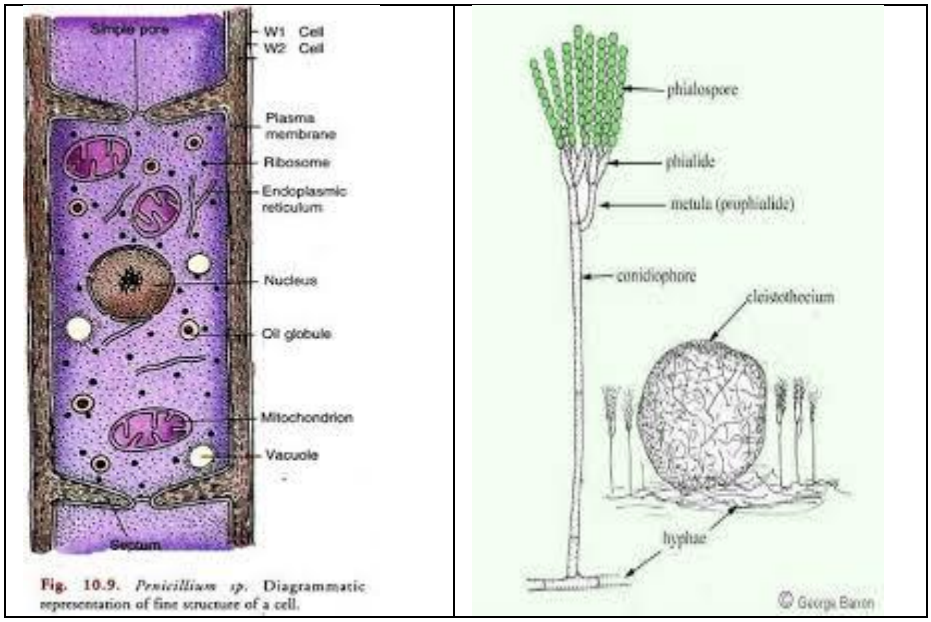


Fig. 10.9. *Penicillium sp.* Diagrammatic representation of fine structure of a cell.

Cell structure: the hyphae are septate and each cell is uninucleate. The cell wall is microfibrillar and in *Penicillium notatum* it is reported to consist of three or four layers, the outer most layer is composed of glucans, the next of proteins, the third of chitin fibrils embedded in a granular matrix, and the inner most of pectic or hemicellulosic material. The plasma membrane surrounds the cytoplasm in which mitochondria, ribosomes and endoplasmic reticulum is embedded.

Reproduction:

Vegetative Propagation: The vegetative reproduction takes place by fragmentation during which the hyphae break up into short fragments, which grow by repeated division into a new mycelium.

Asexual Reproduction: The asexual reproduction is by formation of non-motile, asexual spores, the conidia, and produce at the tip of special, erect, hyphae called conidiophores. Many crops of conidia are produce during a growing season.

Conidiophores: The mycelium produces simple, long, erect, conidiophores that branch two third of the way to the tip, in characteristic broom like fashion. The branches of conidiophores end in a group of conidiogenous cells, the phialides that produce conidia at their tips in chain.

Structure and development of conidia: The conidia are tiny, uninucleate, spore like structures which may be globose to avoid in form. The spore wall is pigmented and is differentiated into two layers, an outer thick, ornamented layer, the exine; and inner smooth and thin layer, the intine. The conidia are detached from the conidiophores and are carried by wind to a suitable substratum where they germinate by forming a germ tube. The germ tube elongates, becomes septate to form a new hyphae.

Sexual Reproduction: The perfect state of *Penicillium* is assigned to two different genera, the *Eupenicillium* and *Talaromyces.* All the species are homothallic. In *Penicilllium vermiculatum* the sexual reproduction is oogamous. The male sex organs are antheridia and female sex organ are ascogonia.

Ascogonium: A mature ascogonium is a long erect, multinucleate, tubular structure with curved upper end. It arises from uninucleate, septate hyphae as a finger like, lateral outgrowth which elongates into an ascogonium. The nucleus of the ascogonium divides many times mitotically to produce 32 or 64 nuclei.

Anteridium: while the ascogonium is developing a uninucleate branch originates from a cell of the same hyphae adjacent to the developing ascogonium, or from neighbouring hyphae. This is the antheridial branch. It grows up band coils around the ascogonium. The tip of the antheridial branch swells up and is cut off from rest of the branches to form a uninucleate antheridium.

Fertilization: The tip of the antheridium comes in contact with the walls of the ascogonium and the walls of contact between the two dissolves to form a pore. The protoplast of the gametangia comes in contact with each through this pore. The antheridial and ascogonial nuclei arrange themselves in pairs. Each pair is called a dikaryon.

Development of Ascus and Ascospores: The stimulus of plasmogamy results in septation of ascogonium into binucleate cells. Some of these segments usually those present in the middle, produce outgrowth called ascogenoum which develop into ascogenous hyphae composed of binucleate cells. The tip cells of these hyphae act as ascus mother cells which develop either simply by elongation, or by crozier formation into ascus. Karyogamy takes place in the ascus mother cell and this diploid uncleus undergoes meiosis to produce four haploid nuclei. A mitotic division results in the formation of eight haploid nuclei. These are transformed into ascospores by free cell formation. The ascus are globose or pear-shaped and the unicellular, uninucleate and lens-shaped with a groove around the edge.

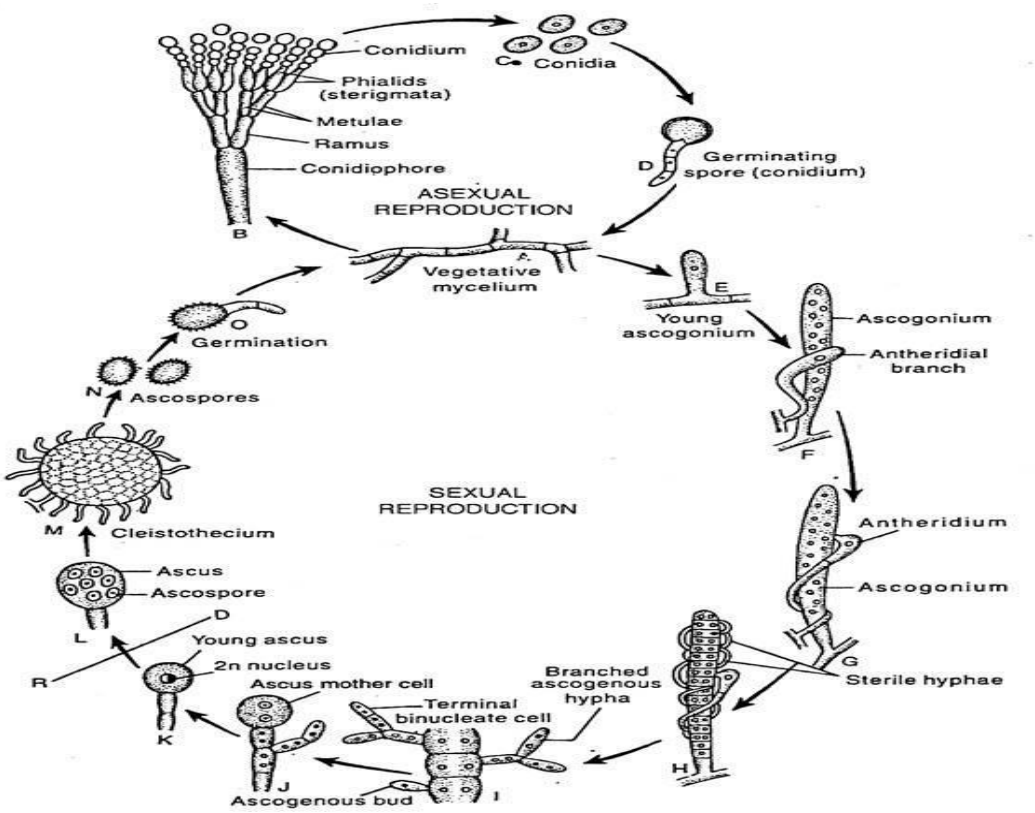


Fig. 4.44 : Life cycle of *Talaromyces vermiculatus* (*Penicillium vermiculatum*)

Class 3: Basidiomycetes

Occurrence: Basidiomycetes are both parasitic (rust and smut) and saprophytic.

Somatic structure: the plant body is a septate, much branched mycelium. The mycelium is usually white, bright yellow or orange coloured. The mycelium of basidiomycetes passes through three stages:

1- Primary mycelium: formed from the germination of haploid basidiospore and it is monokaryotic and the cells are uninucleate.
2- Secondary mycelium: originates from the primary mycelium asa result of sexual reproduction, with only plasmogamy taking place.
3- Tertiary mycelium: at the completion of the life cycle it is produced as a result of karyogamy.

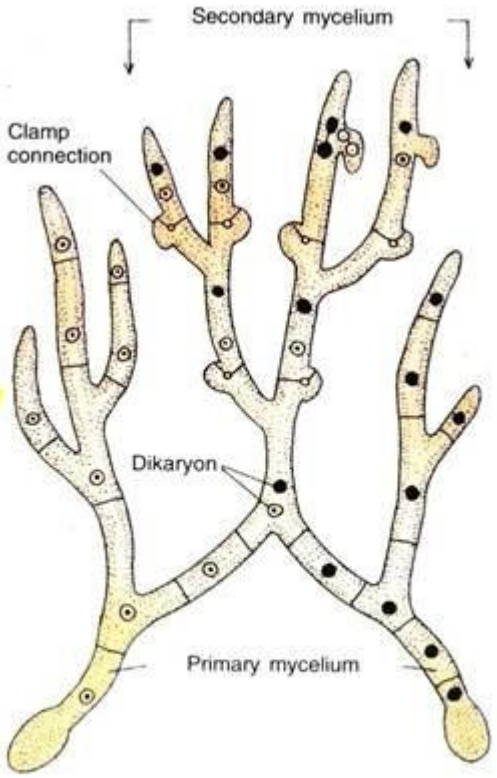


Fig. 13.2. *Basidiomycetes.* Sketch showing the formation of a secondary mycelium from a dikaryotised cell produced by somatogamous copulation between two uninucleate cells of primary mycelia of opposite strains.

The clamp connection: thus simply functions as a bypass. It ensures that the sister nuclei formed by the conjugate division of the dikaryon separate into two newly formed daughter cells. The clamp connections are usually formed on the terminal cells of the hyphae of the secondary mycelium.

Asexual reproduction: takes place by avariety of methods such as fragmentation, budding, conidia and arthrospores

Sexual reproduction: there are four stages

(1) Plasmogamy (2) dikaryotization

(3)karyogamy (4) reduction division

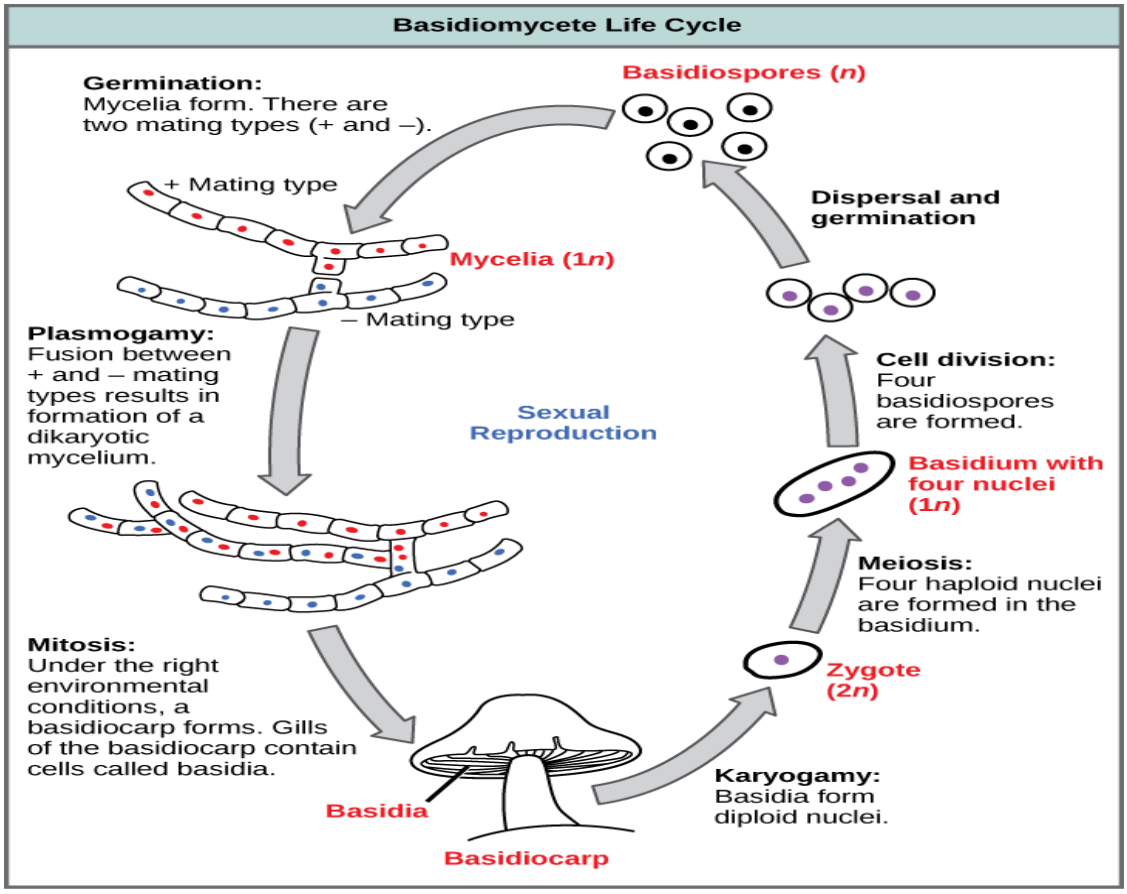
The lifecycle of basidiomycetes includes alternation of generations.

1- Spores are generally produced through sexual reproduction, rather than asexual reproduction. The club-shaped basidium carries spores called basidiospores.

2- In the basidium, nuclei of two different mating strains fuse (karyogamy), giving rise to a diploid zygote that then undergoes meiosis. The haploid nuclei migrate into basidiospores, which germinate and generate monokaryotic hyphae. The mycelium that results is called a primary mycelium.

3- Mycelia of different mating strains can combine and produce a secondary mycelium that contains haploid nuclei of two different mating strains. This is the dikaryotic stage of the basidiomycetes life cyle and it is the dominant stage.

4- The secondary mycelium generates a **basidiocarp**, which is a fruiting body that protrudes from the ground—this is what we think of as a mushroom. The basidiocarp bears the developing basidia on the gills under its cap.



Classification: majority of modern mycologist classified basidiomycetes into two subclasses.

Subclass 1- Heterobasidiomycetidea: is primitive, here no basidiocarp is formed and the basidium is septate. This includes three orders *Ustilaginales*, *Uredinales* and *Tremellales*.

Subclass 2- Homobasidiomycetideae: is advanced here the basidiocarp formed and basidium is unseptate. Depending on the nature of basidiocarp, this is divided into two series:

(1) Hymenomycetes, the basidiocarp is open and the basidia are exposed from the very beginning. This includes only one order: *Agricales*.

(2) Gasteromycetes, the basidiocarp is closed structure. It breaks open only at maturity releasing the basidiospores this includes four orders *Hymenogasterales*, *Nidulariales*, *Lycoperdales* and *Sclerodermatales*.

Basidiomycetes

Agaricales

Agaricaceae

Agaricus

The basidiomycetes are commonly called the higher fungi. The genus *Agaricus* is commonly called mushroom. Agaricus campestris is one of our popular mushrooms which is cultivated for its delicous fruiting body. Not all mushrooms however are edible, some of them in fact are deadly.

Hapitate: It is a saprophytic fungus found growing on soil humus, decaying litter on forest floors, in the fields and lawns, wood logs and manure piles. It grows best in moist and shady places and is commonly seen during rainy season.

Vegetative structure: Vegetative body mycelia consists of septate much branched hyphae. Spore on germination develop into monokaryotic or primary mycelium, either + or- typ. The primary mycelium is short lived and it soon transform into

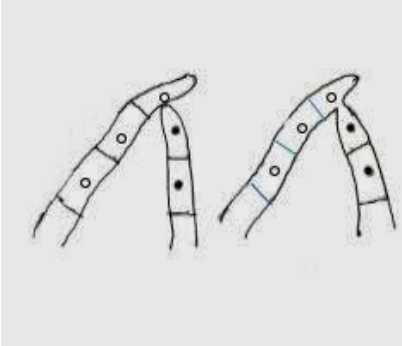
diakaryotic or secondary mycelium by the fusion of two cell of different monokaryotic mycelium following clamp connection. The hyphae of the diakaryotic mycelia interlace twist together to form thick hyphal cord, called rhizomorph which bear the fruit bodies. Agaricus reproduces by all the three means: vegetative, asexual and sexual.

VEGETATIVE REPRODUCTION

It is mostly propagated by vegetative means where dikaryotic mycelium develops spawn, the mushroom seed. The mass spawn divides artificially into small blocks that are grown in soil supplemented with organic manure to obtain fruits bodies.

ASEXUAL REPRODUCTION

It takes place by chlamydospores that are formed rarely during unfavorable condition. Terminal or intercalary chlamydospores are developed on dikaryotic mycelium, which are on germination during favorable condition produce dikaryotic mycelium.

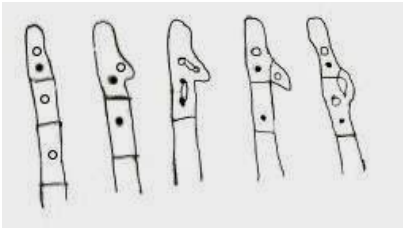


Stages of plasmogamy

SEXUAL REPRODUCTION

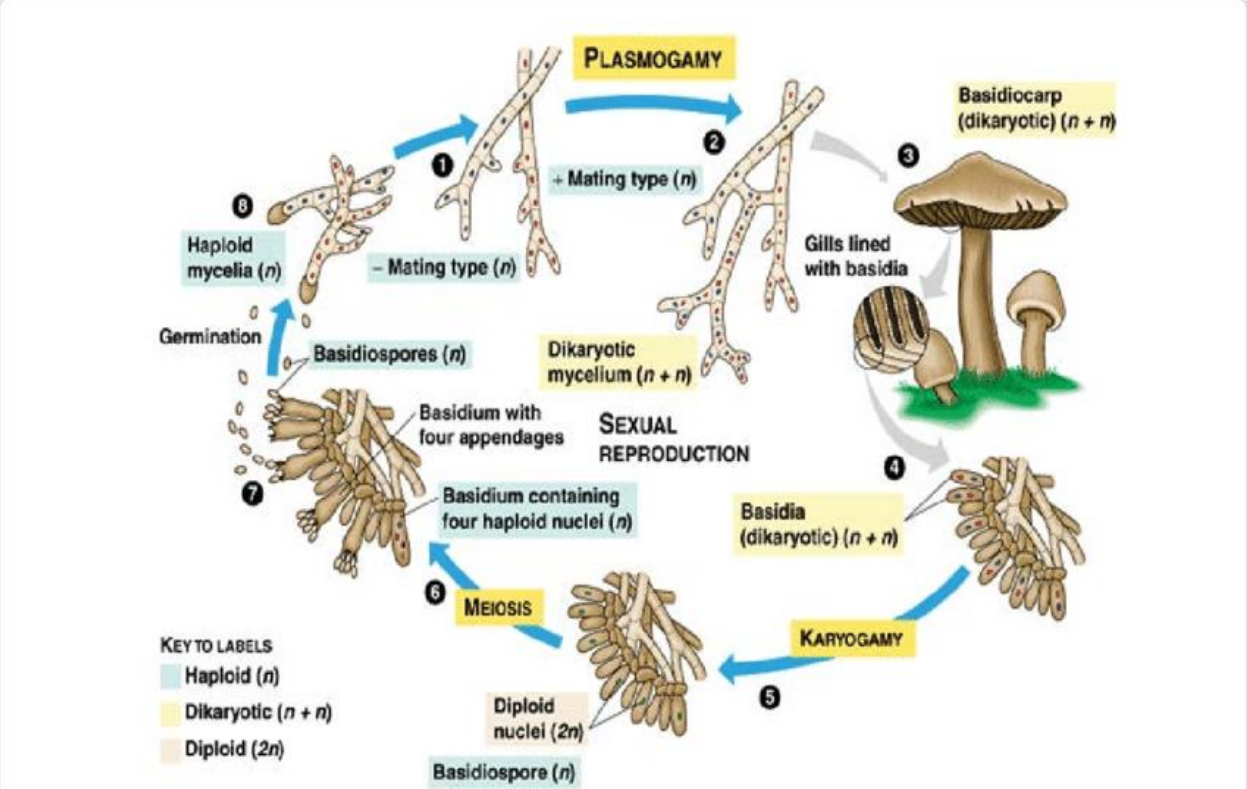
Sex organ are absent in *Agaricus* and sexual reproduction takes place by somatogamy. Most of the species including *Agaricus campestries* are heterothallic. Somatogamy includes plasmogamy, karyogamy, and meiosis. Karyogamy does not take place immediately after plasmogamy, but meiosis follows soon after karyogamy:

1. Plasmogamy: two cells of monokaryotic hyphae of opposite strains (- or +) come in contact with each other. The cell wall dissolve at the point of contact and a dikaryon (n+n) is formed this dikaryotic cell develops into dikaryotic mycelium by regular cell divisions through clamp connection. The dikaryotic mycelia are subterranean and after aggregation at some points they form button which remains dormant before the rain comes during late summer. After rain, the soil become soft and the button develops into fruit body.
2. Karyogamy: it takes place in the young basidium which develops in on gills in the fruit body. Both the nuclei fuse together and form diploid nucleus.

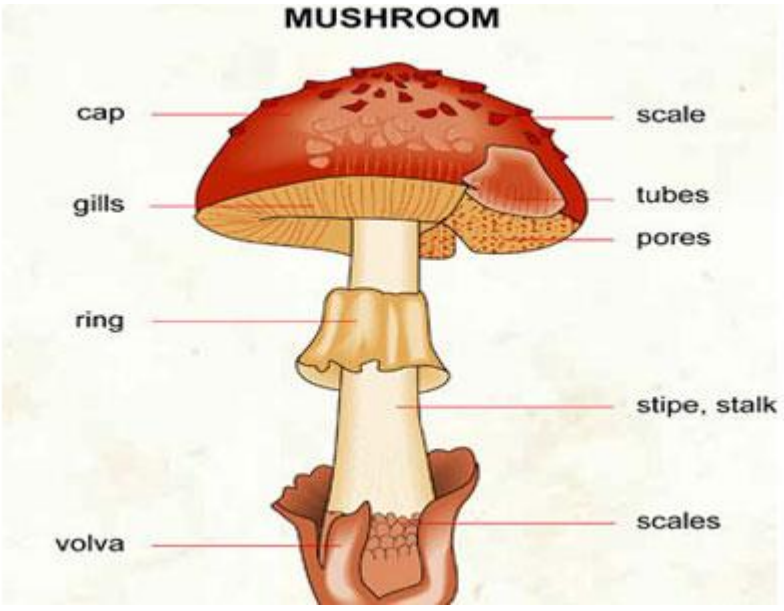


Growth of mycelium by clamp connection

3. Meiosis: it takes soon after karyogamy and forms four haploid nuclei. The basidiospore, thus formed on the sterigma of basidium are haploid and either of + or – type.



Life cycle of *Agaricus*



Class 4: Deuteromycetes (fungi imperfecti)

Deuteromycetes are also called fungi imperfecti. This is an artificial class of fungi created to include all those fungi in which the sexual stage is either absent or not known. Some of the deuteromycetes are unicellular like yeast.

Deuteromycetes have members that belong to both ascomycetes and basidiomycetes.

Reproduction in deuteromycetes is only by asexual spores i.e. conidia formation. They are parasitic or saprophytic in nutrition. Many act as decomposers of litter, thus helping in mineral cycling.

Somatic structure: the thallus of fungi imperfecti consists of a well developed septate branched mycelia. The cells are usually multinucleate. The septa are perforated permitting the streaming of cytoplasm.

Deuteromycetes asexually reproduce by conidia which are borne on conidiophores. The conidiophores may be simple structures or are produced in special structures like Acervulus, Synnema and Pycnidium. In addition to this some Deuteromycetes specially animal and human pathogens produce other types of spores called:

Microconidia are minute conidia.

Blastospores are asexual cells produced as a result of budding or directly from hypha.

Arthrospores are produced by disjoining and isolation of cells. They are otherwise called oidia.

Classification : Deuteromycetes are divided into the following orders

1- Sphaeropsidales conidia are borne in pycnidial cavities
2- Melanconiales conidia are borne in acervuli which are sub epidermal orsub cuticular in the host.
3- Moniliales conidiophores may be simple or branched.

Deuteromycetes

Moniliales

Dematiaceae

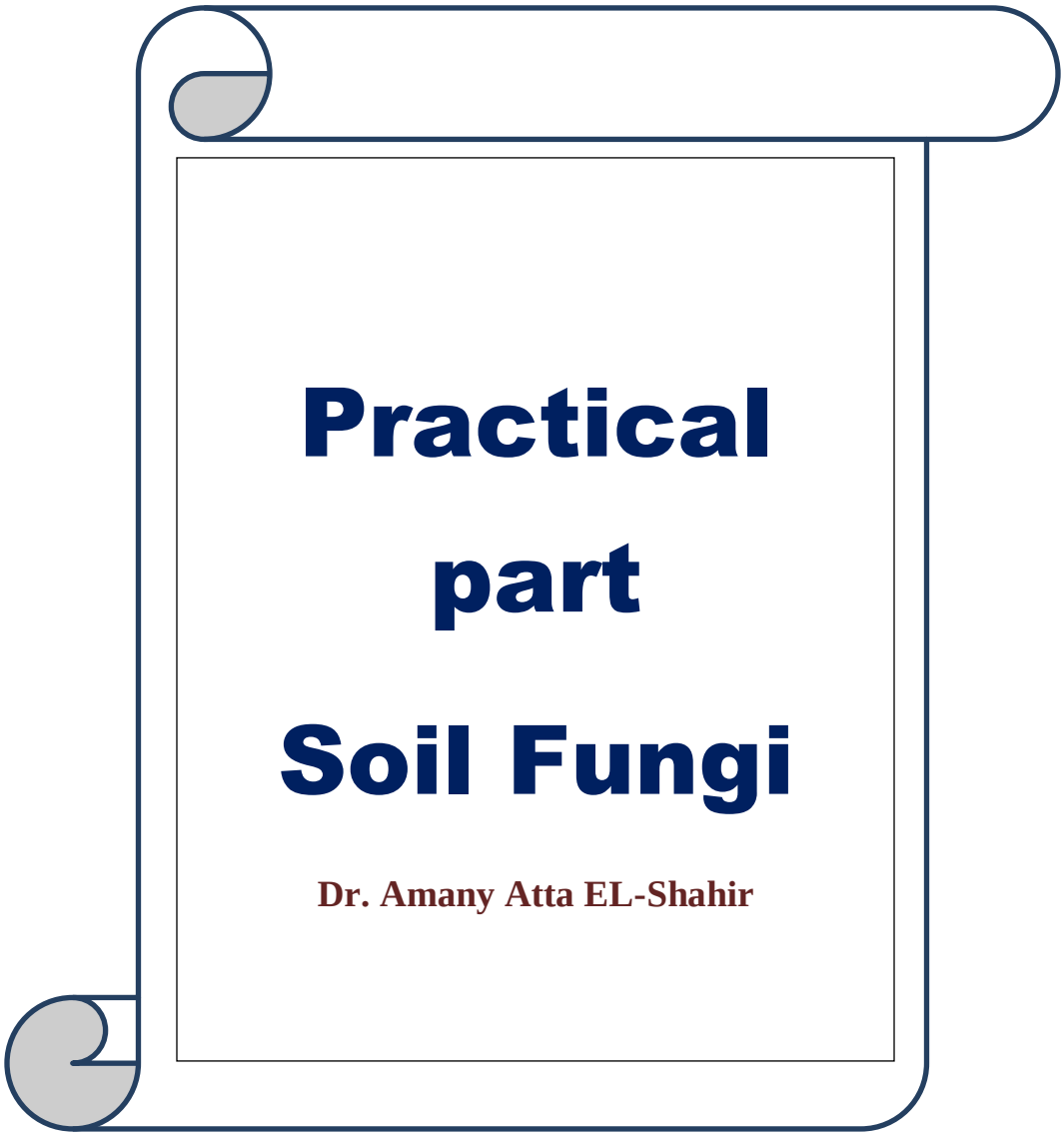
Alternaria

Diseases caused and symptoms:

1- Early blight of potato: this disease is caused by *A. solani* and is widespread in areas wherever potato cultivated.
2- Alternaria leaf spot of cabbage: cruciferous plants like cabbage, mustard, cauliflower and raddish get the attack of leaf spot. *A. brassicae*, *A. brassicola* and *A. raphani* cause the leaf spot disaes. *A. raphani* is specific to raddish only.
3- Leaf blight of wheat: the disease is caused by *A. triticola*.

Vegetative structure: the plant body is a mycelium the mycelium is much branched with septate hyphae. The hyphae are light yellow, hyaline and semitransparentin young conidia. Mature hyphae are of the colour of olive oil.

Reproduction; perfect stage of the fungus are not seen. Asexual reproduction takes place by means of conidia. Conidiophores are simples, unbranched and septate. Conidia are multicellular and have 5-10 cross walls. In some cases the end of conidia have beak like projections. They are dictyosporous, ie., have both transverse and longitudinal septa.



Practical part

Soil Fungi

Dr. Amany Atta EL-Shahir

Safety Procedures for the Microbiology Laboratory

General Laboratory Safety Practices and Procedures

1. If you are **taking immune-suppressants, are pregnant,** or have a known medical condition that would prevent full participation in the laboratory, please contact the course instructor before the first day of lab.
2. Read and understand each laboratory exercise **before** you come to class.
3. Do not eat, drink, smoke, or chew pens in the laboratory.
4. You must wear close-toed shoes while in the laboratory and long pants.
5. No hats of any kind will be allowed in lab, unless allowed by University policy and cleared with the instructor.
6. Long hair should be pulled back to keep it away from bacterial cultures, bacticinerator or open flames.
7. Follow precautionary statements given in each exercise.
8. Personal electronic devices will be turned off and stored while in this laboratory. *The unauthorized use of any electronic device (phone, tablet, computer) in lab will result in a loss of course points.
9. Know where specific safety equipment is located in the laboratory, such as the fire extinguisher, safety shower, and the eyewash station.
10. Recognize the international symbol for biohazards, and know where and how to dispose of all waste materials, particularly biohazard waste. Note that all biohazard waste must be sterilized by autoclave before it can be included in the waste stream.

Figure 1: Biohazard Symbol

11. Keep everything other than the cultures and tools you need OFF the lab bench. Only necessary work material should be at or on the laboratory bench. Coats, backpacks, and other personal belongings will not be allowed on the laboratory bench top. Store them in a place designated by your instructor. This is to prevent cluttering of the workspace and to avoid exposing them to permanent stains, caustic chemicals, and microorganisms used in the exercises.
12. Leave all laboratory facilities and equipment in good order at the end of each class. Before leaving the laboratory, check to make sure the bacticinerator heat sterilizer is turned off.
13. Never, under any circumstances, remove equipment, media, or microbial cultures from the laboratory.

14. No pets are allowed in the laboratory.

Microbiology Specific Laboratory Safety Practices

During the course of the semester in the laboratory you will be taught the methods used in the proper handling of microorganisms. Although you will not be working with any that are human pathogens, exercise caution in handling all material coming in contact with live microbial cultures. All cultures should be handled with respect and proper aseptic technique *as if they were potential pathogens*. This is called **"universal precaution"**. Specific instructions that should be followed:

1. Remember that all bacteria are potential pathogens that may cause harm under unexpected or unusual circumstances. If you as a student have a compromised immune system or a recent extended illness, you should share those personal circumstances with your lab instructor.
2. Wear gloves when working with cultures, and when your work is completed, dispose of the gloves in the biohazard garbage. Lab coats, safety glasses or goggles are also required. These will be stored in the laboratory each week in a ziplock bag.
3. Disinfect your work area both BEFORE and AFTER working with bacterial cultures.
4. Cultures of live microorganisms and any material coming in contact with live cultures must be properly sterilized after use in the laboratory. Your instructor will inform you of specific procedures. Follow the general rules outlined below.

   a. Glassware such as test tubes, bottles, and flasks may be reused and washed after sterilization. These are normally placed on a cart at the front of the laboratory after you have finished an experiment or exercise. BE SURE TO REMOVE LABELS before placing any glassware on the cart. Your instructor will sterilize and then wash these items.

   b. Some materials, such as plastic petri dishes, plastic pipettes, microscope slides, and swabs, are considered disposable. These are used once and if they become contaminated by contact with live microorganisms are sterilized and discarded. All of these disposable contaminated materials should be placed in the designated waste container containing a BIOHAZARD autoclave bag.

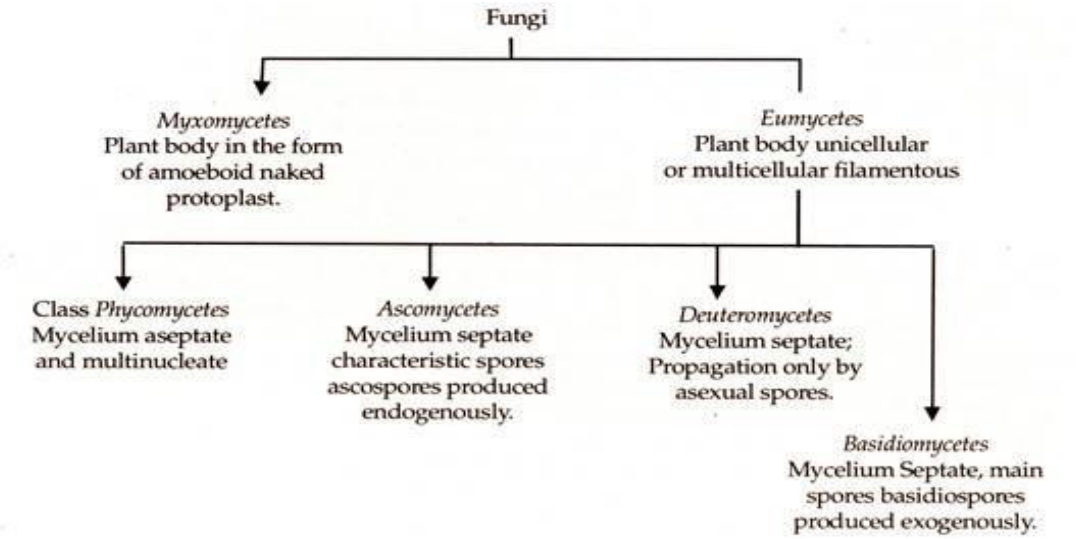
5. Never place contaminated pipette tips (or pipettes), inoculating loop, or any other contaminated material on the bench top. Sterilize loops before and after each use. Place contaminated pipette tips in the orange biohazard buckets on your bench. Place all other contaminated materials in their designated waste containers. Do not place or put anything containing live microorganisms in the sink.
6. Aerosols should be avoided by the use of proper technique for sterilizing the inoculating loops and by performing any mixing of cultures and reagents in such a way as to avoid splashing.

7. Cultures or reagents should always be transferred with an automatic pipettor that will be provided. In no case should one employ mouth pipetting.
8. Always keep cultures capped and in proper storage racks when not being used during an exercise.
9. In the event of an accidental spill involving a bacterial culture, completely saturate the spill area with disinfectant, then cover with paper towels and allow the spill to sit for 10 minutes. Then carefully remove the saturated paper towels, dispose of them in the biohazard waste, and clean the area again with disinfectant. Notify your instructor about the spill. If the chemical is marked "danger" or "caustic" you should notify the instructor who will handle this type of spill.
10. Immediately report all accidents such as spills, cuts, burns, or other injuries to the instructor
11. Make sure that lab benches are completely cleared (everything either thrown away or returned to storage area) before you leave the lab.
12. Clothing worn in the microbiology laboratory should be washed before being subsequently worn in a facility such as a hospital, clinic or nursing home, or in an area of public food preparation.
13. In the event of a fire alarm, follow the directions of your instructor, and meet at the place designated by your instructor.

Classifications

Alexopoulos (1956) places all fungi in the division Mycota. The division Mycota is divided into two subdivisions (1) Myxomycotina (2) Eumycotina (true fungi). Myxomycotina has only one class – Myxomycetes.

Eumycotina has the four classes as shown in the figure.



K. Mycophyta

D. Myxomycophyta

C. Myxomycetes

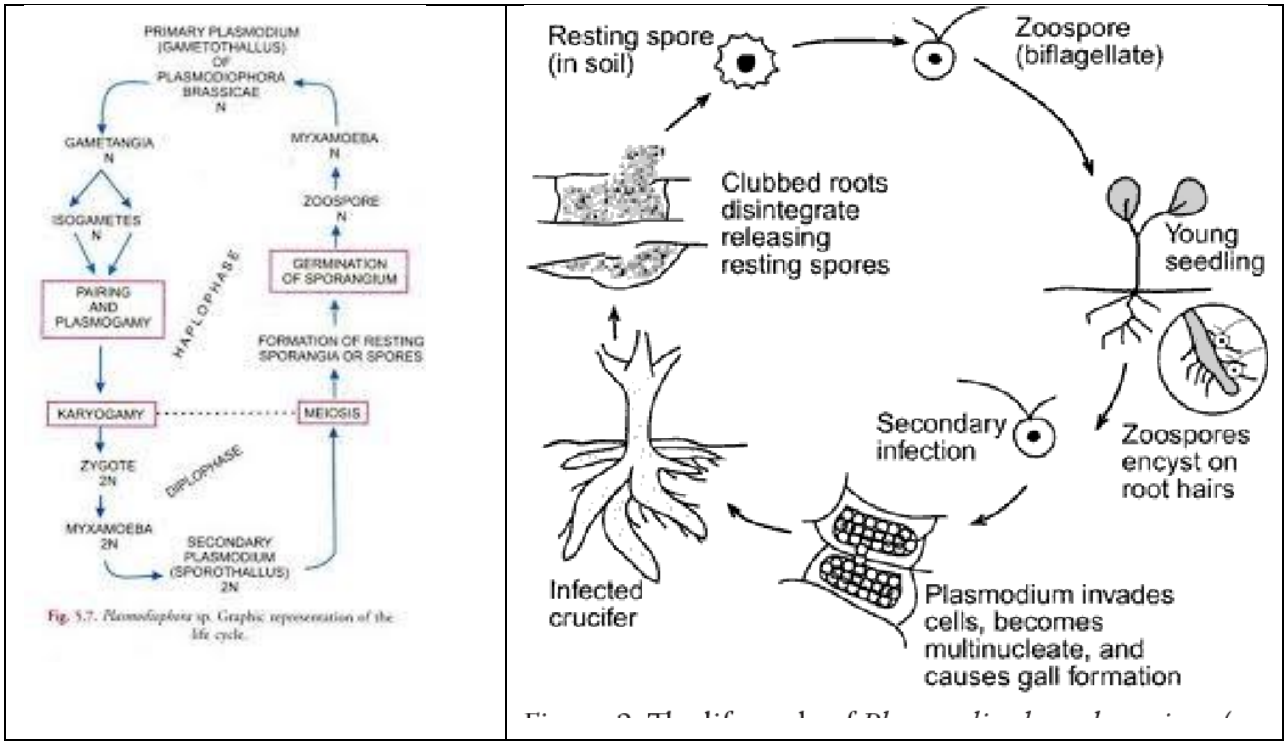
O: *Plasmodiophorales*

F: *Plasmodiophoraceae*

Ex. *Plasmodiophora brassicae*

Please check images in the following link

https://www.shutterstock.com/search/plasmodiophora-brassicae

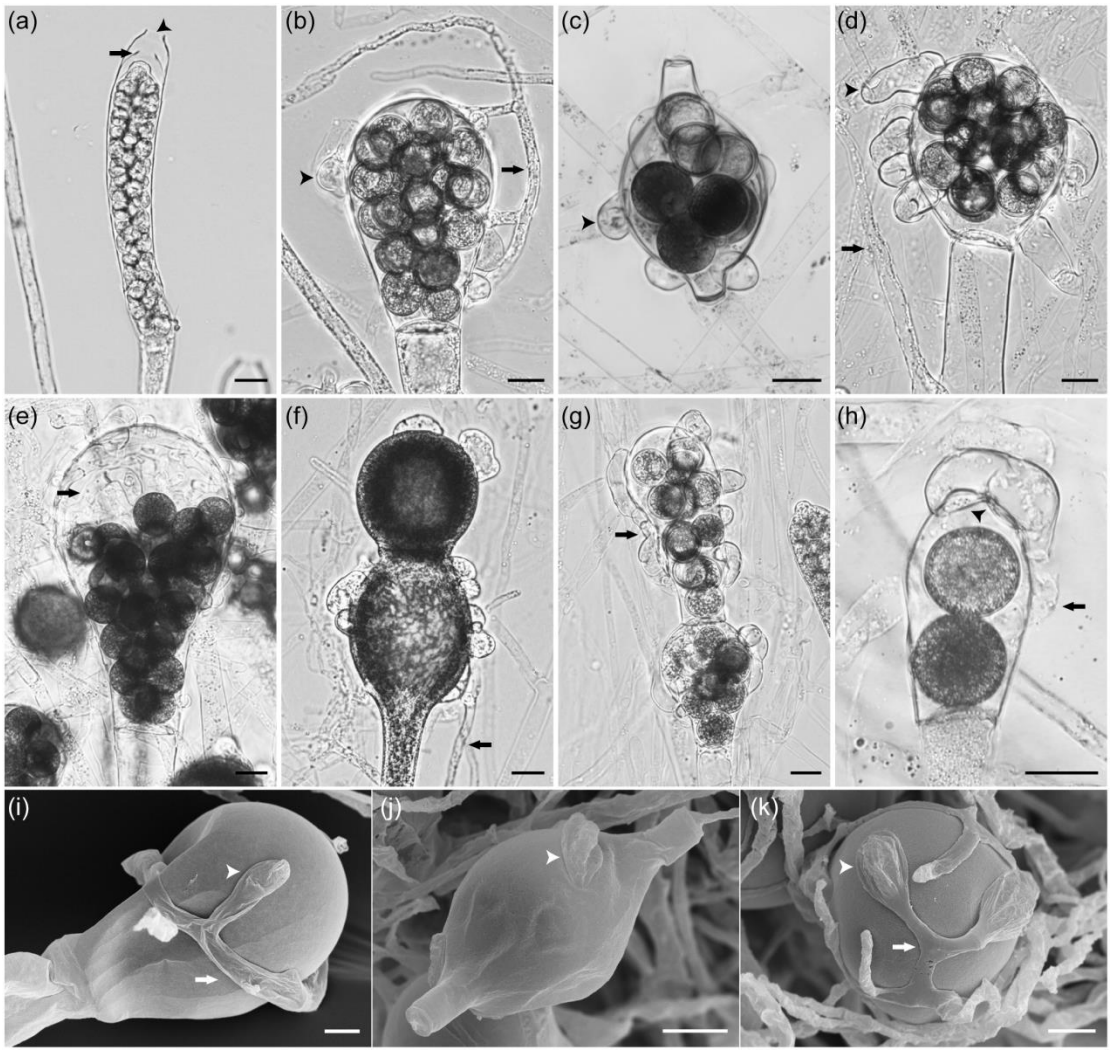


C: Phycomycetes

O: Saprolegniales

F: Saprolegniaceae

Ex.: *Saprolegnia* sp.

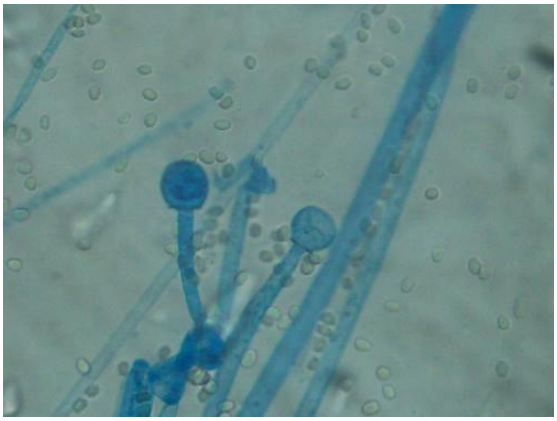


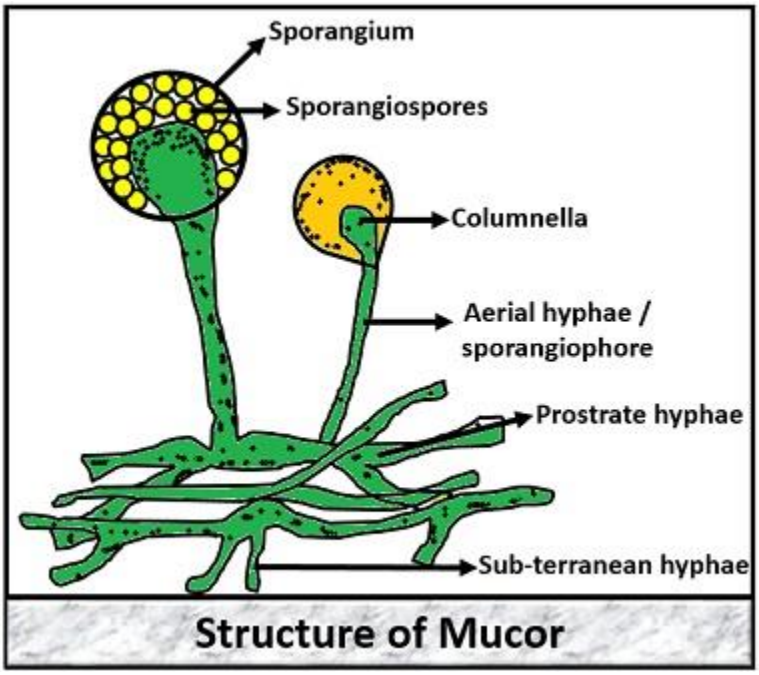
C: *Phycomycetes*

O: *Mucorales*

F: *Mucoraceae*

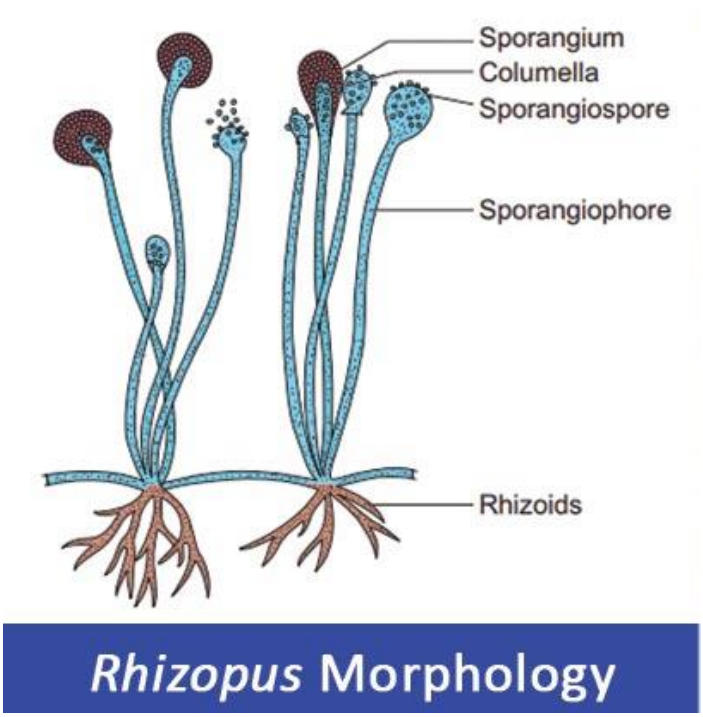
Ex.1. *Mucor*

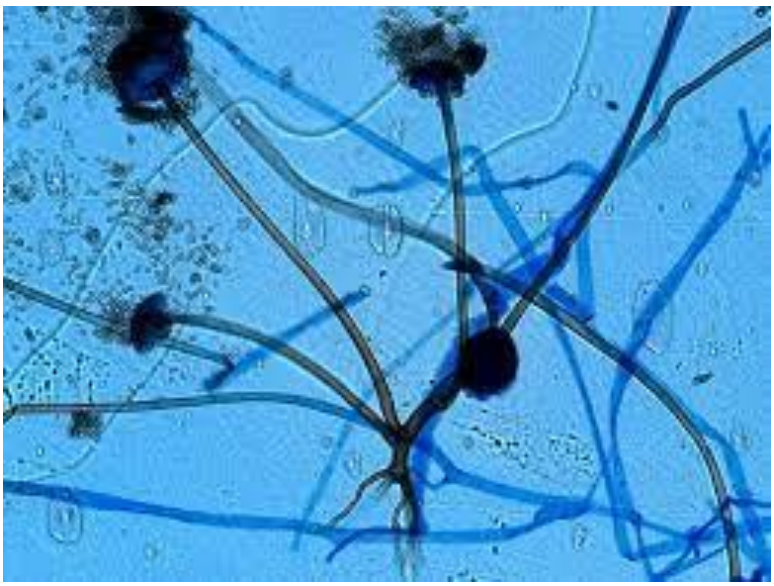




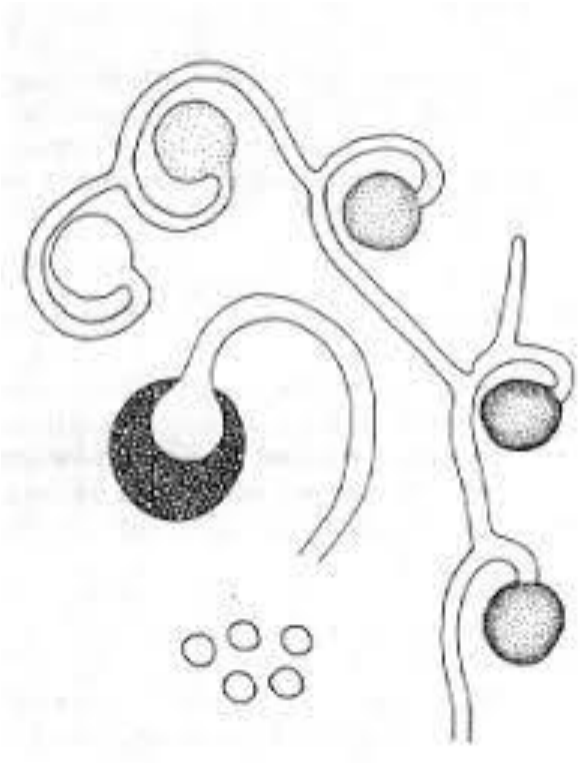
Ex. 2 *Rhizopus* sp.

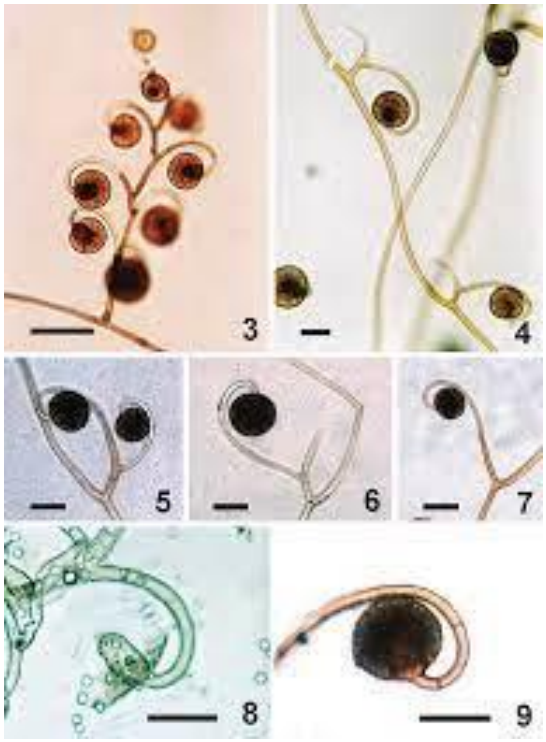
Rhizopus is **a genus of saprophytic and parasitic fungi**. They are found in moist or damp places. They are found on organic substances like vegetables, fruits, bread, jellies, etc. The vegetative structure is made up of coenocytic (multinucleated) and branched hyphae.





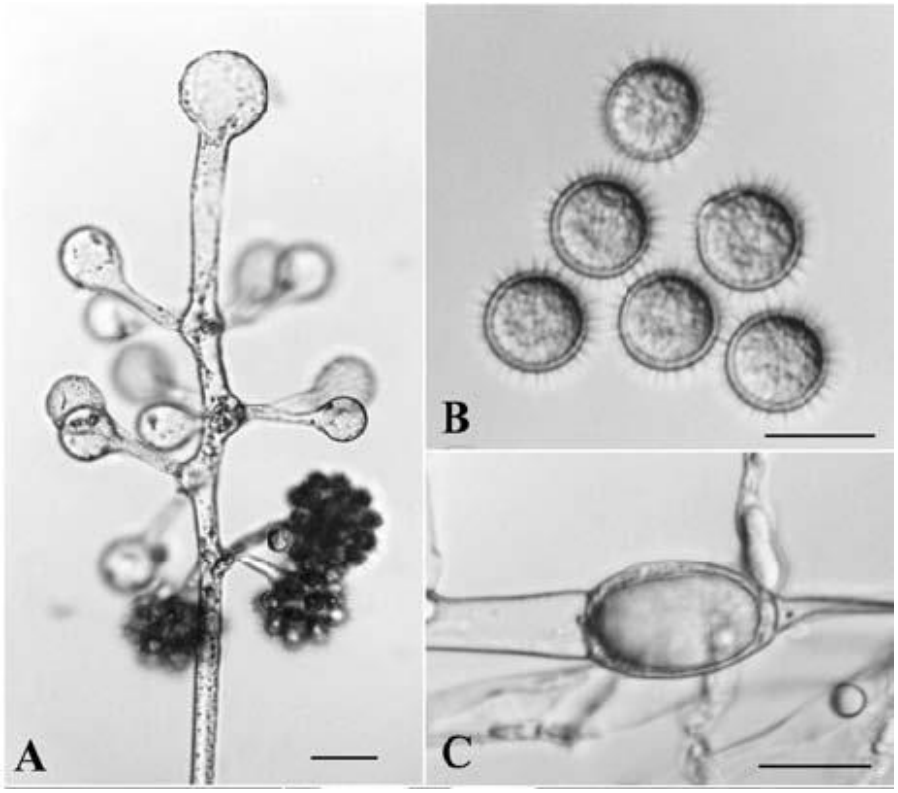
Ex. 3 *Circinella* sp.





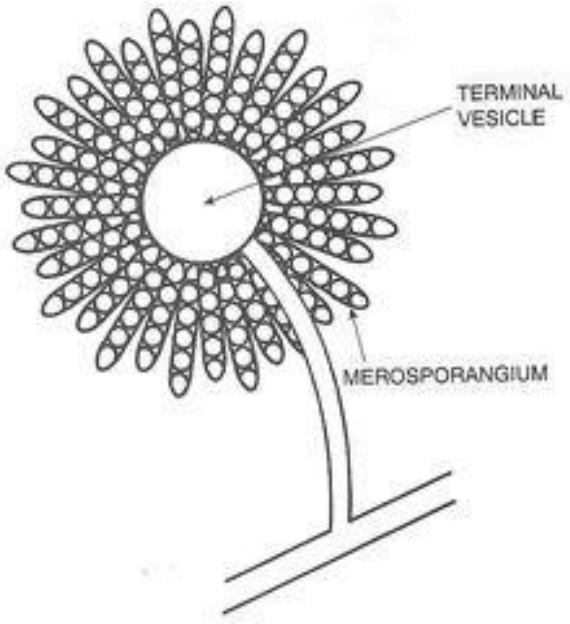
Family 2: *Choanephoraceae*

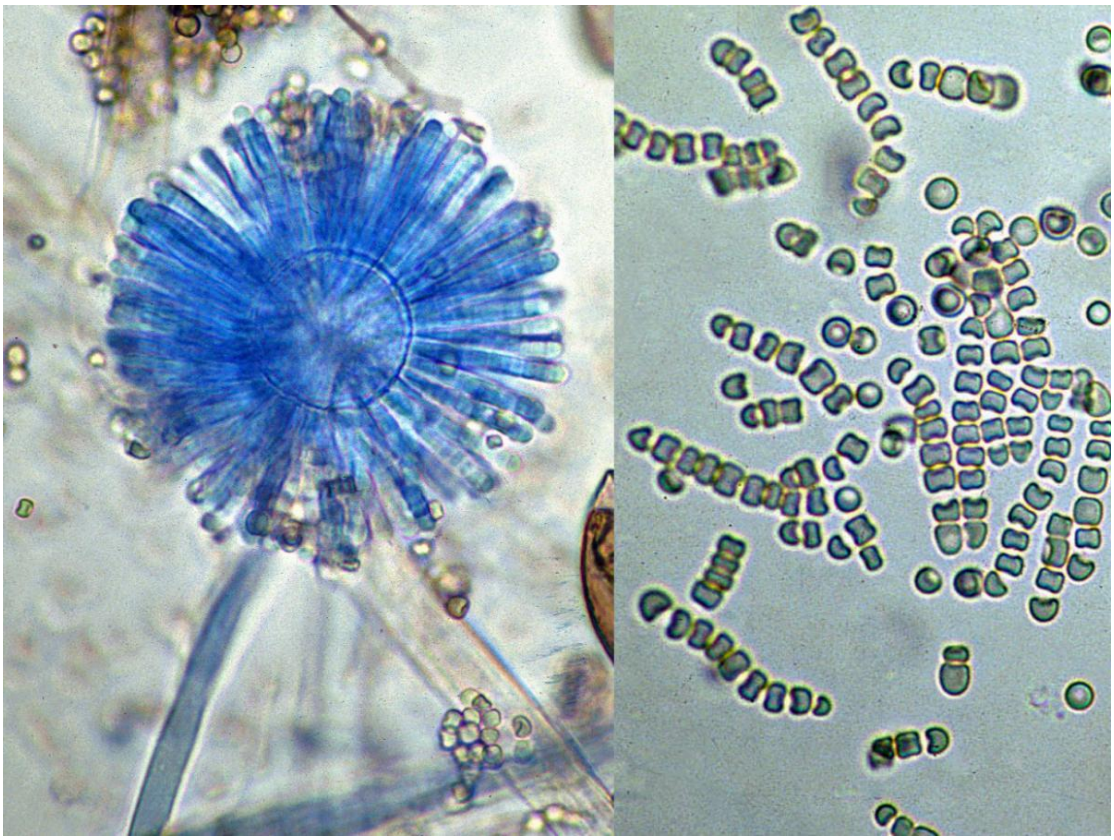
Ex. *Cunninghamella echinulata*



Family 3 : *Cephalidaceae*

Ex. *Syncephalastrum* sp.





Class 2: *Ascomtcetes*

Subclass: *Euascomycetes*

Series: *Plectomycetes*

Order: *Aspergillalaes*

Family : *Aspergillaceae*

Ex.1 *Aspergillus*

General characteristics

1- Colony colour
2- Colony reverse
3- Sterigmata: Biserriate – uniserriate
4- Conidia: globose – subglobose –elliptical – ovate – rough – smooth – hyaline – pigment.
5- Vesicle : globose – subglobose – clavate.
6- Conidial head: radiate – columnar – clavate.
7- Conidiophore: long – short – branched – unbranched – smooth – rough – hyaline – pigment – straight – sinuate.
8- Ascospre
9- Hull cell
10- Sclerotia

Different *Aspergillus* sp.

1	*Aspergillus clavatus*
2	*Aspergillus chevalieri*
3	*Aspergillus fumigatus*
4	*Aspergillus candidus*
5	*Aspergillus flavus*
6	*Aspergillus ochraceus*
7	*Aspergillus niger*
8	*Aspergillus versicolor*
9	*Aspergillus nidulans*
10	*Aspergillus ustus*
11	*Aspergillus flavipes*
12	*Aspergillus terreus*

Ex. 2 *Penicillium*

General characteristics

1- Colony colour
2- Colony reverse
3- Metulae: Present – Absent
4- Penicillin: Monoverticillata – biveticillata – symmetrica – asymmetrica – divaricate – nondivaricata (velutina – lanata – fasiculata).
5- Conidia: globose – subglobose –elliptical – ovate – rough – smooth – hyaline – pigment.
6- Conidiophore: long – short – branched – unbranched – smooth – rough – hyaline – pigment – straight – sinuate.
7- Ascospre
8- Hull cell
9- Sclerotia

Different *Penicillium* sp.

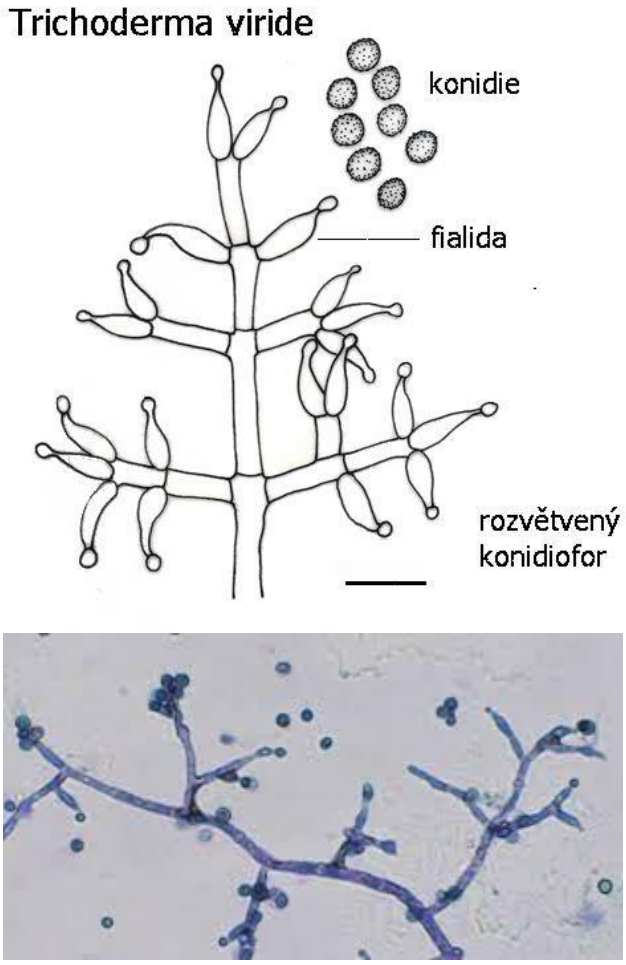
1	*Penicillium corylophilum*
2	*Penicillium duclauxi*
3	*Penicillium funiculosum*
4	*Penicillium chrysogenum*
5	*Penicillium steckii*
6	*Penicillium waksmani*
7	*Penicillium purpurogenum*
8	*Penicillium corylophilum*
9	*Penicillium duclauxi*
10	*Penicillium funiculosum*
11	*Penicillium chrysogenum*
12	*Penicillium steckii*
13	*Penicillium waksmani*
14	*Penicillium purpurogenum*

Class 3 : *Deuteromycetes*

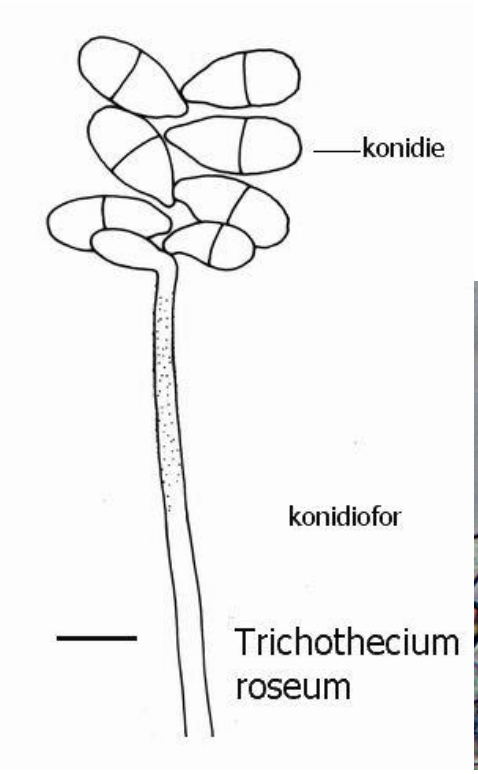
Order: *Moniliales*

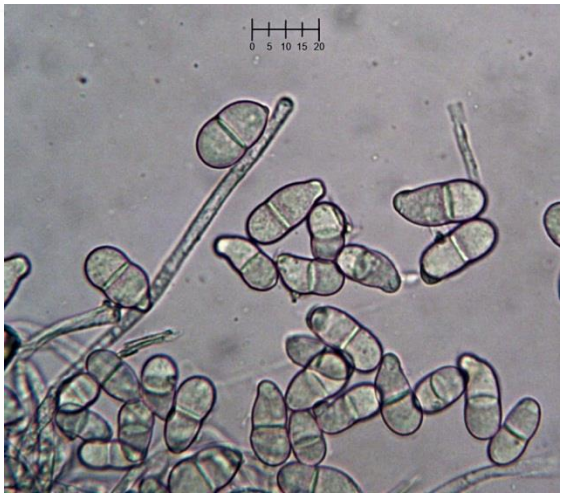
Family: *Moniliaceae*

Ex. 1: *Trichoderma*

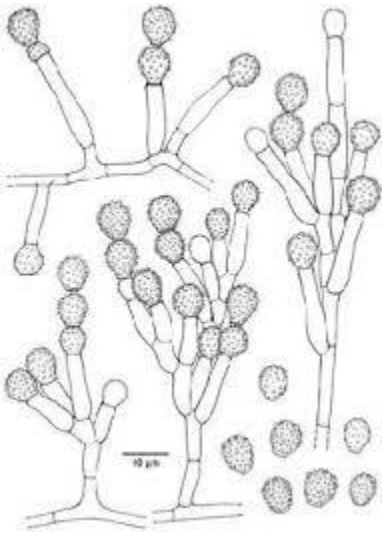


Ex. 2: *Trichothecium roseum*

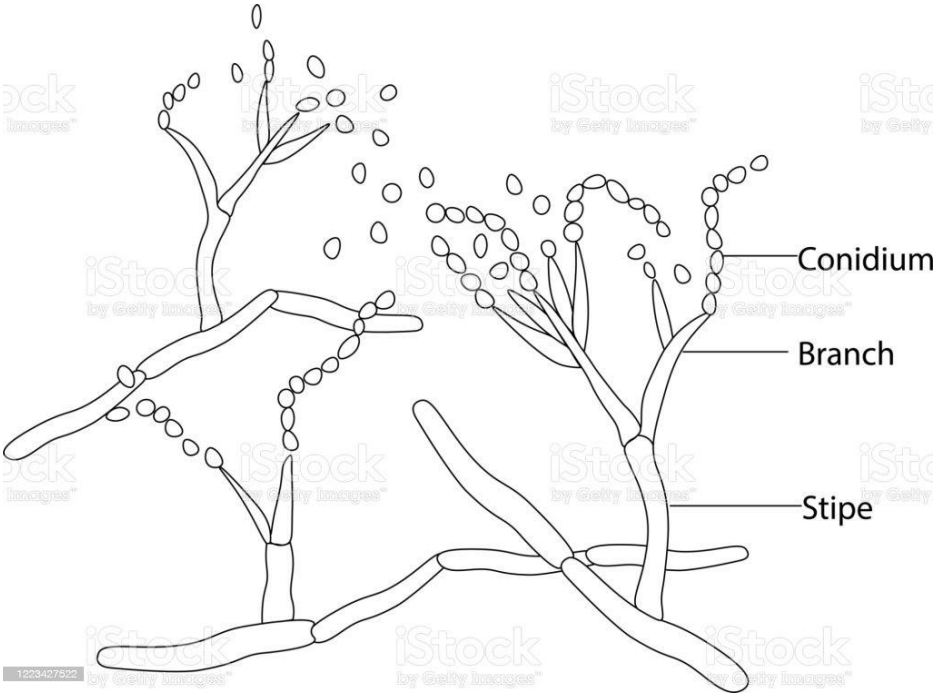




Ex. 3: *Scopulariopsis*

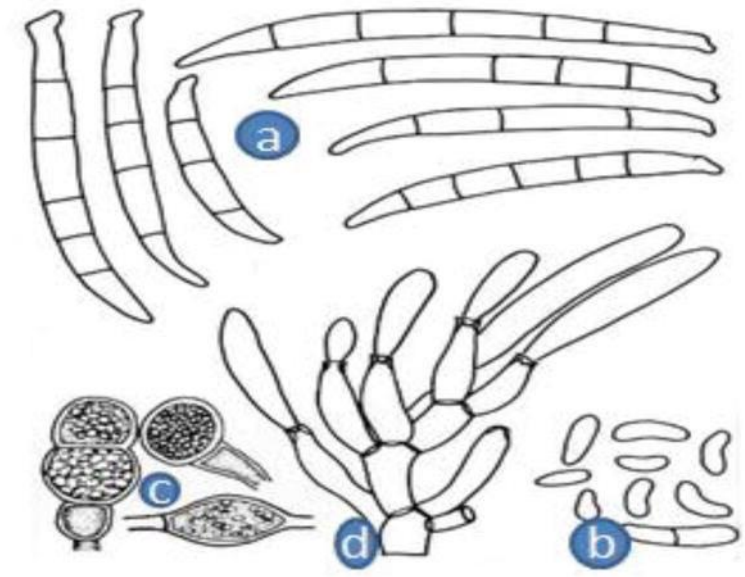


Ex. 4: *Pacielomyces*

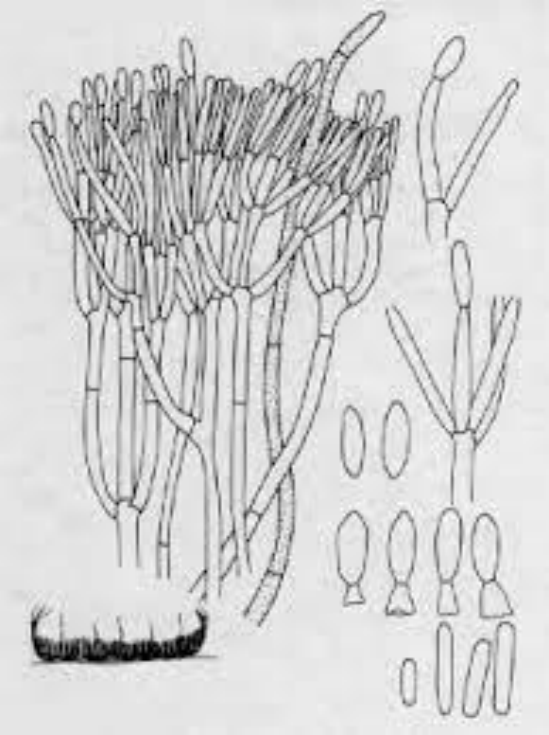


Family 2: *Tuberculariaceae*

Ex. 1: *Fusarium*

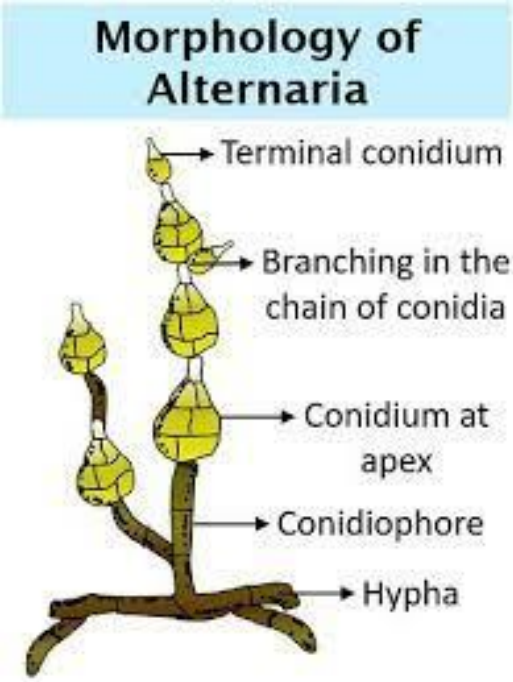


Ex. 2: *Myrothecium*

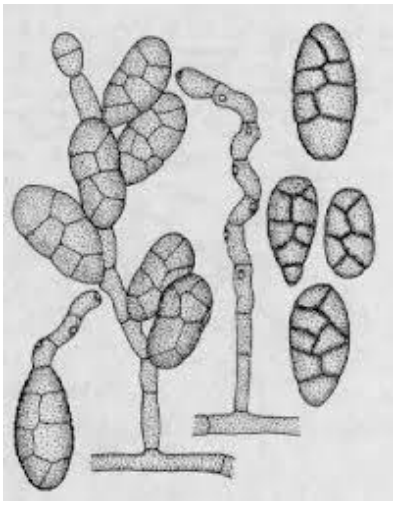


Family 3: *Dematiaceae*

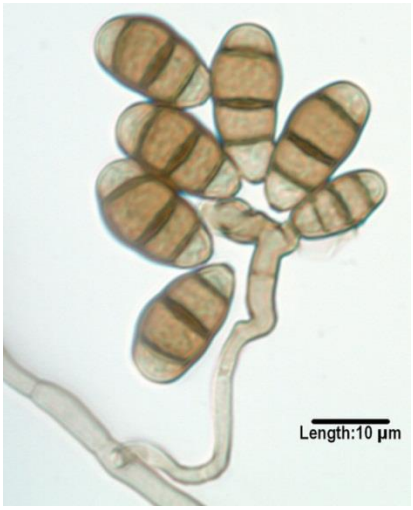
Ex. 1: *Alternaria*



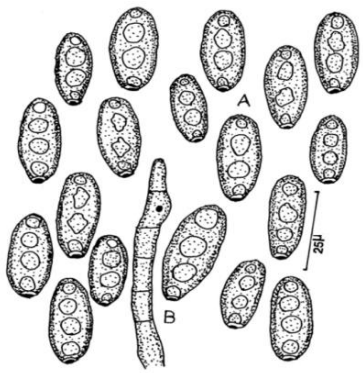
Ex. 2: *Ulocladium*



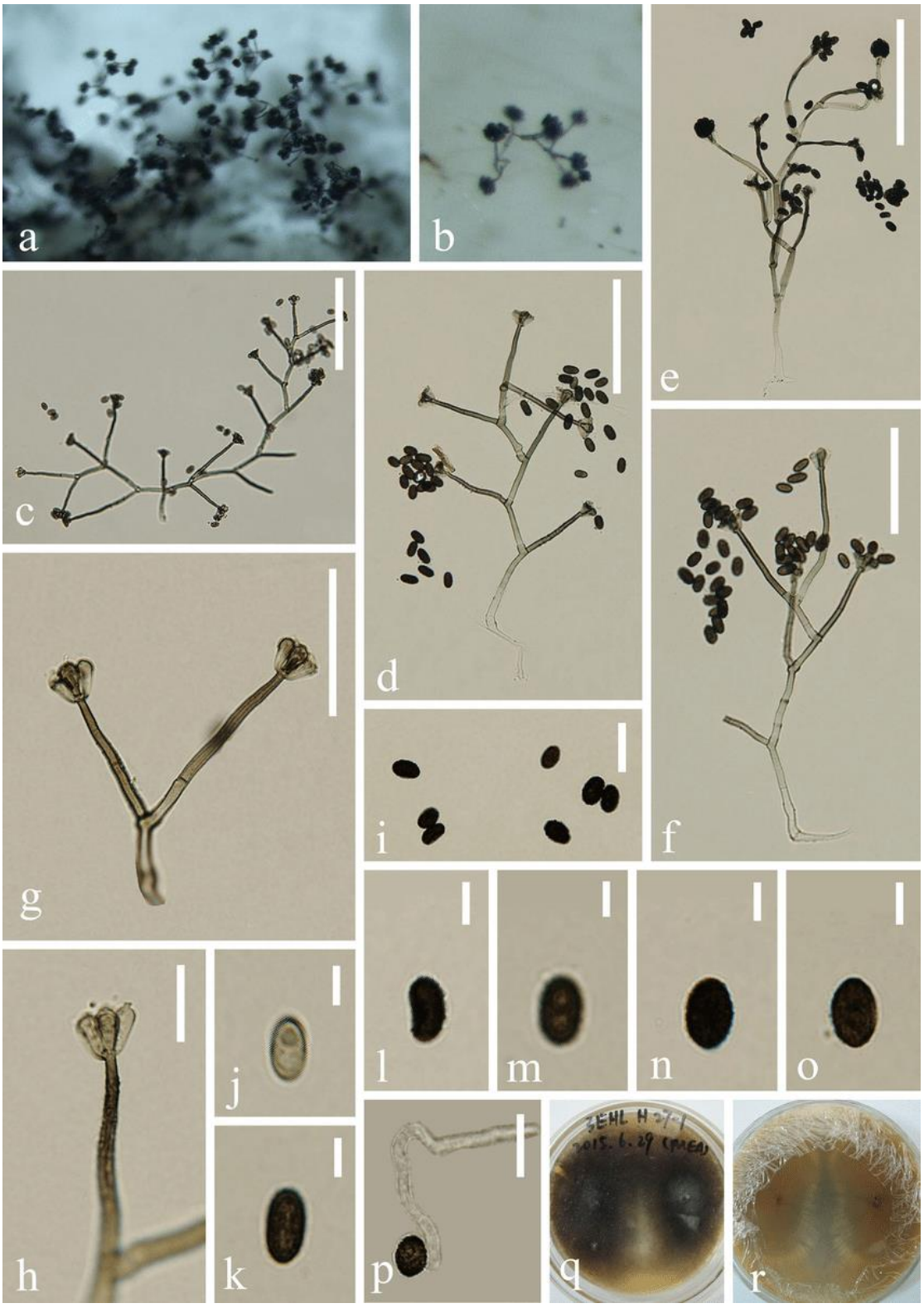
Ex. 3: *Curvularia*



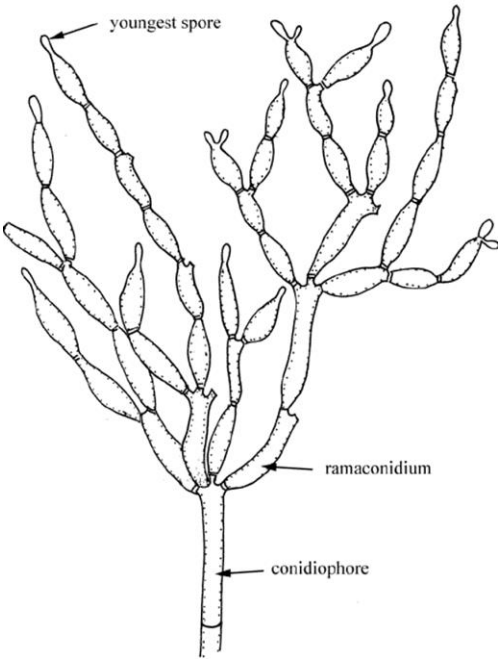
Ex. 4: *Drechslera*



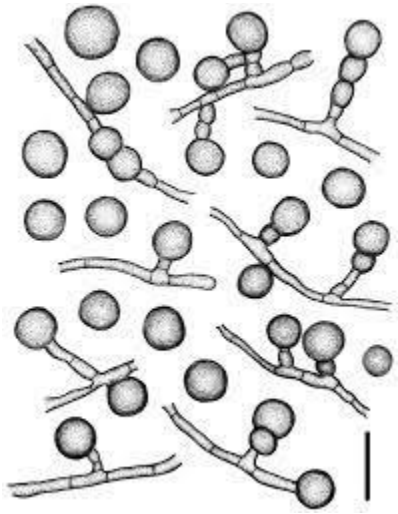
Ex. 5: *Stachybotrys*



Ex. 6: *Cladosporium*



Ex. 7*: Humicola*



Family 3: *Stilbaceae*

Ex. *Trichorus spirales*

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- Introduction to Fungi. First edition. S. S. Rajan (2001).

INDUSTRIAL MICROBIOLOGY

FOR 3rd YEAR STUDENTS

FACULTY OF SCIENCE

BY

DR. MOHAMED A. HUSSEIN

2021- 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 it is 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 secretes.

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:

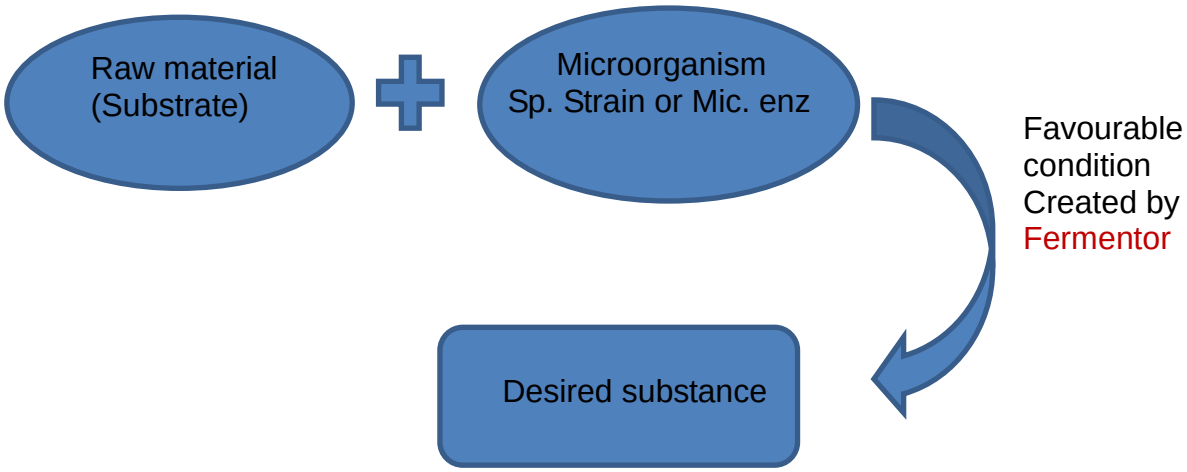
During the production all the suitable nutritional including nutrition elements and environmental conditions including pH, humidity, temperature and aeration (aerobic or anaerobic) should be available.

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 coast of fermented product depends on several factors such as:

1- Coast of raw materials.
2- Coast of additives like enzymes and vitamins.
3- Coast 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_2 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_2).

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_2 analysis.

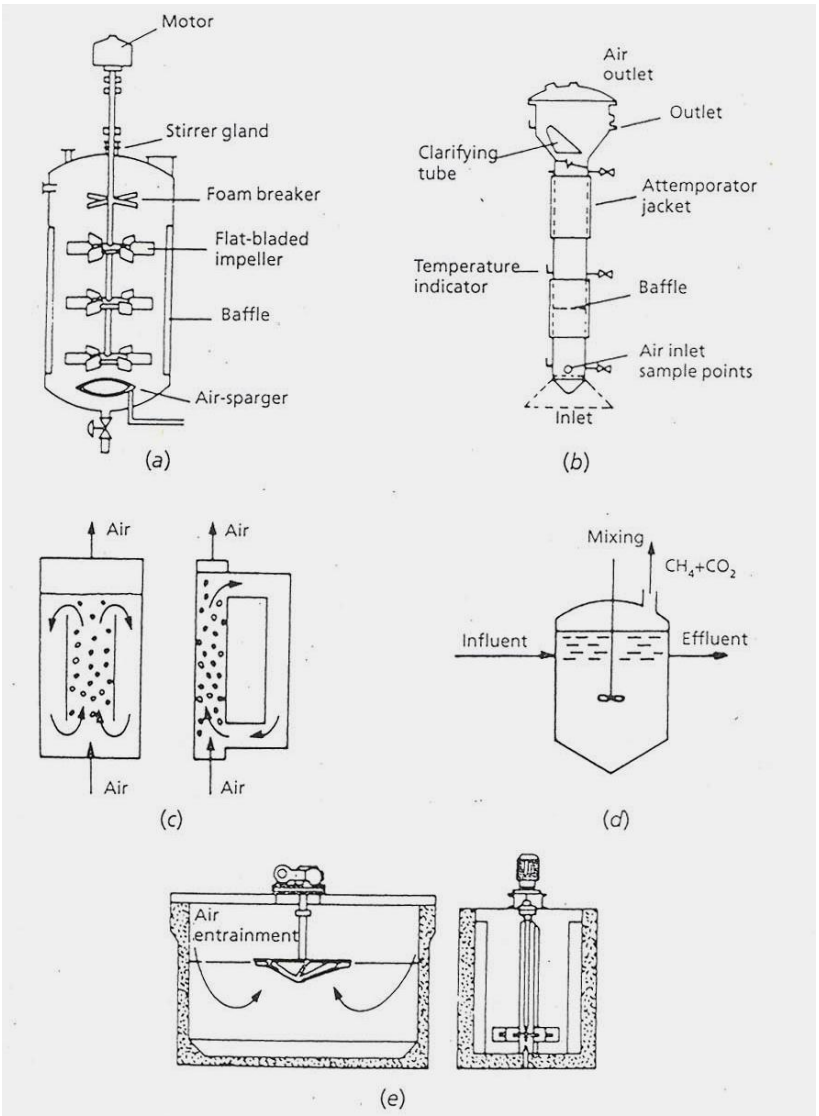


Fig. 3 Various forms of bioreactor. (*a*) Continuous stirred tank bioreactor. (*b*) Tower bioreactor. (*c*) Loop (recycle) bioreactor. (*d*) Anaerobic digester or bioreactor. (*e*) Activated sludge bioreactor. ((*a*) and (*b*) from Kristiansen and Chamberlain, 1983).

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.

Downstream processing operations

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:

Class	Description
Class A	Microorganisms that have never been identified as causative agent of disease in human and that offer no threat to the environment.
Class 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.
Class C	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.
Class D	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.
Class E	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 (*Leuconstoc, Lactobacillus*, and *Propionibacterium*), yeasts and molds; that are only used for some types of surface ripened and mold ripened cheeses. The various combinations of microflora determine what milk product you will end up with.

Different unripened milk products are created by using various starting 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.

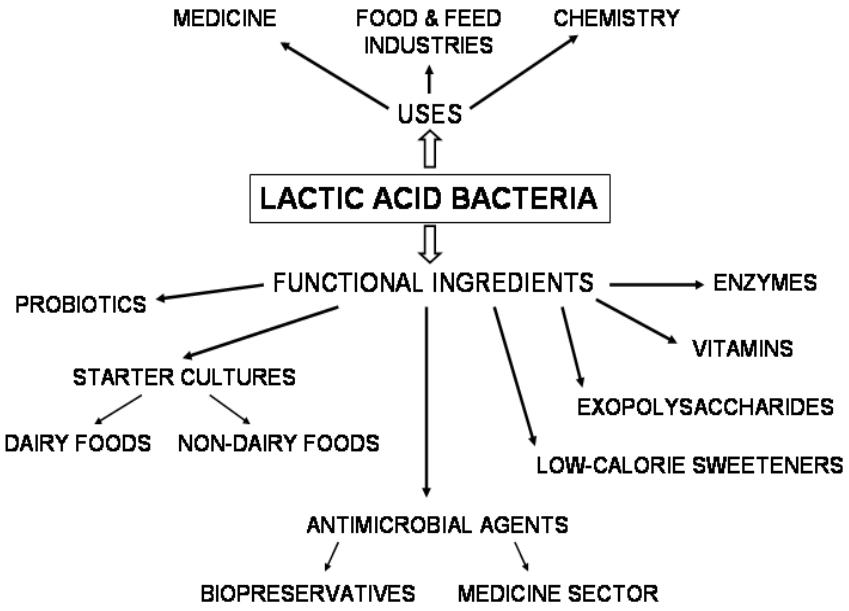
Cheese production:

- 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:

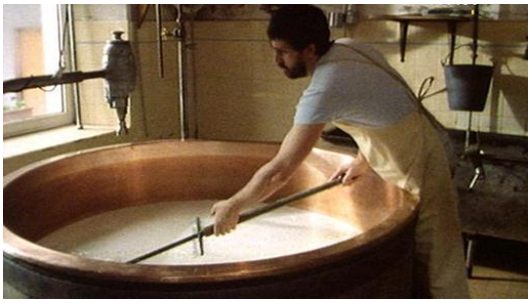
1- Acidification of the milk by the conversion of sugar lactose into lactic acid by the lactic acid bacteria.
2- Coagulation of the casein by a combination of proteolysis and acidification.

- 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.

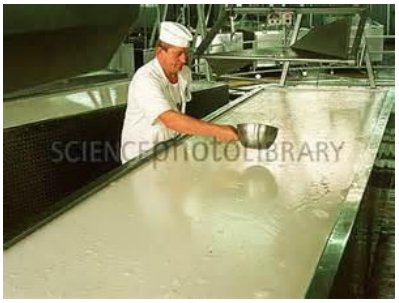
Recent biotechnological cheese productions involve the use of recombinant DNA techniques for chymosin production and commercial use.

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

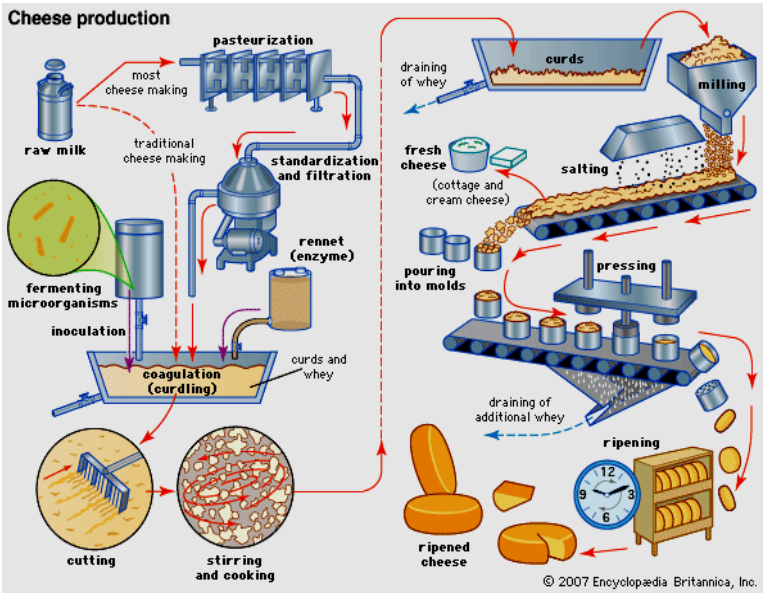
3- **Curd treatment (Secondary microflora)** for ripening the cheese and determine the final texture and aroma of each type of cheeses. For hard ripened cheeses such as Cheddar, curds are further compressed and the bacteria particular for the cheese is added. The Cheddar is

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.

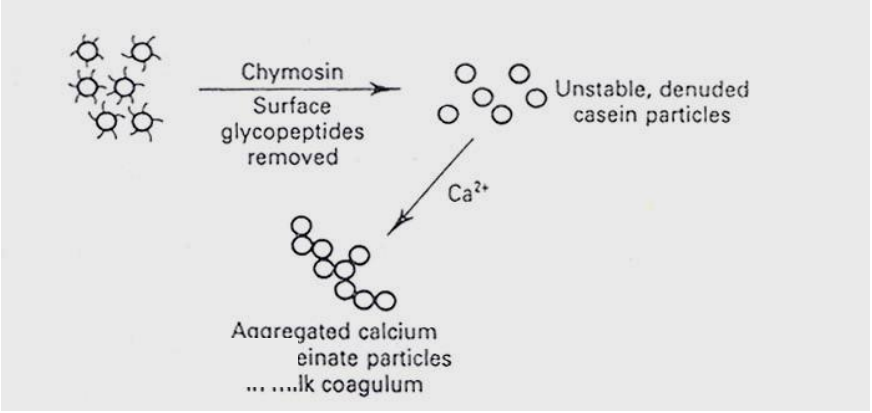
Kinds of Cheese

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)


Some Kinds of Cheese
Soft Cheeses
Brie (French)
Camembert (French)
Cottage (United States)
Cream (United States)
Semisoft Cheeses
Limburger (Belgium)
Munster (German)
Port du Salut (French)
Roquefort (French)
Hard Cheeses
Cheddar (English)
Edam (Dutch)
Gruyère (Swiss)
Swiss
Very Hard Cheeses
Asiago (Italian)
Parmesan (Italian)
Romano (Italian)
Sapsago (Swiss)

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 glutens. These proteins have the characteristic of forming long molecular strings when they are "worked" or "kneaded" that bind the bread together in the sticky mass we call **dough**. Gluten also contributes to the delightful 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_2 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-α), 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_2:

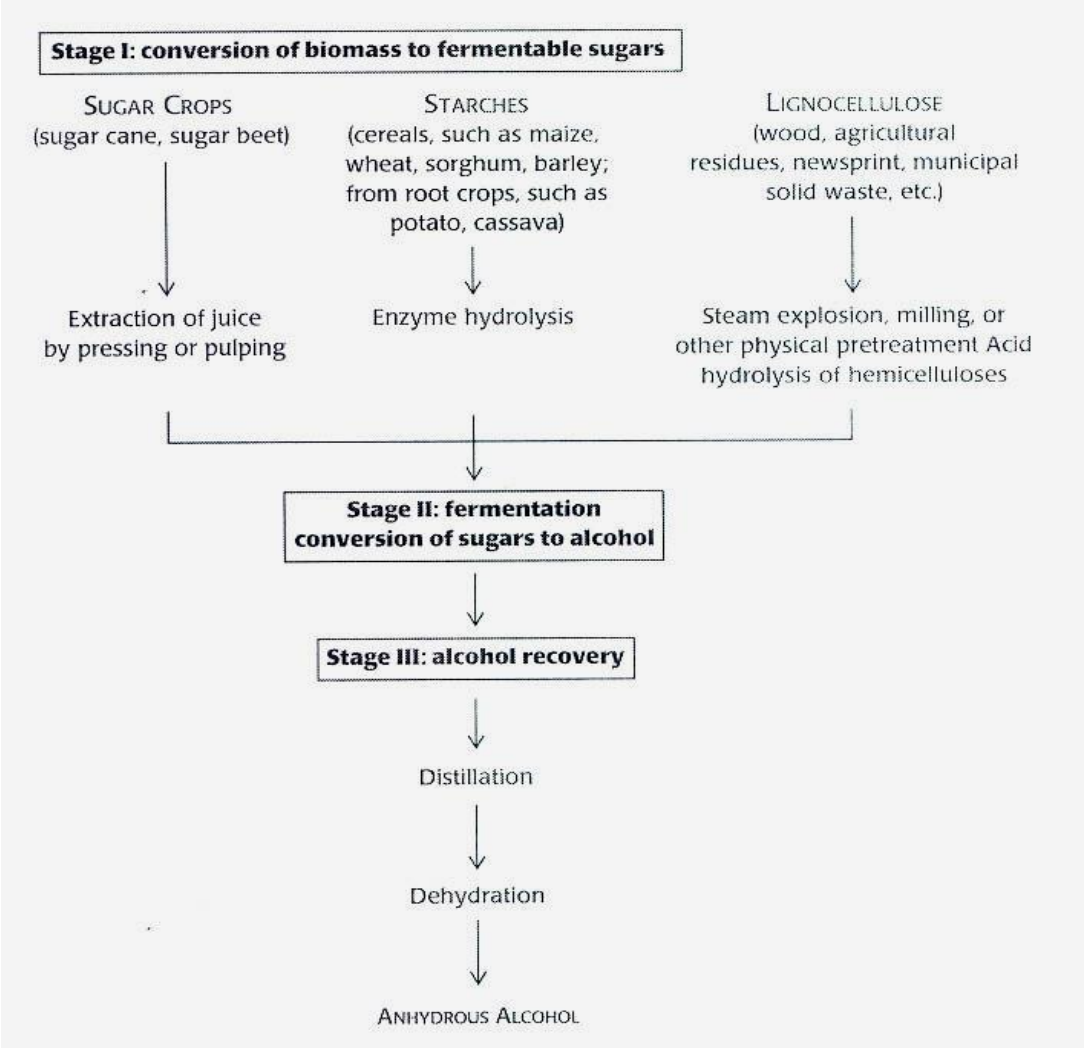
$$\mathbf{C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2}$$

Glucose ⟶ Ethanol + Carbon dioxide

The CO_2 released in the process, dissolves in water and form a carbonic acid. This acid dissociates to form hydrogen carbonate and hydronium ions:

$$\mathbf{CO_2 + 2H_2O \longrightarrow H_2CO_3 + H_2O \longrightarrow H_3O + HCO_3^-}$$

In acidic solutions, the dissolution of CO_2 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_2 released during the fermentation is captured and sold for carbonating beverages and the manufacture of dry ice.

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

$$CH_3CH_2OH \longrightarrow 2HCH_3CHO \longrightarrow CH_3COOH.$$

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

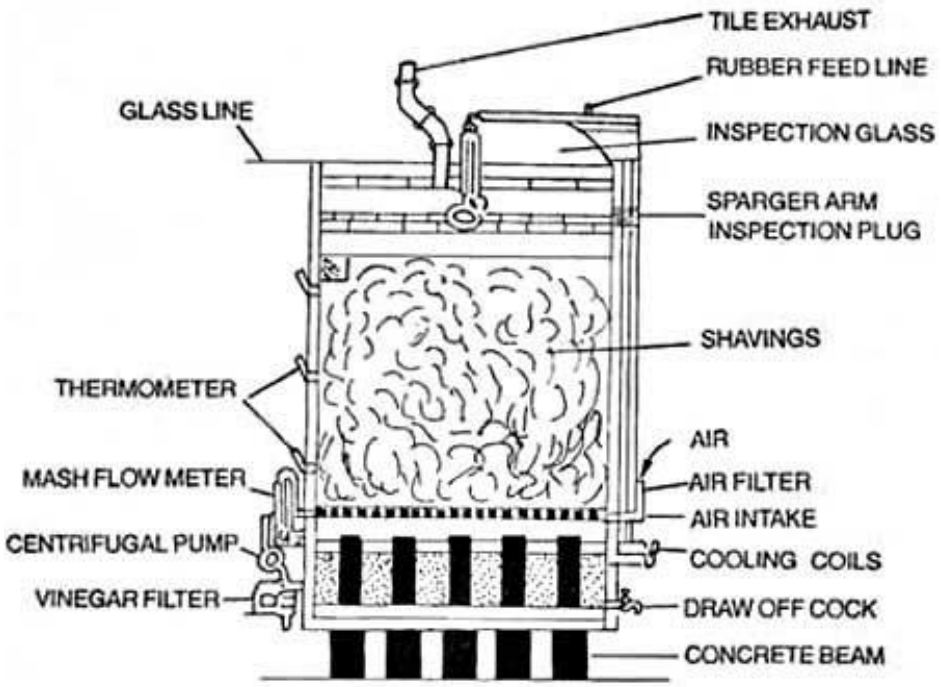
with large stainless-steel tanks called acetators. The acetators are fitted with centrifugal pumps in the bottom that pump air bubbles into the tank in much the same way that an aquarium pump does.

2. As the pump stirs the alcohol, acetozym 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 ($CH_2COOH.COH.COOH.CH_2COOH$), 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 ºC was usually employed for culturing of *Aspergillus niger* but temperature above 35 ºC 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 β-lactam antibiotics used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms. The discovery of penicillin is usually attributed to Scottish scientist Sir Alexander Fleming in 1928.

It is manufactured by growing *Penicillium chrysogenium* 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 *Penicllium 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-α and IFN-γ 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_2, N_2, CH_4, CO_2)	• Reduce oil viscosity and improve flow characteristics • Displace immobile • Sweep oil in place	• Improved oil recovery by gases • Miscible CO_2 flooding
Acids (low molecular weight acids, primarily low molecular weight fatty acids)	• Improve effective permeability by dissolving carbonate precipitates from pores throat. Significant improvement of permeability and porosity • CO_2 produced from chemical reactions between acids and carbonate reduce oil viscosity and causes oil droplet to sweel	• Enhanced oil flooding
Solvents (alcohols and ketones that are typical cosurfactants)	• Dissolve in oil reduce viscosity • Dissolve and remove heavy, long chain hydrocarbons from pore throat (increase effective permeability) • Involved in stabilizing and lowering interf. tension that promotes emulsification • Reduce interfacial tension	• Emulsification promotion for increased miscibility
Biosurfactants	• Reduce interfacial tension between oil and rock/water surface which causes emulsification; improving pore scale displacement • Alter wettability	• Microbial surfactant • Flooding
Biopolymers	• Improve the viscosity of water in waterflooding and direct reservoir fluids to previously unswept areas of the reservoir • Improve the sweep efficiency of waterflood by plugging high permeability zones or water-invaded zones • Control of water mobility	• Microbial permeability modification (selective plugging)
Biomass (microbial cells)	• Physically displace oil by growing between oil and rock/water surface • Reversing wettability by microbial growth • Can plug high permeability zones • Selective partial degradation of whole crude oil • Act as selective and nonselective plugging agents in wetting, alteration of oil viscosity, oil power point, desulfuration	• Same biopolymers

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

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.

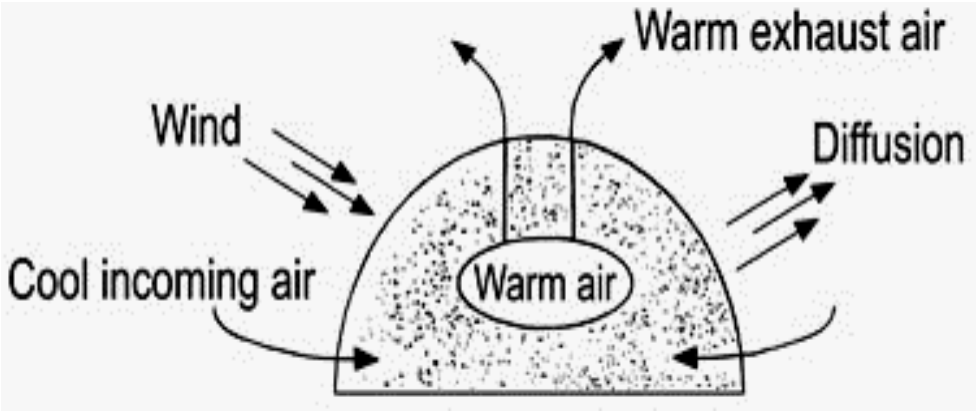
- Minimizes odors from agricultural areas.

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

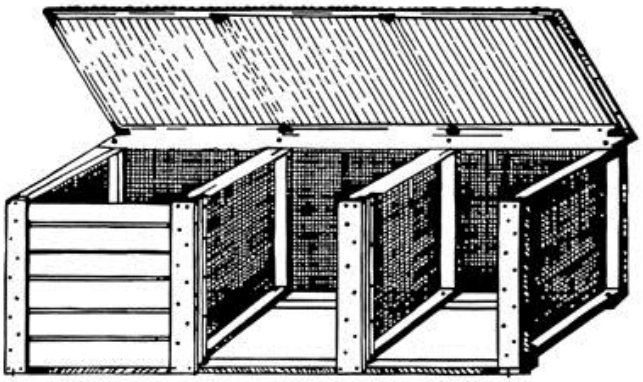
In Aerated static piles air is introduced to the stacked pile via perforated pipes and blowers. This method requires no labor to turn compost but is weather sensitive and can have reliable pathogen reduction due to imperfect mixing.

Aerated piles in the field

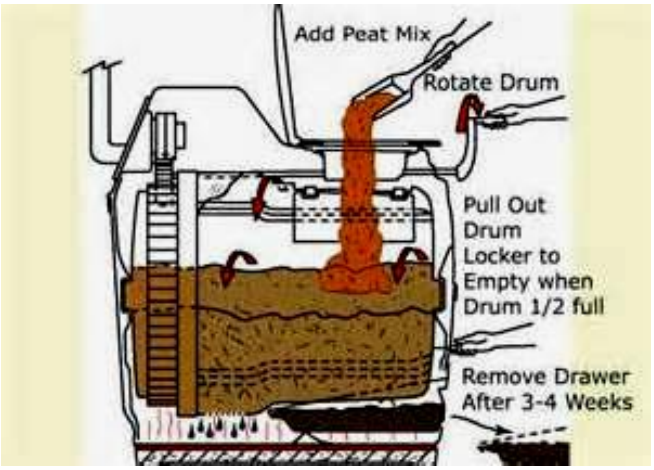
Windrows are long, narrow piles that are turned when required based on temperature and oxygen requirements. This method produces a uniform product and can be remotely located. However, turning the compost can be labor intensive or require expensive equipment. Windrows are typically used for large volumes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

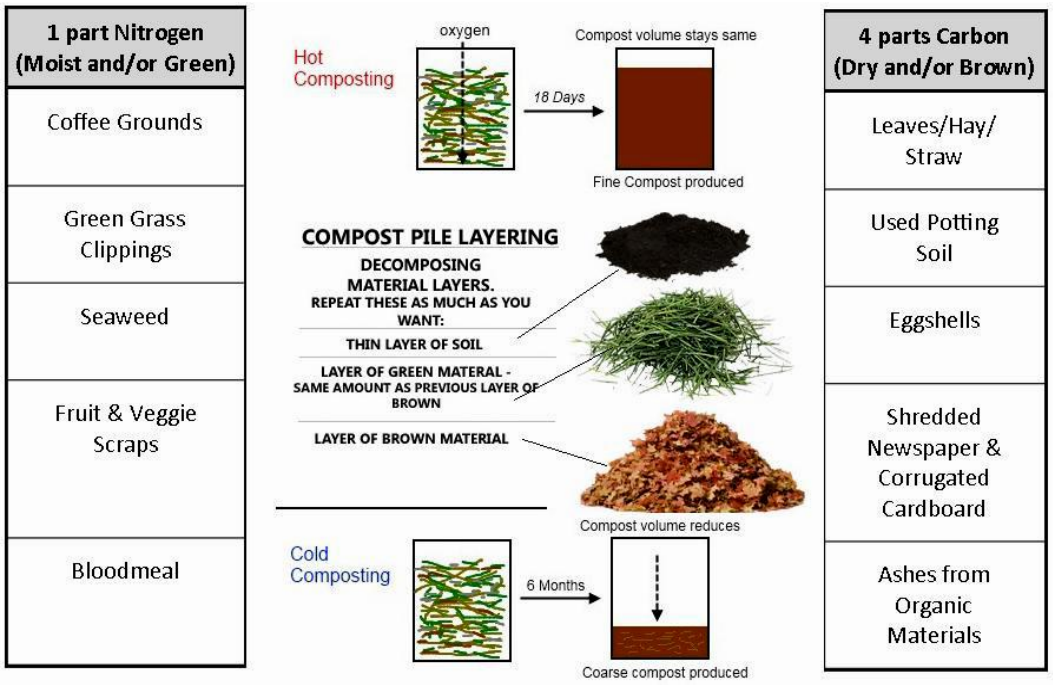


In-vessel systems using perforated barrels, drums, or specially manufactured containers are simple to use, easy to turn, require minimal labor, are not weather sensitive, and can be used in urban and public areas. The initial investment can be high and handling volumes are typically low.



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.

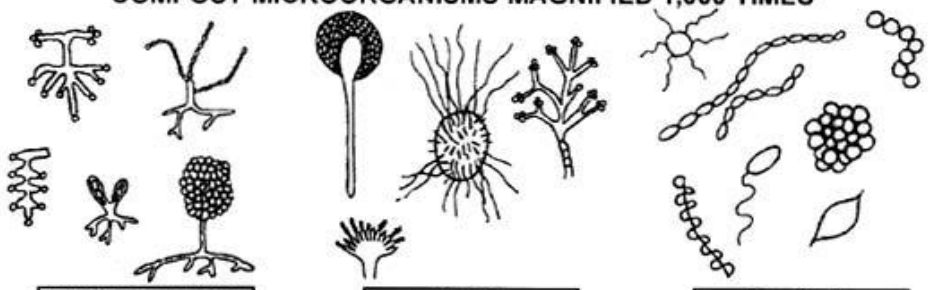
It is very important not to apply unfinished or immature compost, it may have phytotoxins that can kill plants. An inexpensive way to test for mature compost is the watercress test. Watercress seeds will not germinate or grow in immature compost because they are very sensitive to pH and nutrition.

Mature compost

Microorganisms

Figure 3.3

COMPOST MICROORGANISMS MAGNIFIED 1,000 TIMES



Actinomycetes	Fungi	Bacteria
100 thousand - 100 million per gram of compost	10 thousand - 1 million per gram of compost	100 million - 1 billion per gram of compost

Reproduced with permission from On-Farm Composting Handbook. NRAES-54, published by NRAES, Cooperative Extension, 152 Riley-Robb Hall, Ithaca, New York 14853-5701. (607) 255-7654. Quantities of microorganisms from: Sterritt, Robert M. (1988). Microbiology for Environmental and Public Health Engineers. p. 200. E. & F. N. Spon Ltd., New York, NY 10001 USA.

Table 3.6

MICROORGANISMS IN COMPOST

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

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

Uses and 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.

GENERATION OF BIOLOGICAL FUELS (BIOFUELS)

Photosynthesis as a new source of energy.

The world known sources of fossil fuels are coal, natural gas and oil. By applying the current consumption rates, it is assumed that these sources will be depleted within the next 1000, 35 and 15 years, respectively. Approximately 93% of fossil fuel is used for energy production, while only 7% is used by industrial processes such as the production of solvents, plastics and other chemicals.

In the developed countries, many efforts are increasing now for finding and using alternative sources such as solar, hydro, wind, wave and nuclear power.

Biological energy systems are also good alternative in many fields. This depends on biomass production from forests, agricultural and animal residues, and industrial and domestic organic wastes.

- Photosynthetic organisms, both terrestrial and marine, are continuous solar energy converters and are constantly renewable. Plant photosynthesis alone fixes about 2×10^{11} tonnes of carbon with an energy content of 2×10^{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.

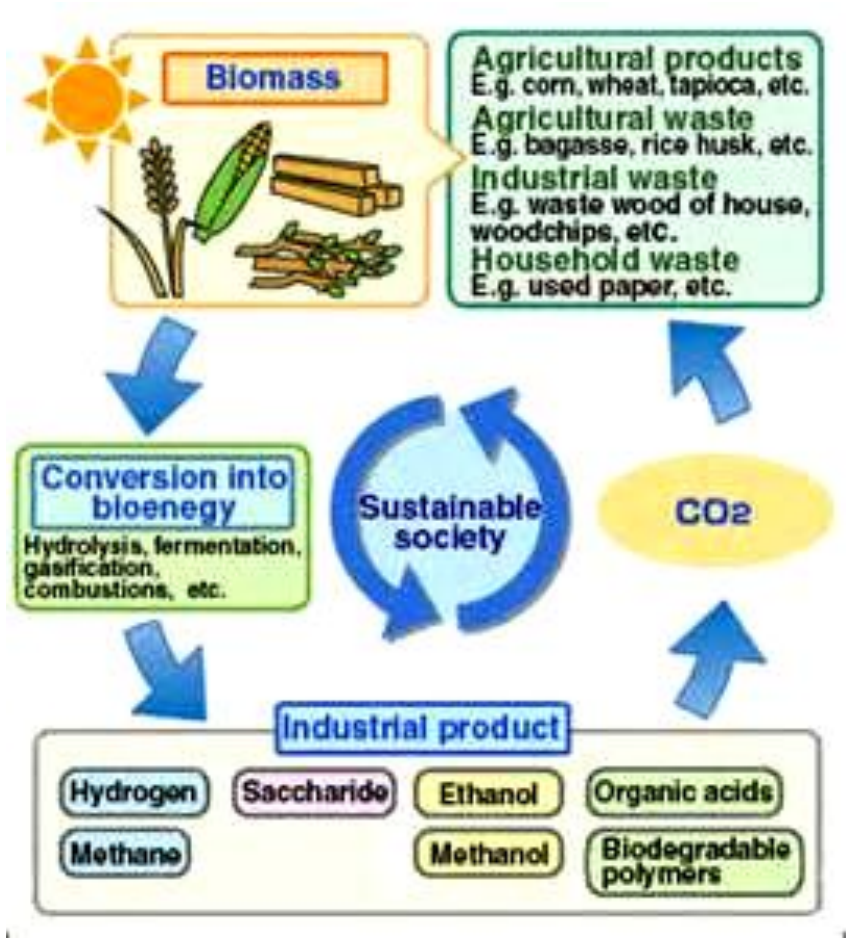
Conversion of the resulting biomass into fuel can be achieved by biological or chemical means or by a combination of both. The two-main end- products are methane and ethanol, although other products may result depending on the initial biomass and the process utilized.

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

$$C_6 H_{12} O_6 \longrightarrow 2CH_3 CH_2 OH + 2CO_2$$

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 evapouration of the fermentation broth.

Tab *al raw materials for fuel ethanol production*

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

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

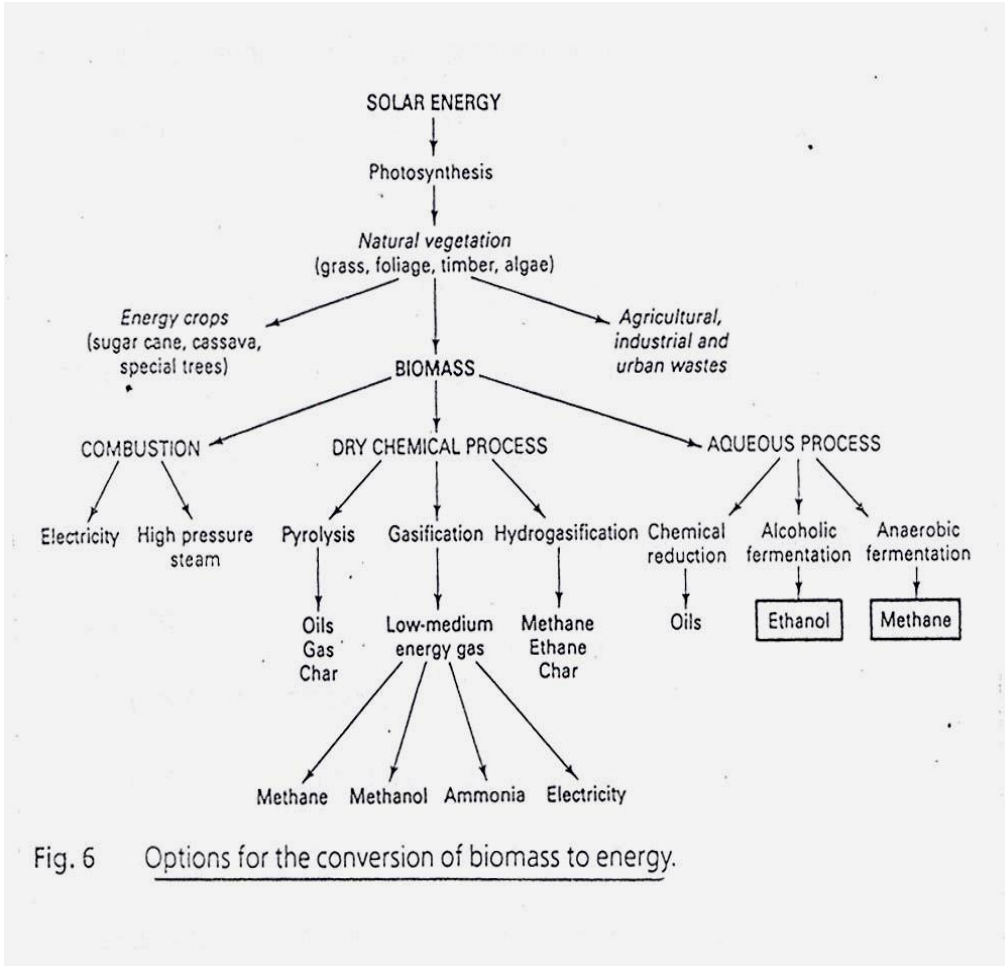


Fig. 6 Options for the conversion of biomass to energy.

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) Evapouration to feed or fertilizers.

b) Mineralization to ash.

c) Anaerobic fermentation to produce methanol.

d) Conversion by microorganisms into single cell protein.

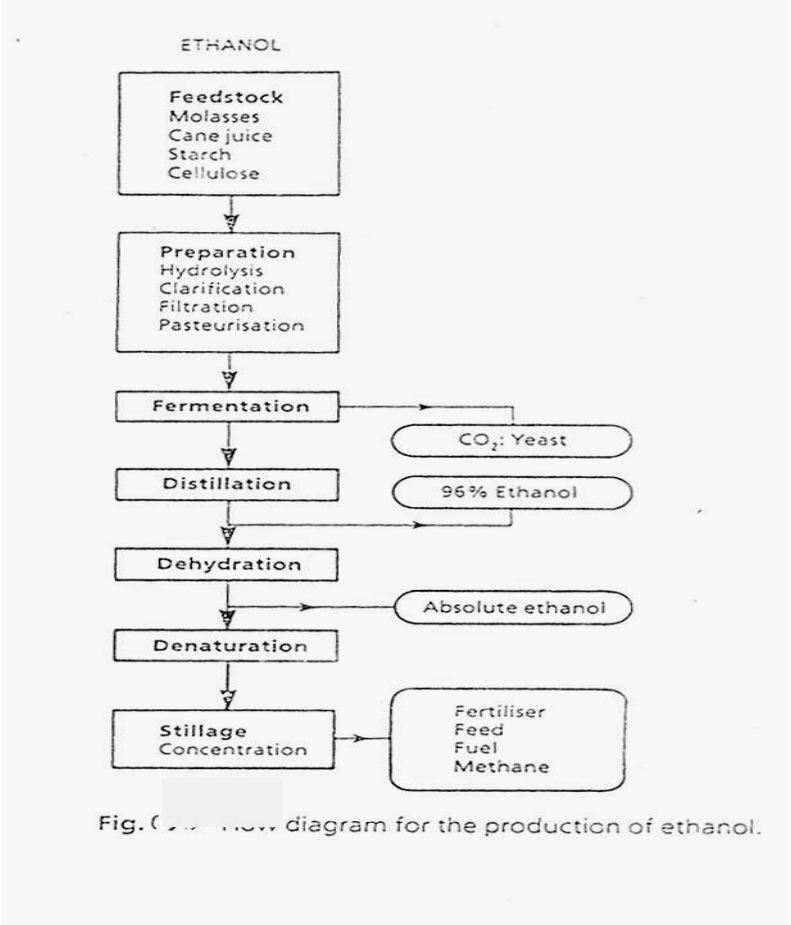


Fig. (... diagram for the production of ethanol.

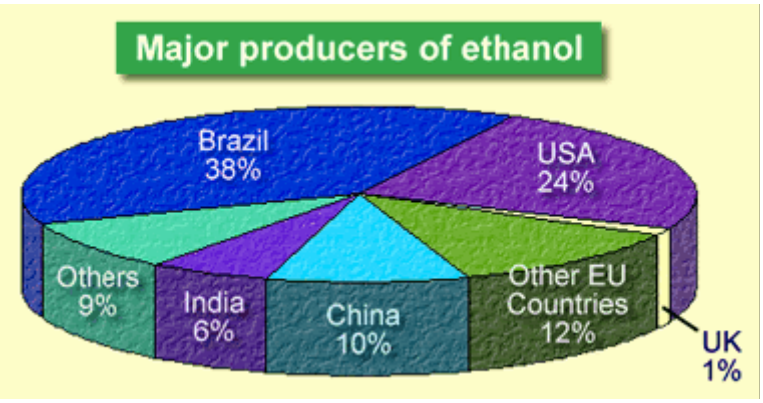
Biodiesel is another biofuel made by combining alcohol (usually methanol) with vegetable oil, animal fat, or recycled cooking grease. It can be used as an additive (typically 20%) to reduce vehicle emissions or in its pure form as a renewable alternative fuel for diesel engines.

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.

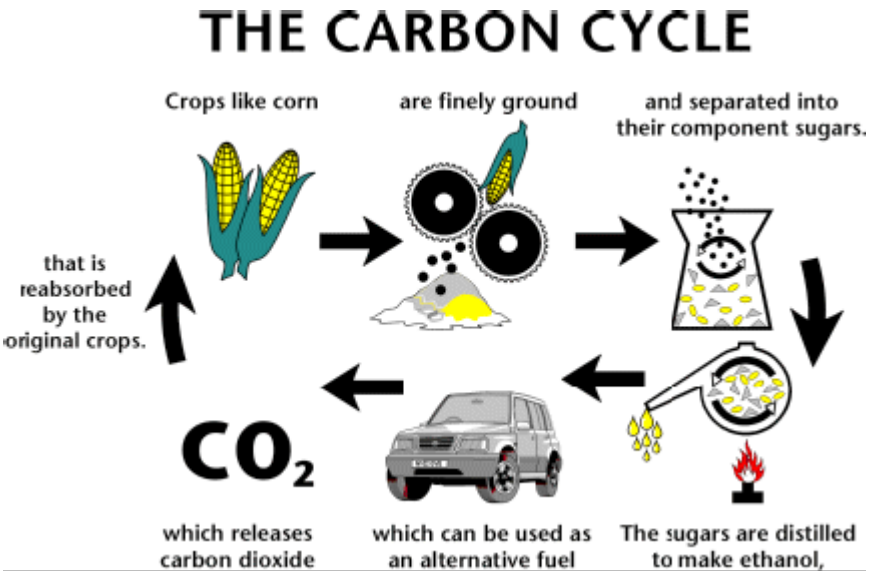


Impact on the environment

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_2, 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_2.

There are several ways for economical production of methane

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

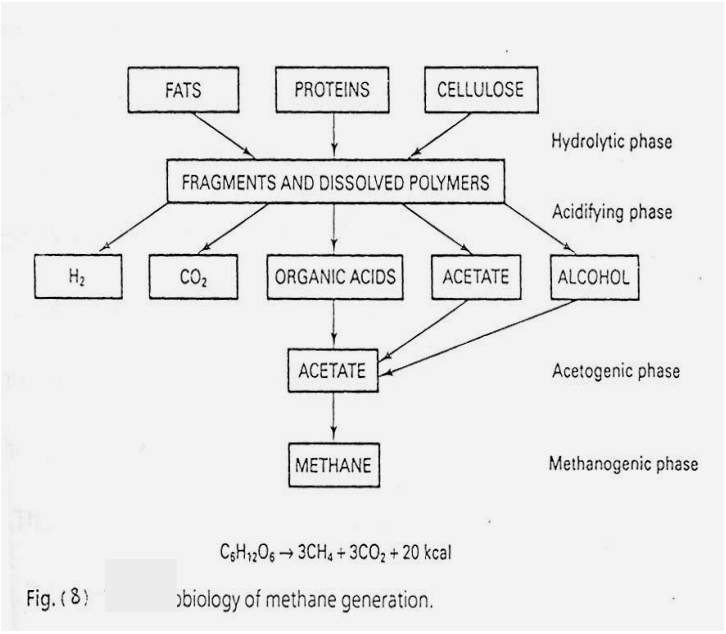
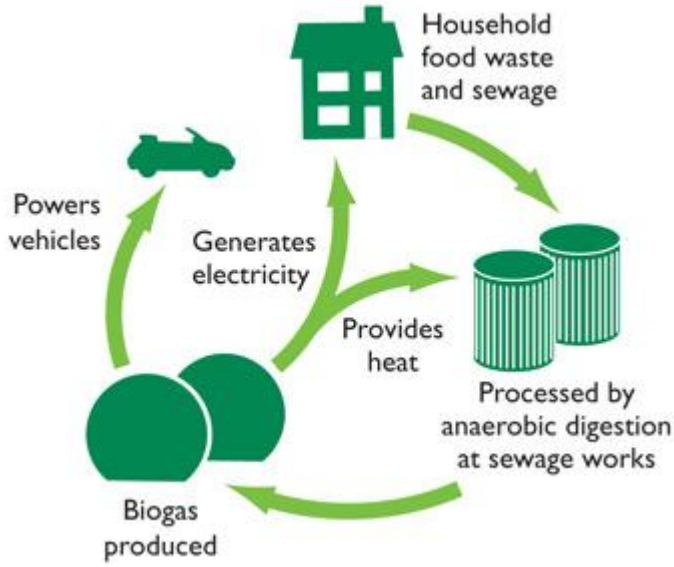


Fig. (8) ɔbiology of methane generation.

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.

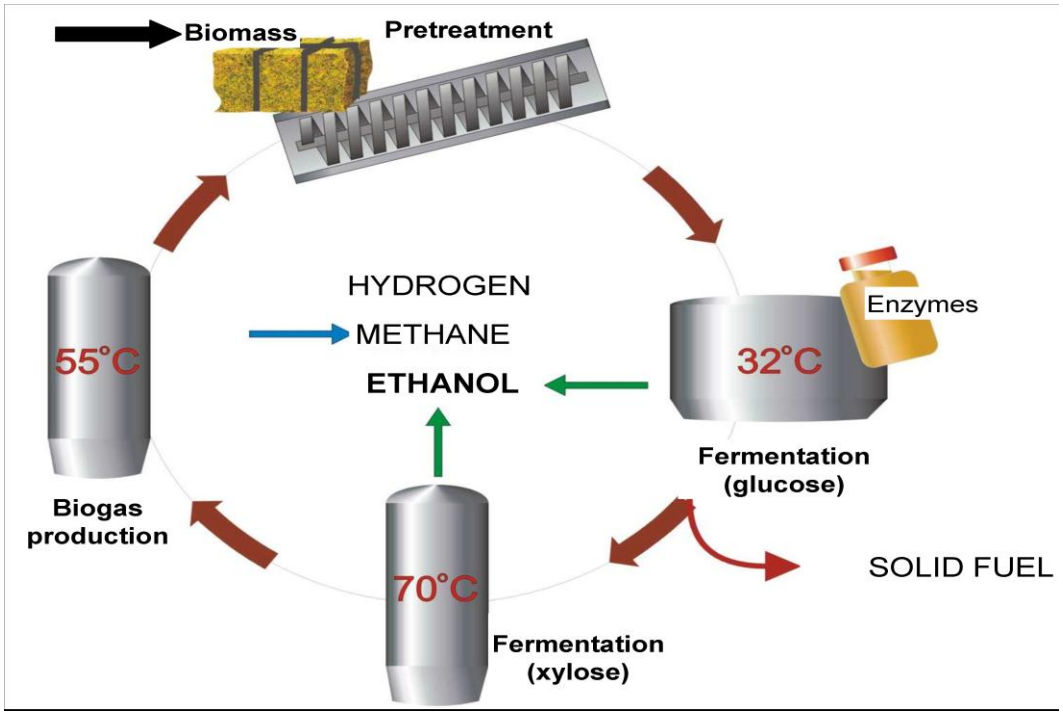
It is possible to convert 30- 50% of urban wastes' energy into methane and with certain vegetable materials and forages, this percentage should be raised to 70%.

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

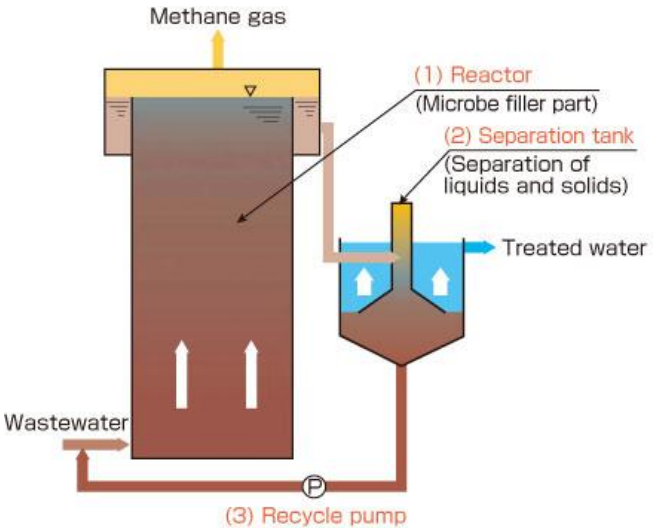
C- Biogas reactors

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_2 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 pratctice 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 $3m^3$ 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.

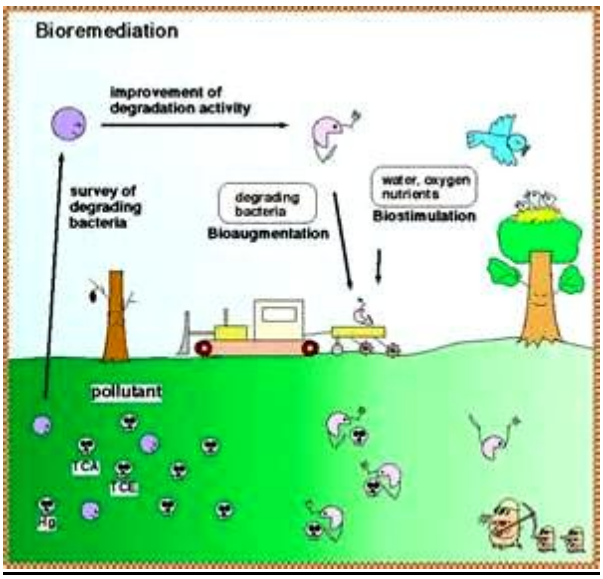
2- The medium is inoculated with soil which is likely to contain a diversity of microbes (e.g. rich garden soil, sewage etc.).

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, leekage 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.