NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 320

COURSE TITLE: MICROBIAL ECOLOGY

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COURSE TITTLE: MICROBIAL ECOLOGY (3UNITS)

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TABLE OF CONTENTS

Unit 1 MICROBES AND ECOLOGICAL THEORY

Contents

UNIT 2 MICRO-ORGANISMS IN ECOSYSTEMS

2.6 Further Readings / References

UNIT 3. PHYSIOLOGICAL, MORPHOLOGICAL AND GENETIC ADAPTATIONS OF MICRO-ORGANISMS TO THEIR ENVIRONMENTS.

3.0 Introduction

3.1 Objectives

3.2 Main Contents

- Microbial adaptations to marine and fresh water environments
- Survival of Micro-organism in soil
- Survival of Micro-organism in air

3.3 Conclusion

3.4 Summary

3.5 Tutor-marked questions

3.6 Further Readings/ References

UNIT 4. BIOCONVERSION

Contents

4.0 Introduction

4.1 Objectives

4.2 Main contents

- Biodeterioration
- Biodegradation

4.3 Conclusion

4.4 Summary

UNIT 5. MICROBIAL INTERACTIONS

Contents

MICROBIAL ECOLOGY

General Introduction

In this course, we shall consider the existence of microorganisms exist in communities. Recent estimates suggest that most microbial communities have between 10^{10} to 10^{17} individuals representing at least 10^7 different taxa. How can such huge populations exist and more over, survive together in a productive fashion? To answer this question, we must know which microbes are present and how they interact. That is, we need to study microbial ecology, a multidisciplinary field which discusses some aspects of environmental microbiology.

Microbes deal with small or minute organisms, while ecology is a branch of biology dealing with organisms, their relationship with one another and that of their environment. There are several approaches to the teaching and learning of microbial ecology. One approach is to consider the activities of the microorganism in nutrient cycles and food chains; another approach is to look at it from the factors which govern microorganisms and their environments.

Generally speaking, microbial ecology is seldom primarily concerned with the organism *per se* rather it is concerned with their activities in terms of the resultant chemical and physical changes they make in the environment and those aspects of nutrient cycles, polluted water sources, diseased patients, biochemical capacities of causative organisms to induce infection and so on are all considered as aspects of microbial ecology.

Some of the important tools and techniques used to study microbial ecology are presented.

General Objectives

The objective of this course is to study microbial community dynamics and the interactions of microbes with each other and with plants, animals and the environments in which they live.

For the purpose of this course the soil, air, water, animal and plant surfaces are considered as specific environments.

UNIT 1. MICROBES AND ECOLOGICAL THEORY

Introduction

In this unit, we shall define some basic terms used in microbial ecology. We shall also consider colonization and succession as natural phenomena in ecosystems. Some specific examples are presented.

Objectives

The objectives here is to equip students with the appropriate use and application of some basic ecological terms; and to enable us understand that colonization and succession are natural events which progress from a freshly exposed surface to a pioneer community and finally terminate in a climax community.

Main Contents

Basic Definition in the Study of Microbial Ecology

1. **Ecosystem**: This is a combination of biotic and abiotic components of a specific environment. Normally, such a unit has a collection of organisms and abiotic components which are unique to it and as such, one eco-system is different from the other.

2. **Community** This is a collection of microorganism inhabiting a given site in the ecosystem (that is the ecosystem without the environmental factors or abiotic factors).

3. **Population**: This is the individual microbial species. Unlike the community, it is more homogenous and specific.

4. **Individual**: This refers to the individual organisms in the population. Each ecosystem has, associated with it certain physical, chemical and biological characteristics. These factors govern the composition of the community by dictating which of the individual microorganisms will be successfully established, and out of those established, some will be dominant while others are limited and other groups are totally eliminated. Therefore, selection by environmental factors is important. Those organisms that are established are those that are better adapted to the abiotic conditions (P^H, temperature, moisture, water activity, oxygen concentration etc) in that particular environment.

Organisms that cannot cope with the prevailing conditions are eliminated. The environment builds the community through selection. It is as a result of this relationship with a community that we have certain Species of micro-organism as dominant.

The Habitat: A habitat is an area having a degree of uniformity in terms of the abiotic components. They are therefore also considered to be of ecological significance e.g. surfaces of plants and animals, soil, open sea, blood, air, nasal passages, alimentary cannal and so on. The size of the habitat is not important because it varies considerably. The important factor is that certain sets of conditions are uniform to that area.

Niche. The ability of microorganisms to make use of resources available in their habitat is varied and this has lead to the idea of niche. The role of a particular organisms in a particular place is the niche.

Microorganism could be divided into types depending on their functions in the different habitats, those with narrow and those with broad range of tolerance. Those with narrow niche are highly specialized and perform single function or role e.g. obligate parasites, autotrophic organisms, while those that carryout a range of functions are said to have a broad niche e.g. heterotrophic organism. Those that occupy a narrow niche are easily eliminated if there is a change in environmental conditions especially as it affects their survival, but they however, flourish luxuriantly when their conditional requirements are met. Examples are photosynthetic micro-organisms which are adversely affected when the source of light is blocked but they grow rapidly when there is light. On the other hand, those that have a broad niche are not severely affected by changes in the environmental conditions.

1.2 COLONIZATION AND SUCCESSION.

When an area is denuded or freshly exposed e.g. when a tissue is freshly exposed or wounded or when we have an earth quake which exposes the earth's surface, a number of microorganism will be deposited on such surfaces. The first microorganisms to arrive on such surfaces are called pioneers. These organisms grow and multiply to form the pioneer community and from the time the exposed areas are occupied by microorganisms, it is said to be colonized.

After the establishment of the pioneer community, they feed on the substrates, produced byproducts and other waste materials or metabolites and so the environment becomes modified. The modified environment paves way for colonization by other organisms, while the pioneer communities are gradually eliminated. Thus we have a situation whereby one community will out-grow and replace another. This phenomenon is referred to as succession.

Climax Community.

When colonization has taken place, succession follows and there is a continuous modification of the environment. However, at a stage the community remains constant for at least some time and the process of succession is stopped; and a community that characteristics that habitat results. This is called the climax community. The species composition at this stage is maintained reasonably constant for a period of time. The stability in composition does not mean that the organisms do not die but the number of organisms dying is quickly replaced and this is a reflection of dynamic equilibrium. The climax community therefore is a self-replicating entity where the microorganisms and the physical environment are in constant equilibrium.

Often, there are alternations in the physical conditions e.g when a large quantity of pollutants is introduced into a stream, the organic matter component is eliminated and the climax community is distorted. However, such disturbances are temporary and the original climax community is restored with time or as soon as the disturbance is removed.

1.3.0. SUCCESSION IN NATURAL HABITATS

1.3.1 Succession of Micro-organisms on Cellophane Film.

Tribe (1960) studied the colonization and breakdown of cellophane film (pure regenerated cellulose). The breakdown of cellophane buried in a number of soils was studied. Though variations existed in the soil type, a general pattern of colonization was observed. The first colonizers were fungi and out of these, based on morphological and vegetative characters, three (3) classes were identified.

(a) Those Characterized by coarse mycelium which ramifies through the surface of the cellophane and rapidly initiates decomposition by lysis of the cellulose adjacent to their hyphae. Such fungi are mostly in the form genus *Rhizoctonia* and were clearly cellulolytic and dominant species at that stage.

(b) Members of the second group are mostly species of the well known cellulolytic genera e.g *Chactomium species* and *Humicola species* which exist as co-dominants. These fungi did not ramify extensively with the cellophane but rather they penetrated the thickness at scattered sites by means of "rooting" hyphae which then branches to form a kind of circular hyphal system.

(c) The third group consists of chytrids which develop on the cellophane pieces. At this stage, the cellophanes are no longer intact but in pieces.

The presence and activities of the initial colonizers (pioneer community) were obviously related to their ability to produce "*cellulases*". Though, other factors must have been involved because the soil contains other cellulolytic organisms which rarely appear on cellophane. Bacteria are relatively uncommon during this initial stage of fungal attack. During mycelium senescence however they rapidly increase and presumably utilize either materials diffusing from the hyphae or the hyphae itself.

2. The second colonizers are the bacteria. In turn bacteria support a population of nematodes and protozoa. After the micro-organisms have colonized the cellophane/cellulose, mites, springtails and other worms become active and the substrate becomes unrecognizable by their passage through the guts of these organisms. Cellophane decomposition therefore involves a wide range of micro-organisms and small animals whose occurrence depends on nutrient availability.

1.3.2 SUCCESSION OF MICRO-ORGANISMS ON DUNG.

If a fresh dung is placed under a bell jar and a fairly suitable humidity is maintained, a succession of fungal fruitifications can be observed. The first to appear are Zygomycetes followed by Ascomycetes and then Basidiomycetes. Each group has been considered to represent sugar, cellulose and lignin utilizers respectively.

Harper and Webster (1964) while confirming the sequence have shown that it is not a succession based on nutritional factors. They grew a number of fungi involved under a variety of conditions and showed that each group had a characteristic minimum time before commencement of growth and appearance of fruiting bodies. If the fungi are listed in the order based on the minimum time, the sequences is also found to be the same as on dung.

According to these authors, the succession of fruiting bodies on dung is connected with the duration of necessary developmental periods rather than different assimilatory abilities.

1.3.3 COLONIZATION OF STERILE HUMAN HAIR.

Griffin (1960) made a sequential study of sterile human hair placed on the surface of various soils. In general, his observations showed that the first colonizers where *Fusarium* species, *Penicillium* species and some *Mucorales*. These species rely on simple sugars. The second group of colonizers includes *Chaetomium cochloides,* *Humicola* species, *Gliocladium roseum* and *Penicillium* species. The first two are cellulolytic while the last two are polysaccharide users.

The third group are keratinolytic members of Gymnoascaccae which are mainly Ascomycetes. In this case, succession is based on nutrition. This is more clearly shown in the late predominance of species utilizing the most resistant component of the substrate (Keratin).

CONCLUSION

Microbial ecology in the study of community dynamics and the interaction of microbes with each other and with plants, animals and the environment in which they live Micro-organism are an important part of ecosystem (self regulating biological communities and their physical environments). Microbial ecologist employ a variety of diverse analytical techniques to understand the critical role of microbes in specific ecosystems and in maintaining life on earth.

SUMMARY

Microbial ecology is the study of microorganisms' interactions with their living and nonliving environments. Micro-organisms function in physical locations that can be described as microenvironments. The resources available in a microenvironment and their time of use by a microorganism describes the niche.

TUTOR MARKED QUESTION

1a. Define the following terms:

Ecosystem

Habitat

Niche

b. What is the order of successions and colonization of cellophane films as described by Tribes (1960)?; and on what basis is this succession taking place?

Answer

a) An ecosystem is a combination of biotic and abiotic components of a specific environment.

A habitat is an area having a degree of uniformity in terms of the abiotic components.

A niche of a microorganism is the role of that microorganism in a particular place or habitat.

b) Tribe (1960) studied the colonization, succession and breakdown of cellophane films buried in a number of soils. He found out that the first colonizers were fungi belonging to three (3) classes. The first group were those characterized by coarse mycelium. They cause lysis of the cellulose adjacent to their hyphae and they were clearly cellulolytic.

Members of the second group are the *Chaetromium* and *Humicola* species which are the well known cellulolytic genera. These penetrate the thickness at scattered sites by means of "roofing hyphae". The third group of fungi are the chytrids which develop on the cellophane pieces as the cellophane is no longer intact.

These groups of fungi are the pioneers and they have ability to produce "cellulases". The next group of colonizers are the bacteria whose population increase during mycelium senescence. In turn, the bacteria support a population of nematodes and protozoa. After the microorganisms, mites, springtails, and other worms become active and the substance becomes unrecognizable by their passage through the guts of these microorganisms.

The succession and colonization of cellophane film is base on expects of nutrition.

FURTHER READINGS/REFERENCES.

Arms, K.; and Camp, P.S. 1979. *Biology.* W.B. Saunders co. New York, Atlanta, Chicago

Curtis T.P.; and Sloan, W.T. 2005. Exploring microbial diversity – A vast below. *Science. 309*: 1331-13333

Delong, E.F. 2002. Microbial population genomic and ecology. *Curr. Opin. Microbial 5*:520-525

Dutta, A.C 2000. *Botany for Degree Students.* 3rd Ed. University press. Calcutta, Oxford, New York.

Griffin, D.M. 1972. *Ecology of Soil Fungi.* Chapman and Hall. London.

Overbeck, J.; and Chrost, R.J editors, 1999. Aquatic Microbial ecology, biochemical and molecular approaches. New York, Springer- verlay.

UNIT 2: MICROORGANISMS IN ECOSYSTEMS

Introduction

In this unit, we shall consider the various environments where microbes can exist. The microbial environment is complex and constantly changing. It often contains low nutrient concentrations (oligotrophic) and exposes microbes to many overlapping gradients of nutrients and other environmental factors. The physicochemical factors that govern the growth and survival of microbes in these environments and the methods employed in their isolation, study and quantification are discussed here.

Objectives

The objectives of this unit are to consider the various microbial environments, survey the effects on microbial growth of individual environmental factors such as water availability, p^H, temperature, oxygen concentration etc; and also consider the techniques for isolation of microorganisms from the soil, air and water.

Main Contents

2.1.0 SOIL MICROBIOLOGY

The soil (as an ecosystem) is the natural medium for terrestrial plant growth. It is composed of varying proportions of organic and inorganic components which arise as a result of interactions between many complex processes such as weathering of rocks, decomposition of plants and animals materials, and redistribution of materials by water movement and human activities. Some of the particles forming soil come together to form aggregates or clumps.

The way in which they are arranged spatially gives the soil a structure.

Like many other types of environments, microorganisms (bacteria, fungi, algae, nematodes, actinomycetes, and protozoa) are also present in the soil and they are known to perform various functions and even contribute to the structure of the soil by producing gums and cement which apart from holding the soil together can also damage the structures by blocking the pores.

Functions of soil Fungi: Fungi are abundant in the soil, however not as bacteria. Different soils have different types of fungal species associated with it depending on the prevailing conditions. Fungi are active in the soil as mycelia and are usually dormant as spores.

1. One of the most important functions of soil fungi is the degradation of complex plant structures like hemicelluloses, pectin, lignin, cellulose etc into simple molecules which are made available to plants as nutrients.
2. They help in the formation of stable soil by binding the soil through hyphal penetration.
3. Fungi are able to breakdown complex proteinous materials, producing ammonia and sulphur compounds which could be used by higher plants.
4. Fungal degradation activities improves soil texture and organic composition

5. Soil fungi may function as parasites in the soil under certain conditions to the disadvantage of higher plants
6. Often, fungi compete with higher plants for nutrients like nitrates and ammonia.
7. Under certain conditions, they are able to trap nematodes and protozoa in the soil (biological control).

Soil Bacteriology: Bacteria are known to be the most abundant type of soil micro-organisms. Hundreds of species of bacteria are present in 1gram of soil and they vary in different soils found in various parts of the world. The nature and extent of soil bacterial population however depends on prevailing environmental conditions, such as moisture, p^H, temperature, aeration and nutrient availability. Soil fertility is largely dependent on bacterial activity, and soil bacteria are essential to all life processes because without putrification and decay, there will be no decomposition of dead plants and animal matter.

Soil bacteria by their activities also make simple chemical substances such as Nacl, $Capo_4$, $NaNo_2$ etc available to plants.

2.1.1 METHODS OF STUDY AND ISOLATION OF SOIL MICROORGANISMS.

The soil serves as a reservoir of many microbial pathogens of plants and animals which play an important role in soil economy. The ability to detect, and if possible isolate these microorganism in pure cultures has become important to soil microbiologists. There are however, various techniques for isolation, many of which are selective. There are some problems encountered too, in the isolation. Two main difficulties are commonly connected with isolation of soil microorganisms. These are:-

(a) The inability of soil microorganism to grow and complete their life cycles in defined synthetic media.

(b) Contamination especially by bacteria and fast growing fungi. The advent of antibiotics has taken care of the second problem while the first is yet to properly solve.

It should be however, noted that there is no single efficient technique of isolation of soil microorganisms, but a combination of many techniques may be beneficia. Results obtained and its interpretations depend on the technique used. The various techniques which can be used in the isolation of soil microorganisms include:-

1. **Soil Plate Dilution Method/Serial Dilution Method**. (**Waksman;1927).**

   In this method, a known amount of soil is taken with a known volume of sterile distilled water; then serial of dilutions are made. Sample volumes of each dilution are then incooperated with just molten agar in Petri dishes, allowed to cool and then incubated. After a convenient interval, the number of colonies resulting are noted, and pure cultures can then be prepared for further identification. From the results, the number of colonies per plate, the species and the amount of microorganism per gram of soil can be calculated.

Advantages

This method describes the soil microorganisms in terms of species and frequency per gram of soil. Therefore, it gives both quantitative and qualitative accounts of the microbial spore load of the soil

Disadvantages

a. The Petri dishes have specific nutrients and specific environmental conditions (p^H, temperature. etc) so the fungal colonies appearing are those favoured by the environmental conditions and those not favoured are inhibited. This problem can be solved by using different media and varied experimental conditions.

b. Because some microorganisms grow faster than others, the fast growing ones may over grow and suppress the slow growers.

c. Colonies arising from soil plate techniques are predominantly from spores. Therefore the technique seems to favour species that spore heavily in soils. Members of *Mycelia sterila* and Basidiomycetes rarely appear.

d. Spores attached to the large soil particles will not grow because they will settle a the bottom of the dish, while those attached to fine particles will be seen growing because those particles will be at the surface.

2. **Soil Plate Method (WarCup; 1951).** In this method, some quantities of soil is dispersed in Petri dishes containing just molten agar and incubated for some days to allow for germination and growth of microbial spores.

Advantages

a. The method gives both the total and the individual species of microorganisms present per gram of soil.

b. It gives a more complete count because both spores attached coarse and fine particles will appear.

Disadvantages

The method is also an indirect method and most of the disadvantages of the dilution method will be applicable.

3. **Rossi – Cholodny Slide (Rossi; 1928).**

According to Rossi, the method consists of pressing slides against undisturbed vertical soil surface. After removal and staining, the picture of microorganisms as they normally occur in the soil will be obtained. Cholodny modified this method by allowing the slide to stay in the soil for five days to three weeks before removal. Presently, this method has been greatly modified and may involve the burial of microscope slides coated with agar in the soil. The measure of the length of hyphae developing on the coated slide is a measure of the relative activity of the microbial species in the soil.

Limitations:

a. The substrates (agar) are not natural to the soil.

b. Introduction of the slide to the soil is a kind of disturbance.

c. The isolation of hyphae for subsequent colony differentiation might be difficult.

4. **Other Indirect Methods** of isolation of soil microorganisms are modifications of the three basic methods outlined above.

a. **Perforated or Immersion Tube** (Chesters and Thorton; 1956).
In this method, agar is protected from direct contact with the soil by pouring it into perforated tubes or by enclosing them in perforated boxes or plates. The aim of this method is to modify the Rossi-Cholody technique so as to isolate those fungal species which are active in the soil rather than those ones existing in dormant forms.

Disadvantage:

The problem of selectivity of the agar medium to different species of fungi.

b. **Direct Method** (Casida; 1939). This method involves examination of soil samples with the microscope. It involves staining of prepared soil films. The method reveals only small percentage or proportion of hyphae and spores present in the soil sample. It also makes identification difficult except where reproductive structures are clearly visible.

c. **Biological Method** (use of Bait = natural medium). In reality, this technique is similar to enrichment culture and mostly living hosts or organic materials are used as baits to attract the particular microorganism one wants to isolate. In many case pure cultures can readily be obtained by subculturing directly from the bait after it has been in contact with infected soil, water or plant material for some time. Known examples of baits and the microorganisms they isolate includes:

Pears = *Erwinia amylovora*
Hair/Nail = Keratinolytic fungi
Cotton = cellulolytic Species
Hemp seed = Saprolegniaceae
Apple/pinaple = *phytopthora cinnamonii*

2.1.2 FACTORS AFFECTING MICROBIAL GROWTH IN THE SOIL (Soil as an environment for micro organisms).

A combination of a number of factors physical, chemical and biological make it possible for microorganisms to survive in the soil. Among these factors are:

a. Soil components (soil type and structure)
b. Soil water/moisture content
c. Aeration
d. Soil temperature
e. Soil p^H
f. Soil profile

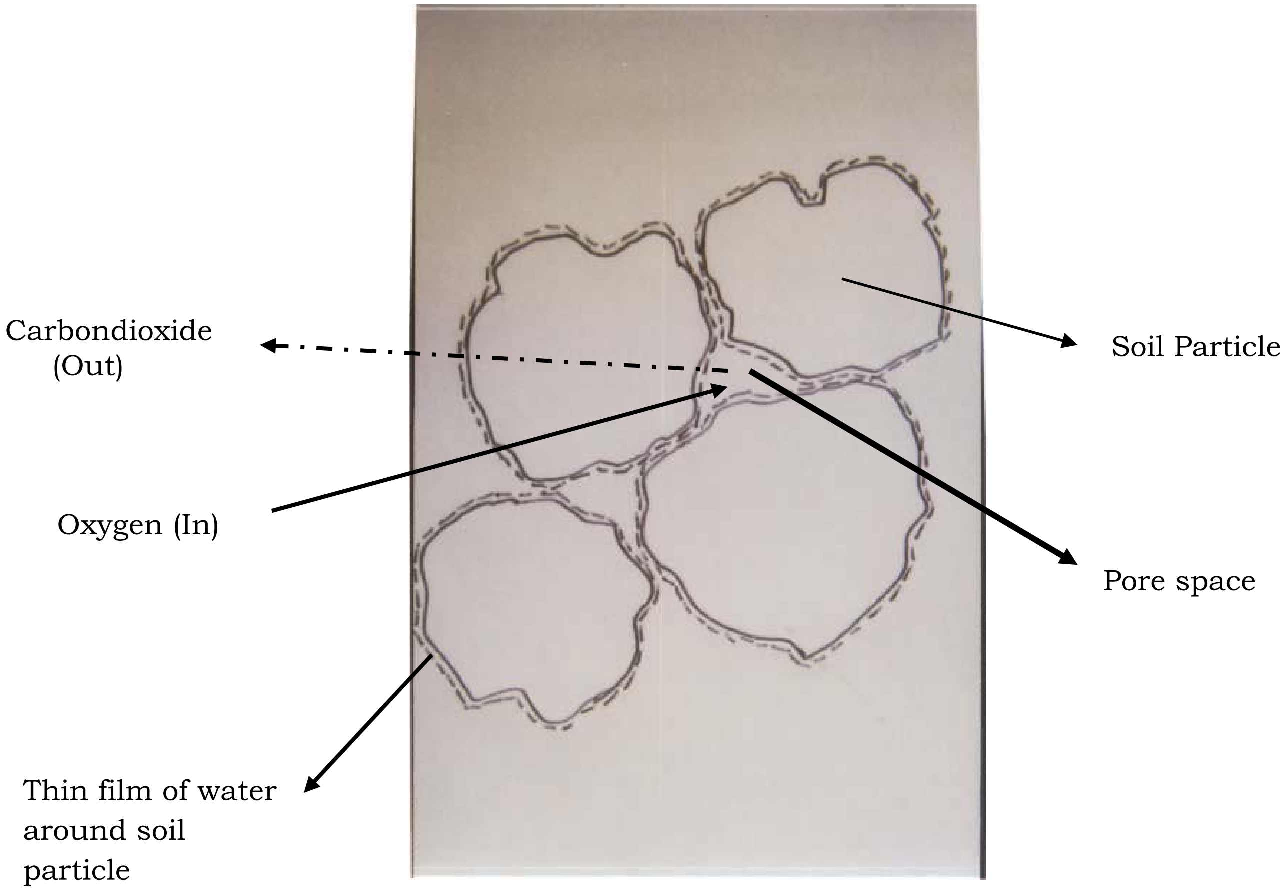
Most of these components are constantly changing, and fluctuations in one component may result in fluctnations in the other factors. Thus, an alteration in moisture content influences aeration, temperature, and chemical reactions in the soil.

Soil Components: The soil consist of a mixture of weathered rock of various sizes. The proportion of the size determines the soil texture. Clay particles are important components of soil environment; and they greatly influence other physical and chemical properties of the soil. Montmorillonite (a clay mineral) when present in the soil in low concentrations reduces the rate of the fungal respiration while stimulating bacteria either at low or high concentrations. This type of mineral preserves a p^H condusive to bacterial growth. Organic matter and humus are formed from remains of plants and animals remains and are constantly supplied to the soil. Organic matter which is not completely decomposed contributes to the formation of amorphous humic materials which is usually important in soil studies. Humus is not a chemically defined entity. It is a complex mixture of many substances the bulk of which is insoluble dark colored materials. Generally speaking, organic materials in the soil decrease with soil dept.

Soil water/moisture content: Disolve a known weight of soil in a known volume of water (usually, soil: water in ration 1:2); dry to constant weight and then:

$$\text{Moisture Content} = \frac{\text{less in weight on drying}}{\text{initial weight}} \times 100\%$$

Soil water is usually present as a thin film around the soil particles.



Aggregates of Soil Particles

Generally, soil water is subject to extreme fluctuations and this has some important effect on soil microbial populations. Many microbial cells are killed by desiccation and only those ones with resistant structure can survive drought. Warcup (1957) found out that fungal species survive in Australian soils as spores, rhizomorphs, sclerotia and resting hyphae. Based on soil water, microbial populations are known to fluctuate with the seasons of the year. Studies have also shown that remoistening dry soil results in the restoration of microbial activities. This is due to the fact that when water is available we have more soluble nutrients becoming available to microorganisms. On the other hand, drying of soil is known to kill some microorganisms which then produce a source of nutrients for the survivors.

Drying could also result in the evolution of certain types of microorganisms which can adapt themselves to low water requirements.

ROLES OF SOIL WATER

- Water is useful in keeping microbial cells turgid.
- Water plays some roles in dispersal of spores.
- Water provides a medium for movement of reproductive structures in some species.
- Water is useful in the diffusion of toxic metabolic wastes away from the cells.
- Soil water is also useful in the distribution of extracellular enzymes in the soil.
- Water is a universal solvent and it is the site and medium for organic reactions.
- Soil microorganisms live in the thin film of water around soil particles.

Soil Aeration

The amount of air present in the soil varies, and this often has some relationship with soil water content. There is also an exchange of gases between the soil and the atmosphere through the air spaces in the soil. The amount of air present the soil varies with the soil type and the different points in the soil e.g in regions of intense microbial activity, especially near plant roots (rhizosphere) the concentration of the air will be drastically altered as a result of metabolism by rhizosphere organisms.

Microbes very considerably in their responses to changes in the concentration of air in the soil and atmosphere. Some bacterial species are strict anaerobes (e.g. *Clostridium botulinum* (food poisioning) while some are aerobic (e.g. *Pseudomonas fluorescence*). Most fungal species are aerobic.

Decomposition of substrates under anaerobic condition by microbes may result in the formation of incompletely oxidized end product e.g. when cellulose is decomposed by bacteria in aerobic condition the end products are $C0_2$ and H_20; but under anaerobic condition oxidation is incomplete and organic acids such as acetic acid accumulates and this in turn affects soil p^H. The concentration of oxygen in the soil also affects the oxidation reduction in the soil while concentration of $C0_2$ affects soil p^H,

- provides a source or environment for autotrophic microbes, and
- have some effect on heterotrophic micro-flora. The deeper you go into the soil there is a decrease in soil aeration.

Soil hydrogen ion (H^+) concentration

Suspend 5 grams of soil in 10 mls of water and mix thoroughly. Take the p^H using a p^H meter or litmus paper. Based on the p^H tolerance of organisms, soil microorganisms have been classified into:

Acidophiles – low p^H lovers

Alkalinophiles – high p^H lovers

Halophiles - grow in high salt concentrations.

The soil p^H has remarkable influence on soil microorganisms. Soil p^H can be affected by a number of factors such as the salt concentration and concentration of $C0_2$ in the soil. Thus as these factors fluctuate soil p^H also changes.

Leaching also affects the soil p^H. As water moves through the soil, bases are leached out and replaced by H^+, therefore constant soil leaching leads to the formation of acid soil. Such changes can also affect other physical properties of the soil.

Majority of soil microorganisms grow well at p^H of 7. However, there are a number of exceptions especially *Thiobacillus* species. This organism is acidophilic and can grow at a p^H of 0.6. Generally, soil bacteria and actinomycetes are less tolerant to acid conditions than fungi. Many species of fungi grow in acid conditions and acid soils such as Pod-sols where the p^H may be as low as 3.0. However, p^H preferences are varied among genus and even species. It should however be noted that the occurrence of a microbe in soil with certain p^H does not mean the particular microorganism is carrying out all its activities at that p^H.

Decomposition and other microbial activities taking place on the soil surface result in the release of acids and ammonia and these in turn results in an increase in the p^H of the soil surface

Soil temperature: Use a good thermometer (Thermocouple) to record temperature. Based on temperature requirements, soil microbes have been classified into

Psychrophiles – cold lovers (0-20^{0}c)

Mesophiles – moderate temperature lovers (20 – 40^{0}C)

- Optimum at 25^{0}c – 37^{0}c

Themophiles = High temperature lovers.

- 20 – 50^{0}c
- Optimum around 40^{0}c

Thermotolerants are mesophilic but capable of tolerating high temperature conditions.

The sun is the major source of soil temperature and several factors are responsible or determine the solar energy absorbed by the soil. Among there factors are:

- The angle at which the sun hits the land.
- The color of the soil; (light colored soils absorb heat less than dark colored soils);
- Vegetation cover; and
- Amount of soil litter

The average monthly temperature on the surface of forest soils varies from 2-19^{o}c, and at 7.5cm depth, it is reduced to 4-14^{o}c. There is a close association existing between the soil moisture content and the heat absorbing capacity. About one calorie of heat is required to raise one gram of water to 10^{o}c while 0.2 calorie is required to raise 1gram of dry soil to 10^{0}c. This implies that the thermal conductivity of wet soils is higher. As soil temperature rises so also the diffusion rate of gases rises. Therefore this results in a complex interaction between moisture, temperature and aeration. The higher its temperature, the more the microbial activities and the more the microbial population. Temperature requirements however varies among the different groups of microbes.

Soil profile/depth. This denotes changes as you go into the soil. Microbial populations in the soil decreases with increase in soil depth. That is, on the soil surface, a lot of microbial activities are taking place. Therefore the population of microorganisms in this zone is usually higher. Other reasons to explain these trends include:

- Presence of substrates which act as food for the microorganisms on the surface layer.

- The effect of sun rays on surface soil temperature has some relationship with enzymatic activities.
- Because of agricultural activities and microbial interactions, soil particles on the surface are not too closely held together and this gives room for aeration

- The water content in soil surface contributes to microbial interactions

PRACTICAL 1&2

Isolation of soil micro-organisms. Measurement of physical and chemical parameters.

Procedure. Collect soil samples (into beakers) at different depths.

(a) Soil surface;

(b) 15cm;

(c) 30cm; from;

(i) Any botanical garden

(ii) Exposed ground (foot path) leading to the Botany garden.

For the different sampling sites, observe and record:-

a. The color of the soil
b. The nature of the soil particles (aggregated, sandy, clayey e.t.c.)
c. The temperature at the surface and at 30cm depth. Record the difference and explain why.
d. p^H of the samples (for the 2 sites) at different depths.
e. Moisture contents

Estimate, using soil dilution technique and soils plate method, the microfloral of the soil at different sites and depths.

(i) What are the disadvantages of your methods of study?

(ii) What are the possible roles of these micro-organisms in soil? Discuss your results and observations.

2.2.0 AEROMICROBIOLOGY.

This is the study of micro-organisms in the air. Like the soil and other environments, micro-organisms are represent in the air and perhaps because of their role in the transmission of some air-borne infectious diseases and the dispersal of microorganisms, the study of aeromicroorganisms has been receiving attention from microbiologists and scientists generally. The components of the aerial environment are varied and mostly include pollen grains, dust particles, fungal spores, bacterial spores, fungal hyphal fraqments, actinomycetes, spores of bryophytes and pteridophytes e.t.c

Usually, micro-organisms in the air are in a state of suspended animation. Many of them are easy killed by desiccation, ultra violent rays and other unfavorable conditions. Therefore the living micro-organisms are either usually resistant or have been in the air for some time. Resistant spores are capable of producing pigments and other means of adaptations.

The dispersal of air – borne particles involves 3 stages

- Liberation and take off into the air
- Dispersion in air current
- Deposition on surfaces at the end of the journey before germination and growth.

LIBERATION:- Before a particle becomes airborne, a number of problems have to be overcome. Example, energy is to required to over come the adhesive forces attaching the particles to the surface of the substrates; also the particle has to be of a size that will be airborne. The degree of adaptation to air borne dispersal varies greatly between different groups of micro-organisms and this is reflected in their relative abundance in the air.

Viruses and bacteria are poorly adapted while fungi have developed many adaptations mechanizing to enable their spores become readily air-borne. While some have long sporophores (stalk) which lift their spores well into the air, some others have ways of forcefully ejecting their spores into the air. Some others rely on passive means. Rain splash, mechanical disturbance and other physical adaptations help a lot in spore liberation into the air.

Dispersion:- The dispersion of air-borne micro-organisms can be considered at two levels.

Fate of individual spores; and the behavior of groups or cloud of spores.

These aspects are related and depend on the physical characteristics of the spores and that of the atmosphere. The important characteristics of spores in this respect are size, shape, degree of surface roughness, density and electrostatic charges; while those of the environment include wind movements, turbulences, layering convention, wind gradient near the ground and the pattern of atmospheric circulation. Because they are heavier than air, spores tend to settle under gravity but because they are also blown by air, they are affected by electrostatic charges. The distance traveled by a spore depends on the interrelationship of the various factors mentioned.

Deposition:- The final stage of airborne dispersal of micro-organisms is deposition. The microbes are returned to the surface layer of plants, animals or soil so that they can no longer be blown by normal wind, though they may still be washed off. Deposition may occur in precipitation or from dry air by several different methods such as sedimentation, impaction, rain washing and so on.

2.2.1 FACTORS AFFECTING CONCENTRATION OF MICRO-ORGANISMS IN THE ATMOSPHERE.

A number of factors can directly or indirectly affect the amount of micro-organisms and their survival structures in air. Such factors may include wind speed, rain, temperature, pigmentation, humidity, vegetation and human activities.

Wind Speed. Fast moving air blows force with it and so readily provides energy for detachment of spores from their vegetative structure and other surfaces. Usually, it is difficult for spores to be deposited at a spot except where there is an obstacle or a wind breaker along the wind course.

Rain Splashes. Rain washes air-borne spores to the soils and as such the amount of rain received by an area may have some influence on the number of air-borne spores and microorganisms. Where an area has a clearly defined raining and dry seasons, the amount of dust particles and spores in the air may vary with the seasons of the year.

Temperature. During the dry season, those air-borne spores that are thin walled are easily dehydrated to the advantage of those with thick warty walls which are more adapted to survive high temperatures. There is also a relationship between temperature and humidity, then temperature and wind movement. Freezing temperature is likely to be encountered by air-borne microbes above 3-5 km in the air (the higher you go the cooler). Depending on how well adapted the structures of the microbes are, the very low temperatures may or may not have a damaging effect on the survival structures of micro-organisms.

Humidity. The water amount in the air is usually referred to in terms of relative humidity that is the ratio of the actual vapour pressure when the air is saturated with water at the same temperature. Vapour pressure varies greatly with temperature and thus relative humidity is low when temperature is high and vice-visa. Relative humidity is a good indicator of the drying effect air on micro-organisms. A wide range of relative humidity may be founds in the atmosphere from 10- 20% in desert regions to saturation or super saturation where cloud formation occurs at ground level resulting in dews or frost.

Different micro-organisms have different relative humidity requirements for their survival in the air. The lower limit of relative humidity for fungal growth is about 65%, while bacterial species require more.

Pigmentation and ultra-violent rays. Ultra violent radiation in the air originate from the sun much of which is reflected or absorbed before reaching the earth's surface. Micro-organisms carried into the higher regions of the atmosphere may be exposed to the damaging doses of U.V radiation. Though ultra-violent radiation of about 265mm is most lethal, other short waves radiation even within the visible region can also be damaging. Pigmented spores especially those of dermatophytes can resist strong ultra- violent rays from the sun. Generally speaking, radiation, desiccation and temperature all interact to affect the survival of micro-organisms in the air. The manner of interaction is not well understood but desiccation and freezing may protect micro-organisms against radiation damage.

Vegetation: This can affect spores in air in two different ways. Leaves can trap spores when washed down by the rain. Some may germinate when conditions are suitable. On the other hand, when the leaves dry off the spores can be blown back into the atmosphere and thus increasing the number of air-borne spores.

Man's Activities: As a result of poor drainage systems, of rubbish dumps and so on, micro-organisms in rubbish dumps and dry feacal materials are blown up into air. Also during activation and soil digging, soil-borne spores are released into the atmosphere. This maybe used to explain why some farmers in some parts of the world suffer from an infection of the lungs and pulmonary tracts caused by *Aspergillus* species, and the disease is called aspergillosis.

2.2.2 METHODS OF STUDY AND ISOLATION OF AERIAL MICRO-ORGANISMS.

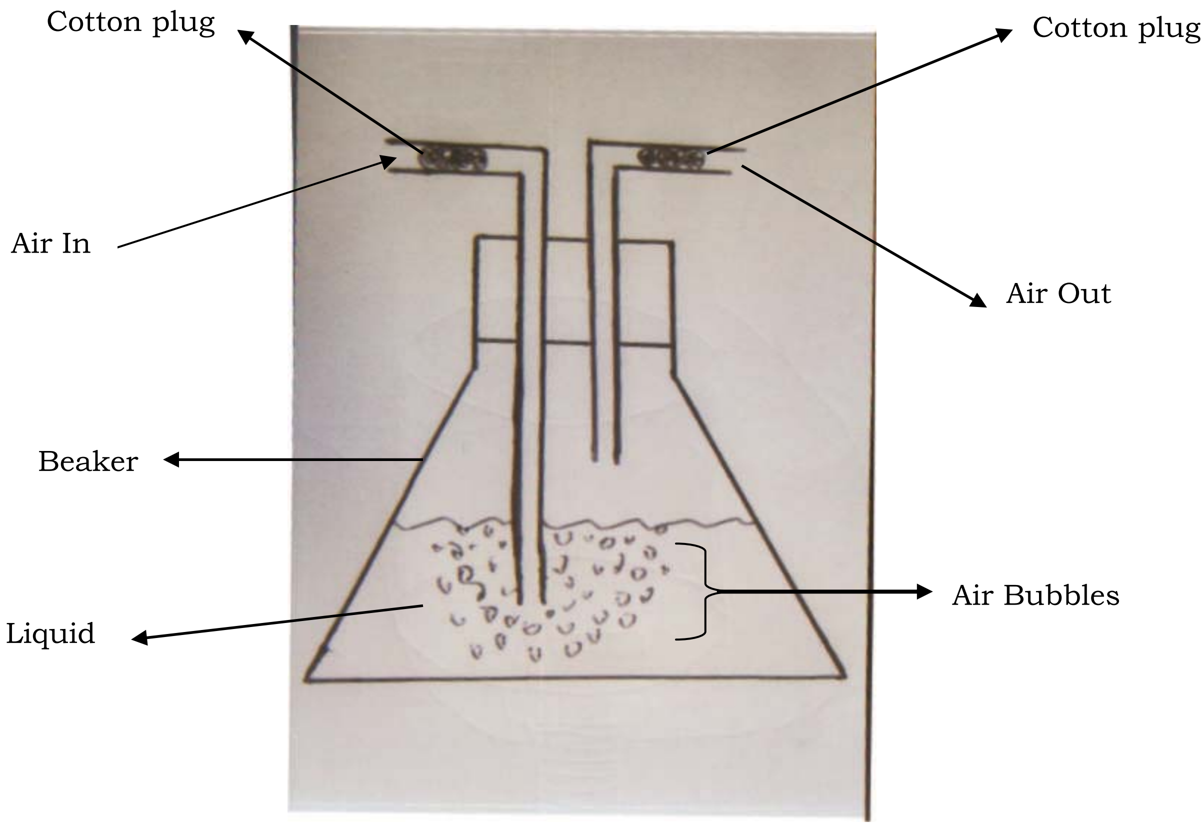
Studies on aerial micro-organisms result from the fact that structures of many disearse-causing organisms are borne in the atmosphere. Different techniques and equipments have therefore been designed and used for the isolation of various types of aerial micro-organisms; but generally speaking, the determination with accuracy of the number of aerial microorganisms is difficult expect with some sophisticated equipment. Simple comparison of aeroflora is however possible with simple equipment. The study of air-borne microbes necessities their separation from air for culturing, microscopic observation, counting and classification. Many air sampling instruments have been designed for industrial hygiene, gravimetrical, chemical and other types of analysis. Most of these instruments however have limited application in aerobiology.

Presently, a wide range of instruments are available for trapping airborne microbes. The different techniques utilize a variety of processes for trapping microorganisms, of which sedimentation, filtration and impingement into liquids and impaction on solid surfaces are probably the most important. It should be noted that the counts of air-borne microorganism vary widely with the time of the day, seasons of the year, prevailing weather conditions and other related factors.

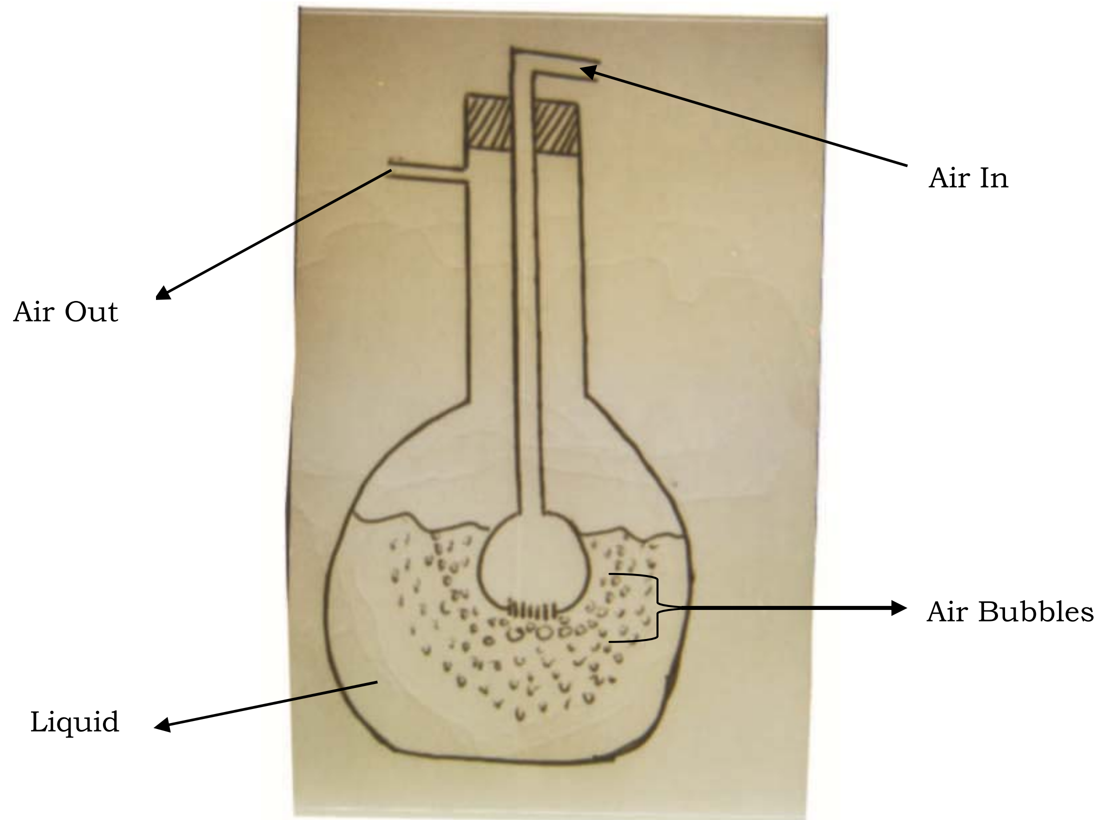
Settle Plate (Simple Gravity Plate) Technique. This is the most widely used method for isolating air-borne micro-organisms. It involves exposing Petri dishes containing nutrient agar medium on a stool outside for a time interval. Particles are allowed to settle on the medium for the said time interval. The dishes are then collected incubated and after a particular time of incubation, colonies are counted and identified. The method is cheap and convenient but the interpretation may be difficult because catches cannot be related to the volume of air sampled, and the rate of deposition varies with terminal velocity, wind speed and turbulence. Larger particles are effectively collected from a larger volume of air than from small volumes of air.
Report have shown that 50 times more of *Puccinia* species spores can be collected than that of *Streptomyces* will be collected at a given time. Also, with this method short sampling periods are only possible so that diunal changes are difficult to detect.

The method gives a qualitative account of air-borne micro-organisms.

Filtration Techniques. This may be filtration using membrane or glass fibers. However excessive desiccation of propagules may limit its usefulness to culturing only resistant cells. Filters may collect cell by sieving, impaction, diffusion or electrostatic attraction; each of these working alone or in combination with another depending on the types of filter. Membrane filters can be cleaned for microscopic examination or propagules can be grown after washing and plating on nutrient media. High volume samples may be useful where organisms in small numbers can be isolated on very selective media. Impingers are used to accelerate air through a Jell or a filter into a liquid which retains the particles. The liquid can be plated out on suitable media and later, the growing micro-organisms are examined microscopically. Since the liquid can be diluted before plating, long sampling periods are possible and also the particles are likely to be broken down to individual cells by agitation of the liquid.

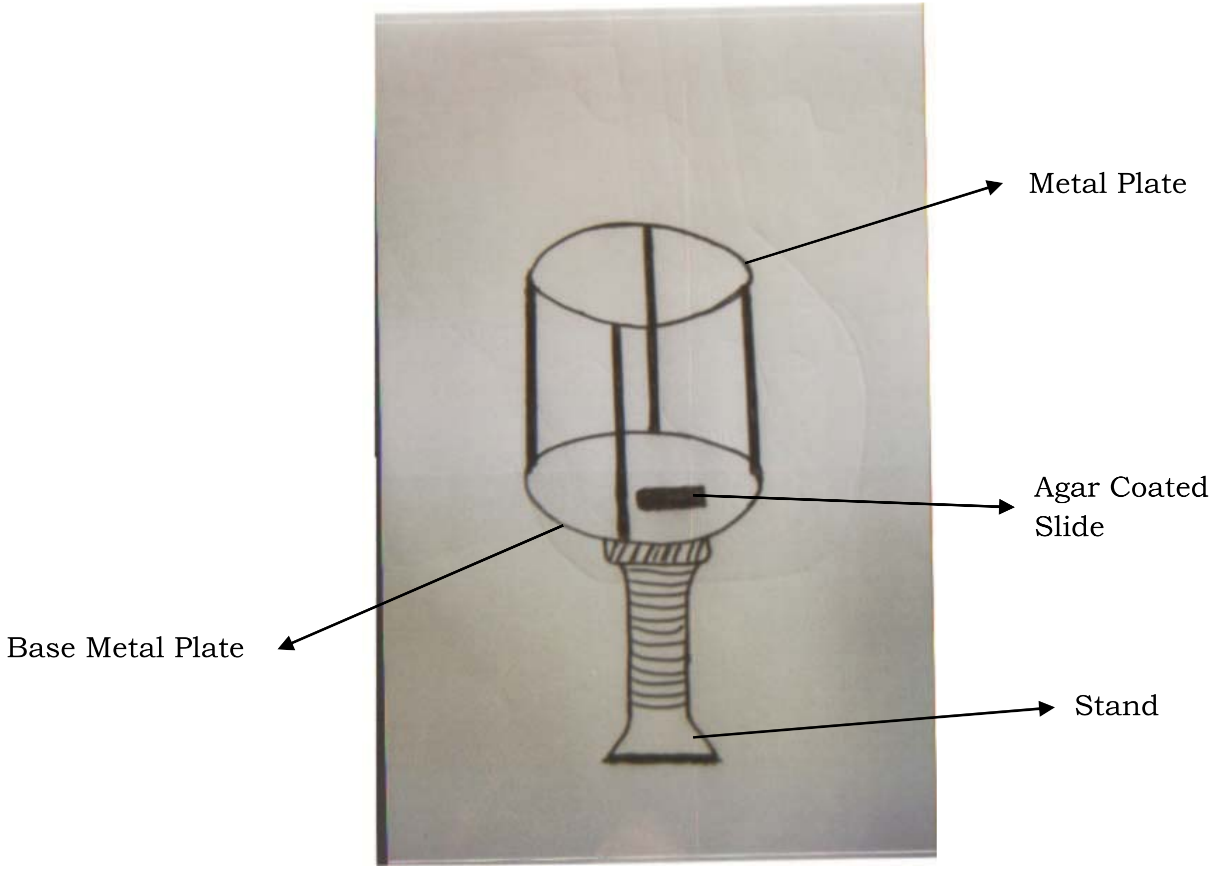


A Simple Impinger

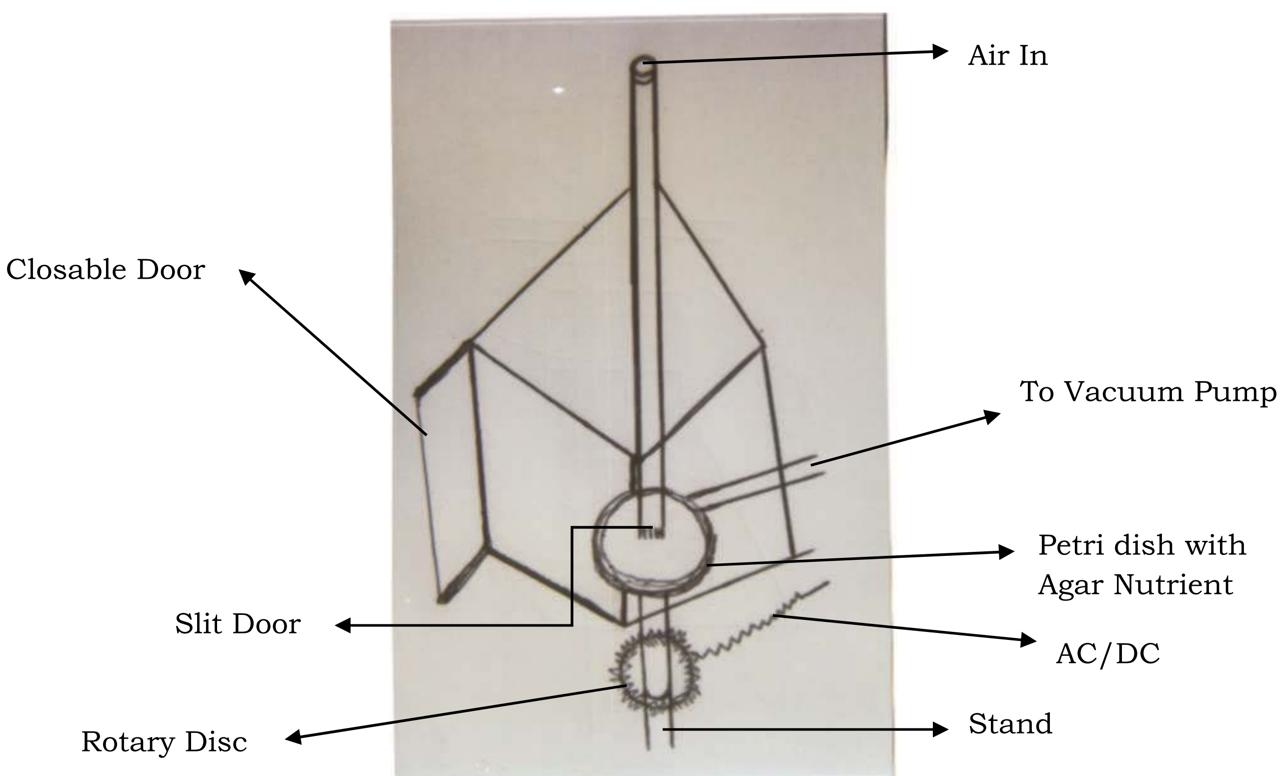


An Aeroscope Bubbler

Impastors have also been widely used in aerobiology. Examples are the Cascade impactors and the automatic volumetric trap which traps spores on coated microscope slides. We have the Anderson slit sampler which has been designed to retain spores on agar media in Petri dishes. It also separates catches to different size fashions, while the automatic volumetric trap traps spore on a moving slide and therefore enable the time of traping to be determined. The impaction method essentially measures the number of particles in the air, although microscopic counting of particles in the air may also be possible.



A Covered Plate of Exposure Platform



Anderson Slit Sampler

CHOICE OF SAMPLING METHOD

No one method is ideal for all needs. A number of questions have to be answered before one or more of the methods are selected. For instance, what are the reasons for investigation? Is it to investigate the total air flora or a particular group? Is it to determine the health hazards or presence the particular organisms in an area? Is it in relation to the occurrence or spread of a disease (epidemiology)?.

WHAT INFORMATION IS REQUIRED?

- Is it the total number of air-borne micro- organisms or the viable ones?
- Is it the size of the particles or changes in concentrate with time?
- Are the microbes distinct microscopically or culturing is necessary for identification?
- Is selective identification/ isolation method required?

Most methods allowing microscopic assessment are the least selective and most suitable for studies of total air spora, particularly of pollen grains, cryptogams and spores of algae. However spores of a few fungi can be identified to species level, although those of many plant pathogens can at least be identified to genus level. Also bacteria may be carried on dust particles that obscure them on slides.

Isolation in culture enables specific identification, but the use of media and incubation conditions are specific for specific groups.

One problem with most of these methods is that obligate pathogens do not grow and some fungi may not sporulate in culture.

PRACTICAL APPLICATION AND IMPORTANCE OF AEROMICROBIOLOGICAL STUDIES.

The application of aero microbiology is of great importance in disease epidemiology, forecasting and modeling. Epidemiology is the study of incidence, distribution and control of diseases within populations or the sum of factors controlling the presence or absence of a disease or pathogen. Epidemiology therefore concerns the interaction between the pathogen, its host and the environment. Aerobiology can make valuable contributions to the epidemiology of animal as well as plant diseases. Sinnecker (1976) recognized six types of diseases with aerogenic transmission depending on:

- the resistance of infective agents in droplets;
- the drop's nuclei or dust;
- the ability to infect from secondary aerosols formed when dust is re-suspended;
- the host range;
- whether there are alternative means of transmission;
- and whether the infective agent can survival for long in droplet form. Even non-infectious diseases can occur epidemically as a result of air-borne infection e.g. coccidiomycosis and histoplamosis, when a population is exposed to large number of causal fungal spores. Aerobiological methods can be used to

detect the presence and quantity of infective agents in primary and secondary aerosols. Their persistence in air and relationship with infection can also be known.

In plant pathology, aero microbiological methods have been widely used to determine the number of spores of a plant pathogens in the air. Aero microbiological studies can also be used in forensic and theft detection by plating out pieces of cloths from suspects and matching the microbial isolates with those of the aerial environment where the theft was carried out.

2.3.0. AQUATIC MICROBIOLOGY (Micro-Organism in Natural Waters)

Most microbial environments are aquatic, in that sometime, the vegetative organism live in aqueous media such as animal and plant fluids, soil, water and many other related habitats. Water is also usually required for the movement of various reproductive structures. Aquatic microbiology however refers to that micro-organism which live in the earth's natural waters ranging from the small ponds to great oceans. Most of the earth's water is contained in the oceans (97.1-97.6%), while smaller quantities are maintained in polar regions as ice and glaciers (2.1%) and ground water (0.3-0.8%) and in land waters

Lakes – 0.009% and

Rivers - 0.00009%

Despite the wide range of conditions encountered in the various aquatic habitats, micro-organisms are found in all types of aquatic habitats.

TYPES OF AQUATIC ENVIRONMENTS

Aquatic environment are often individual into oceans and inland waters. Inland waters can be classified into ground and surface waters. Ground water is that water contained in permeable rocks below the water table. It accumulates as water in the soil perculates through it. Many of the nutrients are therefore filtered out. Because of this, ground water is only able to support a limited population of micro-organisms.

Surface water comprises of lotic or running waters consisting of springs, streams and rivers. The second group are lentic or standing waters comprising of lakes, ponds, swamps and bogs. Springs can occur where ground water breaks the surface and thus like ground water, they are nutrient poor near the origin. Specialized springs occur in certain regions such as hot springs in regions of volcanic activities. We can also have mineral springs and this depends on the surrounding geological conditions. They may occur when water accumulates dissolved mineral as it passes through geological formations. Example hard water and springs found in many lime stone areas. Springs eventually become rivers as their flow is supplemented by tributaries, land run offs and precipitation. A number of factors however determine the rate of flow. Such factor include gradients, transverse conditions of the river valleys etc. Lentic waters differ in size ranging from the smallest ponds to the deepest known lake. Often, distinction between lakes and ponds are not clear but generally, the bottom of ponds are colonized by submerged plants whereas lakes are too deep to be colonized by plants.

The oceans cover approximately 70% of the earth's surface and the depth is variable ranging from 200m to 10000m or more. Because of this great depth much of the sea is in near total darkness. Of great importance to abiotic and biotic components of oceans is the salt content which varies 32% to 38% with an average of 35%. Because of the additional dissolved salts in the sea, the specific heat capacity is high and the freezing point temperature is affected. Salinity is the major phenomenon which is often used to distinguish between water bodies. On this basis, Moore (1961) classified water bodies based on the salt content as follows:

0.5% = fresh water

0.51% = 29.9% = brackish water

30.0% and above = marine (sea and ocean) water

SPECIAL HABITATS IN AQUATIC ENVIRONMENTS

Just like any other habitat, important special habitats can be found in aquatic environments and the micro-organism inhabiting such habitats have special features of survival in such areas. Such feature may be structural or physiological. One of such habitats in any water body is found in sediments and particulate matter comprising the bottom. These vary considerably from sand to mud and silt. These can be classified into three major components according to the origin.

1. **Lithogeneous Components:-** those primarily derived from rock, soil and volcanic ash.
2. **Biogenious Components:-** those composed of skeletal remains of macro-,and micro-organisms e.g. diatoms
3. **Hydrogenous Components:-** those components resulting from inorganic and chemical reactions occurring in the water. Bottom sediments may be a particularly important reservoir of organic and inorganic nutrients and when highly reduced they may provide habitat for anaerobic organisms. Another importance special habitat in aquatic environments is provided by surfaces of plants, animals and non biological structures such as stones. Enough bodies of water surface may be heavily colonized by micro-organisms and physical and chemical conditions occurring at the surface may provide micro-environment much like that of the surrounding water.

Physical and Chemical Factors in aquatic environments

Light. is a critical factors in determining the amount of carbon fixed into an inorganic compound through photosynthesis. Much of the sun's radiation does not reaches the earth's surface since it is absorbed and scattered by atmosphere and cloud cover. Some of the radiation which reach the water surface is reflected back to atmosphere and the small amount that penetrates, suffers further the attenuation through scattering and differential absorption. In pure water, approximately 53% the total light is dissipated as heat and quenched at the first meter of water depth.

In most natural waters, light extinction may be considerably affected by the amount of dissolved solids and suspended materials; and in water with appreciable dissolved and particulate materials, the general effect on light penetration is that there is a general decrease is transmittance. The intensity and specific composition of light penetrating water surface and to any depth has a profound effect on all resident microbial activity since it is the available light which largely determine the potential for productivity.

Generally, photosynthesis increase in relation to light intensity until the photosynthetic system becomes light saturated. Turbidity measurement have been used as a measure of light penetrance, and transparency disc is often employed for this purpose.

Temperature: The amount of heat entering a body of water through insulation depends primarily on the latitude and the water conditions; while the distribution of heat within the water body is dependent on it morphological characteristics and mixing by wind turbulence. In shallow waters such as streams and ponds, there may be large temperature changes. In most case, it is the surface water that is temporarily variable, while deeper waters show alternations seasonally. Such differential heating often can lead to thermal stratification e.g. in a deep lakes, two stratified layers can be recognized viz:

The warm (upper) layer called epilimnion

The cool (deeper) layer called hypolimnion.

However, in shallow lakes there is no stratification because there is a steady mixing of the water. The range of temperature in oceanic surface water is from – 1.7^{o}c in polar regions to 25- 30^{o}c in tropical and subtropical waters. Where as in deeper waters, temperature remains fairly constant at a few degrees above freezing point.

In the oceans and inland waters in cold climate areas where predominant water temperatures are less than 5^{o}c, the resident bacterial are mainly psychrophiles and they can grow event at 0^{o}c. In warmer inland waters, mesophilic bacteria and fungi are the predominant species. Usually if temperature is more than that required for the organisms, the cell components are destroyed or denatured and this results in death. Whereas dropping in temperature below the normal range may not necessarily kill the organisms, it slows down metabolic activity. Within a given range, bacterial metabolism increases with increase in temperature and so there is increase growth with increase temperature, but at temperatures higher than the optimum, growth rate is reduced. Temperature has a profound effect on enzymatic activity.

PRESSURE. Hydrostatic pressure is not an important factor in inland waters but can be of considerable importance in oceans at great depths. It increases at approximately one atmosphere with 10m increase in depth. Increase pressure affects dissociation constants of carbonic acid and therefore results in a decrease in pH. The pressure requirements of organisms differ greatly even among the species. The term barophilic is used to describe bacteria which grow preferably or exclusively at high pressures; While those which survive at high pressures without injury but are normally active at atmospheric pressure condition are called barotholerant or baroduric.

A number of barophilic bacteria have been isolated from deep seas and from brines in deep oil wells. Most bacteria from surface waters are adversely affected by high hydrostatic pressures but not all bacteria are affected to the same extent. In experiments dealing with bacterial growth rate at high pressures, the relationship between pressure and temperature was established. It was found out that high pressure tend to raise optimum temperature for growth.

DISSOLVED GASES. The two most important dissolved gases in aquatic environments are oxygen and carbon dioxide. Oxygen is impotent because of its importance in aerobic bioprocesses and in relation to oxidation – reduction potentials; while carbondioxide is important for photosynthesis and pH equilibrium. The concentration of oxygen in water is dependent on the water temperature, partial pressure, salinity and biological activities. oxygen concentration in water is usually expressed in terms of milliliters of oxygen is one liter of water. Carbondioxide it is very important in aquatic environments since it aids the regulation of hydrogen ion concentration. In water, an equilibrium is established between carbondioxide, carbonic acid and bicarbonate as follows:

$$CO_{2+} H_2O = H_2CO_3 \rightleftharpoons HCO_3 + H^+ \rightleftharpoons CO_3^{2-} + 2H^+$$

The solubility of carbondioxide in water is affected by temperature, salinity and other related factors. Apart from these two gases, a number of other dissolved gasses also occur in natural waters e.g. nitrogen which is not utilized by many aquatic micro- organisms but is involved in special microbial processes (nitrogen fixation). Other gasses produced by microbial activities are methane, H_2, HN_3, H_2S, NH_4 e.t.c.

Hydrogen Ion Concentration (P^H). The optimum P^H for most aquatic bacteria is between p^H6.5-8.5 and this corresponds with the P^H of most large water bodies. The approximate P^H of sea water usually lies below PH 8-8.3 while those of lakes is approximately p^H7; although considerable fluctuations may occur.

There are usually proportionally more fungal species in acid waters compared with neutral and alkaline waters. The effect of p^H is also related to enzymatic activities, and drops in p^H beyond the organism's normal range can affect its physiology, and when extreme, can lead to death. Morphological changes can also occur such as enlargement of cells and irregular swelling and branching.

Photosynthesis is affected by p^H through its influence on carbon dioxide and carbonic acid equilibrium; p^H varies inversely with dissolved carbondioxide concentration and directly with bicarbonate concentration. In aquatic environments, all photosynthetic plants use free carbondioxide with undissociated carbonic acid for photosynthesis. However lack of carbon dioxide at high P^H is not a limiting factor for photosynthesis since plants have enzymes which convert bicarbonates to carbondioxide. Some plants such as *Scenedemus quadricanda* can utilize bicarbonates directly, while *Chlorella pyrenoidosa* can not.

SALINITY. Though salinity is not itself a physical factor, changes in salinity can have profound osmotic effect which sometimes can be lethal and can also be toxic through denaturation of cellular components. Most marine bacteria are halophytic and some have specific requirements for sodium ions and some will not tolerate too high salt concentrations. Moderate changes in salinity may have morphological and physiological effects e.g. rod-shaped bacteria may form filaments with an increase in salinity and the microbial oxidation of organic acids and sugars are affected

MICROFLORA IN AQUATIC ENVIRONMENT

Micro- organisms present in aquatic environment are mainly phytoplanktons. They include bacteria, algae and fungi; others are protozoa and viruses.

Bacteria: - Usually, the bacterial flora of a river may show a peculiar close relationship with the surrounding terrestrial population because of constant injection of soil, water run offs and organic matters. Some aquatic bacteria are phototrophic while the chemotrophic ones are also widely distributed, but generally the distribution and abundance of the majority of the heterotrophs is largely controlled by the concentration of available organic materials, therefore in a nutrients poor spring, the bacteria present are mainly of Gram negative rods such as *Gallionella, Pseudomonas* etc. Along the cause of the stream, as nutrient content increases, there is a general increase in the number of species particularly those bacteria belonging to the families psendomonaceae, bacillaceae and enterobacteriaceae. Further down the cause of the river, some other species start to appear. These may include *Azotobacter, Vibrio, Spirilium, Thiobacillus, Streptomyces, Nocardia Spirochaetes* and nitrifying bacteria.

Generally, rivers have more fluctuating conditions because of inflowing tributaries and sewage outflows; and bacteria populations are variable depending on local conditions. As for the lakes, they are more selfcontained than streams and rivers and in clean waters, most of the bacteria encountered are Gram negative such as *Pseudomonas, Flavobacterium, Micrococcus, Streptrococcus* etc.

The vertical distribution of bacteria varies seasonally with stratification and nutrient distribution. Bacteria are usually high in sediments where organic materials are abundant. In the marine environment, the bacteria species present are only slightly halophilic as compared with the true halophiles such as *Halobacterium* species. Most bacteria present in this kind of environment are Gram negative rods which are motile and facultative psychrophiles. Their numbers are slightly high where organic nutrients are available particularly near water stored sediments. Some of the common genera are *Micrococcus, Corynebacterium, Spirillum, Norcardia, Streptomyces etc.*

Microalgae: - The aquatic microalgae play an extremely important role in aquatic environments as primary producers of organic compounds. They are basically autotrophic, but a few of them can be heterotrophic. In small bodies of water, such as ponds, much of the primary productivity may be carried out by benthic algae but in large bodies of water e.g. large lakes and oceans, algal phytoplanktons are the main producers. In the fresh water; the most in important phylla are the blue green, yellow-brown algae and diatoms. Whereas in the sea, the most important representatives are the diatoms and the dinoflagellates. The distribution of algae is determined by environmental factors such as temperature and nutrients supply. In fast flowing habitats, micro-algae are usually found on stones or living in bottom sediments but in calm water where conditions are favorable, growth of plantonic algae may be very rapids resulting in large accumulation of cells called “algal blooms” e.g. blue-green algal blooms which occurs in neutrophic fresh waters.

Fungi: - All fugal species are heterotrophic. The algae are the producers. Aquatic fungi are important in decompositionbecause they are able to break down fat, cellulose, hemicellulose, liqnin, chitin, proteins and carbohydrates etc in water. Fungi are more important than bacteria in the breakdown of complex materials. su

Representatives of four major classes of fungi are found in aquatic environments, either free living or more often growing on surfaces. But strictly speaking, the real aquatic fungi are the primitive phycomycetes such as blastocladiales, chytridiales and saprolegniales. The distribution of members of the saprolegunales is dependent on the presence of plant and animal materials which they parasitize or consume when dead. They are therefore rare in ground water and springs, because of low nutrients contents, but in rivers, the lower fungi are important. While yeasts may be quite common in sewage out flows, in lakes and oceans, phycomycetes may be common particularly those belonging to chytridiales and saprolegniales. Marine fungi are both halophilic and halotolerant. Many of the phycomycetes are parasite of plants and animals.

CONCLUSION

Water bodies all over the planet support larger and diverse microbial populations. In addition, microbial communities in marine and fresh water sediments make a significant contribution to the earth's total biomass. Microbial communities in fresh waters and marine ecosystems are greatly influenced by complex interactions between dissolved gases and nutrients flux. Gas solubility especially that of oxygen has a profound impact on microbial activities. The marine environment is well buttered by the carbonate equilibrium.

The soil is a complex environment offering a variety of micro habitats. This explains why microbial diversity in the soil is much greater than that found in aquatic environments. Microbes inhabitat the pores between soil particle; others live in association with plants. The rhizosphere is an important site for microbial growth and activities.

SUMMARY

Oxygen solubility and diffusion rates in surface waters are limited. Water are low oxygen diffusion rate environments in comparison with soils. Carbondioxide, nitrogen, hydrogen and methane are also important gasses for microbial activities in waters. The carbonate equilibrium system keeps the oceans buffered at $_{P}{}^{H}$ 7.6 to 8.3. Penetration of light into the surface water determines the depth of the photic zone. Warming of the surface water can lead to the development of thermocline. The nutrient composition of the ocean influence the C.N.P ratio of the phytoplankton which is called the "red field ratio". The ratio is important for predicting nutrient cycling in oceans

Tidal mixing in estuaries, a characteristic of salt wedge, is osmotically stressful to microbes in this habitat. Thus they have evolved mechanisms to cope with rapid change in salinity.

Terrestrial environments are dominated by the solid phase, consisting of organic and inorganic components. In idea soils, micro-organisms function in thin films of water that have close contact with air. Soil organic matter (SOM) helps retain nutrients and water and maintains soil structure. They are either humic or non humic materials.

Microbial degradation of SOM occurs in three phases starting with the degradation and consumption of soluble compounds, followed by the intracellular attack of more resistant materials such as cellulose and finally the slow degradation of structurally complex molecules such as lignin. Micro-organism play major roles in the dynamics (production and consumption) of greenhouse gasses (carbon dioxide, nitrous oxide, nitric oxide and methane).

TUTOR MARKED QUESTION

Q1. Discuss the stages involves in the dispersed of air-borne particles and aerial micro-organisms

Answer:

The dispersed of air-borne particles and micro-organisms involves three (3) stages

- Liberation and take –off into the air;
- Dispersion in air current; and
- Deposition on surfaces at the end of the journey before germination and growth.

Liberation. Before a particle becomes air–borne, a number of problems have to be overcome. Example, energy is needed to overcome the adhesive forces attaching the particles to substrates surfaces. The particle has to also be of a size that can be air-borne. Fungi have developed many adaptations mechanizing to enable their spores becomes readily air-borne. Some have long sporophores (stalks) which lift their spores well into the air, other have ways of forcefully ejecting their spores into air. Some others rely on passive means such as rain splash, mechanical disturbance and other physical adaptations.

Dispersion. This is at two levels; the fate of individual spores and the behavior of groups or cloud of spores. These two aspects are related and depend on the physical characteristics of the spores and that of the atmosphere. The important characteristics of the spores is in respect of size, shape, degree of surface roughness, density and electrostatic attraction. Those of the environment are wind movements, turbulence, layering convention, wind gradient near the ground and the pattern of atmospheric circulation.

Deposition. This is the final stage of airborne disposal of micro-organisms. The microbes and particle are returned to the surface layers of plants, animals or the soil so that they can no longer be blown by normal wind, through they can still be washed of by rain etc.

FURTHER READINGS /REFERENCES

Allen, M.F.2000. *Mycorrhizae.* In Encyclopedia of Microbiology. 2nd ed. Vol 3. J. Lederberg, editor – in- chief: 328-336. Sandiego. Academic press.

Burges, A. 1958. *Microorganisms in the Soil.* Hutchinson. University Library. London.

An International Symposium. Liverpool University Press 1960

Dubin R.D 1961 Techniques for Observation and Isolation of Soil Microorganisms *Botanical Review. 27*:528-60.

Chester and Thorton. (1956). A comparison of Techniques for Isolating Soil Fungi. *British Mycol Soc. 39*: 301 -313

Casida L.E (1969) Observation of Microorganism in soil and other Natural Habitats. *Applied microbiology 18*: 106-1071

Burges, A.; and Nicholas. 1961. Use of Soil Sections in Studying Amount of Fungal Hyphae in Soil. *Soil Sci. 92*: 25-29.

Somerville, D.A.; and Marples M.J. 1962. The effects of soil enrichment on Isolation of Keratinophilic fungi from soil samples. *Sabouradia 6:* 70- 76

Ecology of Soil Fungi. An International Symposium. Liverpool University press. 1960.

UNIT3. MORPHOLOGICAL, PHYSIOLOGICAL AND GENETIC ADAPTATIONS OF MICROORGANISMS TO THEIR ENVIRONMENTS.

Introduction

Aquatic, terrestrial and aerial ecosystems all over the planet support large and diverse microbial populations. In this units, we shall considered the various adaptations of micro-organisms that enable them to survive in these ecosystems.

Objectives

The objective of this units to make the audience understand and comprehend the various ways and mechanisms micro-organisms have developed or evolved either physiologically, morphologically or genetically to be able to withstand extreme conditions in their environments.

Microbial adaptation to Marine and Freshwater Environments.

Marine and freshwater environments have varied surface areas and volumes. They are found in location as diverse as the human body, beverages and the usual places one would expect- rivers, lakes and the oceans. They also occur in water- saturated zones in materials we usually describe as soils. These environments can range from alkaline to extremely acidic, with temperatures from -5 to -15^{0}c at the lower range, to at least 121^{0}c in geothermal area. Some of the most intriguing microbes have come from the study of high temperature environments, including the now classic studies of Thomas Brock and his co-workers at Yellowstone National Park.

Their work led to the discovery of *Thermus aquaticus,* the source of the temperature-stable DNA polymerase which makes Polymerase Chain Reaction (PCR) possible. In addition to temperature, the penetration of sunlight and the mixing of nutrients, oxygen and waste products that occur in freshwater and marine environments are dominant factors controlling the microbial community.

Water provides an environment in which a wide variety of micro-organisms survive and function. Microbial diversity depends on available nutrients, their varied concentrations, the transition from oxic to anoxic zone and the mixing of electron donors and acceptors in this dynamic environment. In addition, the penetration of light into many anoxic zones creates environments for certain types of photosynthetic micro-organisms.

One of the adaptation of marine micro-organisms is how small most oceanic microbes are. They are so small that not until the development of very fine filtration systems and the application of direct counting methods (such as epiflourescence microscopy) that the abundance of ultramicrobacteria was discovered. The small size is an adaptation because microbial cells must assimilate all nutrients across their plasma membrane.

Cells with a large surface area relative to their total intercellular volume are able to maximize nutrients uptake and can therefore grow more quickly than their larger neighbours. Thus, the majority of microbes growing in nutrient- limited or oligotrophic open oceans are between 0.3micrometer and 0.6micrometer Such microbes have evolved to maximize their surface area to volume ratio to oligotrophic conditions.

At the other extreme is an usual marine microbe found off the coast of Namibia in West Africa. *Thiomargarita namibiensis,* which means the "Sulphur pearl of Namibia" is considered to be the world's largest bacterium. Individual cells are usually 100-300 micrometer in diameter. Sulfide and nitrate are used by the organism as electron door and acceptor respectively. In this case, nitrate from the overlying seawater, penetrates the anoxic sulfide-containing mud' only during storms. When this short term mixing occurs, *Thiomargarita* takes up and stores the nitrate in a large internal vacuole which occupies 98% of the organisms volume. The vascular nitrate can approach a concentration of 800mili micron. The elementary sulfur granules appear near the cell edge in a thin layer of cytoplasm. Between storms, the organism lives using the stored nitrate as an electron acceptor. These unique bacteria are important in sulfur and nitrogen cycling in these environments.

Another critical adaptation of micro-organisms in aquatic systems is the ability to link and use resources that are in separate locations or that are available at the same location only for short intervals such as during storms. One interesting bacterium linking widely separated resources in *Thioploca* species (the spaghetti bacterium) which lives in bundles surrounded by a common sheath. These microbes are found along the coast of Chile, where oxygen poor, nitrate rich waters are in contact with sulfide – rich bottom mud. A similar situation obtains in the coast of West Africa. Such bacteria form filamentous sheath structures, and the individual cells can slide 5-15cm deep into the sulfide- rich sediments.

Again, Zoosporic organisms adapt to life in the water by having asexual reproductive spores with a single whiplash flagellum e.g. chytrids. Another important group is the filamentatous fungi that sporulate under water. These hyphomycetes include the “Ingoldian fungi” named after C.T Ingold in 1942. The ecology of these aquatic fungi is very interesting. They produce a unique tetraradiate conidium on the vegetative mycelium which grows inside decomposing leaves. When the vegetative hyphae differentiate into an aerial mycelium, conidia are released into the water. The released conidia are then transported and often are present in surface foams. When they contact leaves, the conidia attach and establishe new centers of growth.

It is important to note that about 97% of the Earth’s water is in marine environment (estuaries, open oceans and dark cold high pressure benthos) much of which is in the deep sea. The surface waters have been studied more intensely by microbiologist, because this is where the photosynthesis that drive all the marine ecosystems takes place. It is only recently that scientist had the capacity to probe the deep sea sediments and the subsurface (the benthos).

An estuary is a semi enclosed coastal region where a river meets the sea. They are defined by tidal mixing between fresh-water and salt water. They feature a characteristic salinity profile called a “salt wedge”. The salt wedges are formed because salt is denser than freshwater, so sea water sinks below the overlying freshwater. As the contribution from the freshwater increases, and that of the ocean declines, the relative amount of seawater declines with the estuary’s increased distance from the sea. The distance the salt wedge intrudes up the estuary is not static.

Most estuaries undergo large scale tidal flushings and this forces organisms to adapt to changes in salt concentrations on a daily basis. Microbes living under such conditions combat the resulting osmotic stress by adjusting their intracellular osmolarity to limit the difference with that of the surrounding water. Most protists and fungi produce osmotically active carbohydrates for this purpose; whereas prokaryotic microbes regulate internal concentrations of potasium or special amino acids (ecoine and betaine). Most other microbes that inhabit estuaries are halotolerant, distinct from halophilic. Halotolerant microbes can with-stand significant changes in salinity, halophilic micro-organisms have an absolute requirement for high salt concentrations.

Estuaries are unigue in many respects. Their calm nutrient-rich waters serve as nurseries for juvenile forms of many commercially important fish and invertebrates. However, they are the most polluted marine environments. They are the ultimate receivers of wastes that are dumped in rivers, and pollutants discharged from industries.

CONCLUSION

There is great diversity of morphology and chemical ability found in micro-organisms. These are extra-ordinary attributes which enable them to have or at least survival in a remarkable variety of habitats or environments. Micro-organisms are thus present on earth where animals and plants exist as well as in much less hospitable places such as the deep ocean sediment or upper layers of the atmosphere. It appears that some can survive the condition in space and they have been transported to places like the moon where they were found to survive outside the space craft. A large number of studies have been conducted for many years on factors of the environment which effect survival of micro-organisms. Many of such studies have focused on understanding the physiological basis for the survival of micro-organisms as well as on the effects of chemical and physical factors on microbial survival *per se.* The environmental factors considered include temperature, p^H, types and amount of nutrients available, moisture content e.t.c. Many variations in these factors have been studied and the results show that micro-organisms adjust to these changes in order to survival and where the variation persists for a considerable length of time, it might lead to selection of types which are capable of growing at such extremes of environment.

SUMMARY

An organism subjected to the effect of lethal conditions impose by variations in its environment can react in several ways. These adaptive measures for survival and growth differ from one organism to another and largely depends on a number of factors including.

- nature of the variable
- nature of the organisms
- stability of other factors which also influence growth.

In a nutshell, some of the extreme changes in the environment, the damage caused and the organisms that resist such changes can be illustrated as presented in the table blow.

Environment Extreme	Damage caused	Resistant forms
1. Temperature		
a. high temp.	Enzyme denaturation	Thermophiles
b. Low temp.	Loss of regulation, Decrease in membrane permeability as a result of crystallization of major cytoplasm components.	Psychrophiles
2. Low ^{a}w	Dehydration, Inhibits enzyme activity.	(a) Halophiles (b) Osmophiles (c) Xenophiles
3. Low p^{H}	Protein denaturation, inhibits enzyme activity.	Acidophiles
4. Ionizing radiation	Free- radical or photon induced damage to DNA &protein	Radio-tolerant microbes
5. Extreme hydrostatic Pressure	Inhibits reactions of biosynthetic pathways through enzyme denaturation.	Barophiles

TUTOR-MARKED QUESTION

Qa). Define salt wedge and explain it's influence on marine organisms.

Answer:

A salt wedge is a salinity profile characteristic of estuaries (semi enclosed coastal regions where a river meets a sea). They are formed because salt is denser than fresh water, so sea water (salt water) sinks below the overlying fresh water.

Their calm, nutrient rich water serve as nurseries for juvenile forms of many commercially important fish and invertebrates.

b) Describe some morphological adaptations of micro-organisms in the water environment.

Answer:

The size (small) of most oceanic microbes is an adaptation because microbes must assimilate nutrients across their plasma membrane. Cells with large surface area relative to their total intercellular volume are able to maximize nutrients uptake and can grow faster than their larger neighbours. So microbes that grow in nutrient limited or oligotrophic open oceans are below 0.3 micrometre – 0.6 micrometre in size. They maximize their surface area to volume ratio as an adoptive strategy in oligotrophic conditions.

At the other extreme in the large "sulphur pearl" of Namibia, the largest bacterium yet known. The cells range between 100-300 micrometre in diameter. Sulphide and nitrate are used by the organism as electron donor and acceptor respectively. The nitrate from the overlying sea water penetrates the anoxic sulphide-containing muds only during storms. When this short term mixing occurs, this bacterium, *Thiomargarita* takes up and stores the nitrate in a large internal vacuole which occupy's 98% or the organism's volume. The vascular nitrate can approach a concentration of 800 milimicrons. The elemental surfur granules appear near the cell edge in a thin layer of cytoplasm. Between storms, the organism survives using the stored nitrate as an electron acceptor.

Another adaptation of aquatic microorganisms is the ability to link and use resources separate locations. Example *Thioploca* species (the spaghetti bacterium) which lives in bundles surrounded by a common sheath, along the loast of Chile and West Africa. Here, oxygen-poor, nitrate-rich waters are in contact with sulfide-rich bottom mud. The bacterium forms filamentous sheath structures and the cells can glide 5 – 15cm deep into the sulfide-rich sediment.

Zoosporic organisms adopt to life in water by producing a sexual reproductive spores with single whiplash flagellum e.g chytrides. Again, the "ingoldian fungi" a hyphomycete can sporulate under water.

FURTHER READINGS/REFERENCES

Arrigo, K.R. 2005 Marine Microorganisms and global nutrient cycles. *Nature.437*: 349-355.

Edwards, K.J., Bach, W. and McCollom, T.M. 2005. Geomicrobiology in oceanography; microbe-mineral interactions at and below the sea floor. *Trends microbial. 13*:449-456.

Schippers, A; Neritin, L.N; Kallmeyer, J;. Ferdelman, T. G; Cragg, B.A; Parkes, R.J; and Torgenden, B.B 2005. Prokaryotic cells of the deep sub-sea floor biosphere identified as living bacteria. *Nature. 433*:861-864.

Willey, J.M; Sherwood, L.M. and Woolverfon, C.J 2008 *Microbiology.* McGrawHill Co. Inc. New York.

Cammack, R.H., 1955. Seasonal change in 3 common constituents of air spora of southern Nigeria. *Nature. 176*:1270-1272.

Dransfiled, M. 1960. The fungi air spora at Sammara, Northern Nigeria. *Trans. Brit Mycol. Soc. 49*(1):121-132.

UNIT 4. BIOCONVERSION

Introduction

In this unit, we shall consider the technical distinction between the two aspect of bioconversion-biodeterioration and biodegradation. Bioconversion is an aspect of microbial ecology which deals with biodeteration and biodeterdation.

In the present time, it seems appropriate to consider afresh the awkward, but irreplaceable, terms biodeterioration and biodegradation. The attempt here is not primarily to find a neat form of words as a definition, for definitions follows usage, but rather to explore what the current usage is, and to satisfy us that the terms usefully describe a group of phenomena which have important, practical and philosophical features in common.

For a word to be useful, it must stand for a concept which itself serves a useful purpose. In practice, if the concept of biodeterioration is to be useful, it must include a group of phenomena which have practical features in common especially methods of study and control. The same considerations apply, quite equally, though with a different emphasis, to instances of biodegradation which are usually not ‘problems’.

Objective

The objective of this chapter is to technically distinguished between the two aspect of bioconversions – biodeterioration and biodegradation with appropriate, and as many example as possible in each case.

Main Content

Biodeterioration has been defined as being the deterioration of materials by economic importance by organisms. While this definition certainly embraces many of the phenomena which we recognize as biodeterioration, it excludes some which must logically be included because the appropriate method of study and control are in principle the same as those concerned with the deterioration of materials. To accommodate these other phenomena we must add to materials the words processes or activities and also constructions. To illustrate this, consider three examples; bird strike of aircraft, invasion of highways and paths by higher plants and the blocking of filters by algae and bacteria. Each of these is generally recognized as a form of biodeterioration, but the damage to materials is quite insignificant compared to the major damage caused. The significant feature which these phenomena have in common is the fact that the causative agent is, in each case, a living organism so that the methods of study and control are those appropriate to organisms. A convenient expansion of the first definition to include these ideas is deterioration of materials, constructions or processes of economic importance.

THE IMPORTANCE OF BIOLOGICAL CAUSATION

The decay of wood by organisms, a form of biodeterioration, can be countered by, for example, lowering the water activity, exclusion of oxygen, exclusion of the organisms, or by the use of very small quantities of particular substances known as biocides. A somewhat similar form of decay of wood can be caused by chlorine in the atmosphere, but none of these methods (except exclusion) would be appropriate to prevent chlorine attack. In deciding how best to prevent the biological decay, it will be useful to know something of the physiology, ecology and biology of the organisms concerned, and to know how they are distributed (which may have little in common with physical laws). The knowledge required to deal with the chlorine problem is completely different in kind and complexity.

The three following examples will serve to emphasis the importance of the common factor of causation by organisms.

Birds may cause a severe nuisance and pollution in a food factory. To counter this, it will be necessary first to understand the behaviour of the birds, know where they breed, find what physical or chemical treatments could be expected to repel them, to introduce predators, or to make use of biocides which will be conveyed to the birds in ways which require knowledge of their behaviour. The biocides themselves, and also any predators, may cause serious contamination of the premises. A similar degree of nuisance and pollution might be caused in the same premises by smoke or fumes from a chemical works, or even from the firm's own heating system. The methods to counter this form of nuisance would have nothing in common with the biological techniques demanded by the bird infestation.

Damage and loss of value may be caused to a shipments of grains by infestation with insects. Damage closely parallel in value and extent could occur in another shipment by physical contamination with coal, glass, or other physical impurity which is difficult to separate from the grain. In the former instance, the counter measure will include identification of the insect concerned, so that its habits and physiology may be known from literature records, manipulation of water activity and/or temperature, and perhaps cautious use of biocides, bearing in mind the limitations which are imposed by the fact that the grain is to be used as food. To overcome the physical contamination problem, engineering methods are used to produce sophisticated separating machines.

These three examples may be multiplied many times, and in every instance the common factors in the biological problems are:

1. The need to understand the biology of the causative organism (or the group to which it belongs),
2. To manipulate physical conditions,
3. To exclude entry, and

4. To use biocides with due regard to possible contamination of the environment or of the material being protected.

The foregoing. and comparable examples, seen to justify the concept of biodeteriomation and the simple definition already given. But further examination shows that this definition requires further amplification.

The word ‘deterioration’ implies that there has being some loss of economic value. That is, from the human point of view, the action is negative. Such deterioration may be caused by mechanical action, where on organism removes or distorts a material or construction; or by chemical action where an organism assimilates at a molecular level all or parts of a material, usually as a foodstuff for itself, or dissimilates at a molecular level, substances whose presence deteriorates a material or construction. But in addition, there may be soiling, or fouling, where the presence of the organism, or parts of the organism cause aesthetic damage or loss of function or value.

The word ‘material’ is commonly defined as a substance used in man’s economy; thus it includes food stuffs, although the more highly processed the foodstuff, is the less important biodeterioration becomes relative the likelihood of the food becoming a carrier of human pathogens and thus becoming a public health matter.

Materials are usually defined as being dead. But from the biodeterioration standpoint, some materials are assumed to be dead, even if they are technical living (e.g. grains and tubers) if their viability is of no concern economically. But in this area of the living and non-living, there are some difficult boundaries to be defined, and it is useful to see biodeterioration as a counter part of pathology. In pathology, living organisms attack other living organisms; but in biodeterioration, they attack materials which may be organisms, or parts of organisms, which were once living.

Interestingly there is not always in practice, a clear boundary between pathology and biodeterioration. Thus an organism which attacks a crop before harvesting (an example of a plant pathogen) may well continue to damage the crop after harvest. When the viability is not important, it is properly a biodeteriogen. But it could well be more convenient if the pathologist follows up such an extension of plant pathology which is often termed ‘post-harvest decay. This is especially true of the high moisture content crops such as fruits and vegetables in which pathogens are at ease in the storage environment. But in the case of drier materials where pathogens are usually less well favoured, damage by organisms is usually regarded as biodeterioration even though the materials are still alive. In fact in the case of stored grains, a different flora is concerned in store (the storage fungi) from those which infest the grains in the field (the field fungi).

The distinction between grains, tubers, and similar resting plant stages which depends on their intended use has practical value. Such crops may be stored and treated quite differently depending upon whether they are destined as food or industrial raw materials on the one hand, or plant propagulcs on the other.

The blurred boundary between pathology and biodeterioration exists also when a food approaches consumption. Here, however, the boundary is not between the pathology of the plant and biodeterioration of its product, but between the possible pathology of the consumer of the food and its biodeterioration. The closer a food comes to ultimate consumption, and the more highly processed it is, the less the significance of biodeterioration and the greater the potential danger of the food becoming a carrier of disease to the consumer. This brings the problem strictly into the realm of pathology and public health hygiene and the food industry should be equipped and well organized to handle these problems. However, biodeterioration problems strictly defined, such as rat attack on foodstuffs and packagings, microbial decay of wall point and photochromic lenses may very well have a public health implication due to the contamination with human disease-causing organisms.

In this blurred boundary, as in that at harvest, these distinctions are in practice useful. In biodeterioration problems, we look for changes in a foodstuff which are relatively ‘easily’ measurable, whilst with a public health problem, the pathologist must be equipped to detect and deal with a few disease organisms which may be dormant and quite negligible in biomass and quantitative effect *in vitro.*

"Construction" is an important word in the definition of biodeterioration because the proper operation of constructions in the widest sense is of great importance to human beings and this may be impaired by living organisms quite apart from effects on the materials of which they are composed. The type of damage is usually blocking, distorting, defiling or disfiguring; it ranges from total inhibition of function, as when a filter is blocked, to purely aesthetic damage as when a building is disfigured by fungal or algal growth. The techniques for control of this type of biodeterioration are the same as those appropriate for control of damage to materials.

"Processes" or "activities" may be interfered with by organisms in ways closely related to their effect on constructions as, for example, when the activity of playing tennis is interfered with by weeds growing in the tennis court; but sometimes the organism damages an activity without necessarily affecting the construction associated with it; for examine, when birds interfere with the activity of flying without much direct effect on the aircraft. Similarly, gross proliferation of water weeds will interfere with boating or swimming, and bacterial slime can interfere with the impregnation of timber with preservatives.

BIODEGRADATION

We have seen that biodeterioration is a concept which depends on man's economy. But the study of biology, and of the science of materials, can both exist without reference to man's economy. Therefore we can view deterioration as an area in the biosphere defined solely in terms of the requirements of human beings. Viewed in this way, biodeterioration is simply that part of the entire world cycling process which man desires to be temporarily arrested, or removed from his experience. It is the unwelcome aspect of the recycling of materials in nature.

But in due course, human beings no longer desire particular materials, constructions, or activities. Then, men are glad to be rid of them; they are glad to see the biological cycle renewed and happy to see wild life return where factories and airfields formerly required its exclusion. Surplus or redundant materials are best disposed of, that is, degraded, by biological agencies which are usually cheap and almost automatic, and they have some merit in keeping many molecules intact rather than the total mineralization which results from burning.

Hence arises the term 'biodegradation'. It is biologically exactly the same process as biodeterioration, but it is positive from man's point of view because the outcome is desired. It is especially desirable when the material disposed of is a dangerous waste which would otherwise have had harmful effects on the environment. Those who have the responsibility for ensuring that these wastes do not linger in the environment welcome biodegradation as their ally.

The safe use of biologically active and dangerous molecules such as those of biocides, and in a less degree, those of undesirable molecules such as detergents, depends very largely on biodegradation of these molecules in the environment. The biodegradability of such molecules is an important criterion of their safety in use. Those with a concern for the environment welcome biodegradable forms of products.

Thus, although biodegradation may be seen by human beings as a direct opposite of biodeterioration, it is impossible to make a scientific distinction between them. Indeed, they are usually the same processes, changed in meaning and significance solely by human need.

Human need is often expressed in terms of value; often this is monetary value but not invariably, for example, it is difficult to put a monetary value on the improvement of the environment. But the concept of value is readily understandable even when it is difficult to quantify and it is a concept which may be used to clarify the distinction between biodeterioration and biodegradation.

Biodeterioration is a process which reduces value, biodegradation is one which enhances it. Therefore, it is usually materials, process or constructions of reasonably high value which will attract attention when they are subjected to biodeterioration and consequent loss of value. Materials of low or negative value (in the sense that they are an embarrassment and will cost money to be disposed of) are obvious candidates for biodegradation and consequent value enhancement. In the particular example of waste disposal the net value might still be negative after a biodegradative process even though there may be a saving in the cost of disposal.

From this point of view, it seems easy to define biodegradation, but there are some difficult boundaries when it is compared with other forms of biotechnology. Thus, the degrading of straw, provided it is a waste (i.e. of negative value) is a process which rids the farm of unnecessary bulk and therefore increases net value even if there is no product. But it happens that it is usually possible to degrade straw to produce a more valuable product, an animal feed, for example, or a fertilizer. Biodegradation in this instance becomes a process of 'bioenhancement'. This is not unusual; there are many instances when the biological disposal of one material can yield a more valuable product. But biodegradation to enhance value can occur higher up the value scale such as, for example, when a flax plant is selectively degraded to yield isolated fibers in the process known as 'retting'.

The idea of biodegradation operating higher up the value scale leads to a difficult boundary with biosynthesis and biological processing generally. Where the value of the product exceeds that of the original raw material, the disposal aspect becomes negligible and the use of the term biodegradation becomes inappropriate.

Conventional use thus confines the term biodegradation to those instances where the original material is of relatively low or negative value and there is an element of disposal in the process. If there is a valuable product, this is a bonus.

The term seems etymologically inappropriate because degradation' does not appear to be compatible with value enhancement. The explanation lies in its history. The term was coined before the enhancement aspects of the process were clearly recognized. "Degradation" in this context refers to the breakdown of complex molecules to mostly smaller and simpler ones, the original complex molecules being often environmentally objectionable. The use of the term as now conventionally extended and limited, is to be welcomed because it defines a valuable and realistic concept.

Biodegradation is sometimes described as a process of 'harnessing' the degradative powers of microorganisms to achieve desirable objectives. The idea of harnessing, however, implies the deliberate bringing together of substrate and chosen organisms; this certainly happens in some instances but very often the organisms are those occurring by chance in the environment and the "bringing together" is simply a matter of providing suitable conditions for degradation to occur. The deliberate use of biodegradation comes close to the indefinite boundary with bio-processing and biotechnology from which it is usually most easily separated by use of the idea that low value substances can be the subject of biodegradation.

Much effort is devoted to the design and production of biodegradable substances. These are substance which when they become wastes (for example disused containers or surplus detergents) are able to be degraded by the typical organisms, and in the typical conditions of the environment.

CONCLUSION

We have noted that blurred boundaries with concepts are common with biodeterioration and biodegradation. It is perhaps a natural consequence of a concept being recognized solely in practical human terms that blurring of boundaries should result. Many scientific activities have observational and philosophical bases and are precisely defined; one can, imagine that the definitions came very early in their development and that later observations and ideas have been fitted in afterwards. But when the concept is one which derives from practical human need, a reaction to a human problem, is not surprising that it should impact on existing definitions with little regard for existing boundaries.

This enquiry began by saying that we are less concerned to find a definition than to explore the present usage of the terms biodeterioration and biodegradation and the practices which they present. We must conclude that although precise definitions are impossible, their usage is now sufficiently widely agreed for practical purposes.

SUMMARY

The usefulness and current usage of the terms biodeterioration and biodegradation are discussed. Biodeterioration is seen as a process which decreases value, and biodegradation as one which increases it; the distinction being based solely on human needs. Conventionally, biodeterioration refers to damage to materials, constructions, and processes of relatively high value, whereas biodegradation refers to materials only, and is distinguished from biological processing mainly because it applies to materials of low or even negative value.

TUTOR MARKED QUESTION

Q. Using examples, state the technical boundary between the two aspects of bioconversion.

Answer

The two aspects of bioconversion are biodeterioration and biodegradation. Biodeterioration is the deterioration of materials, processes and constructions by economic value; while biodegrading is the enhancement of the value of an otherwise valueless material. The technical distinction between them is that biodeterioration refers to materials, processes and constructions; while biodegradation refers to materials only. From the point of view of man's economy, biodegradation enhances value while biodeterioration reduces value e.g. bird strike of air craft, invasion of highways and paths by higher plants, blocking of filters by algae and bacteria, contamination of foodstuff by microorganisms etc. are all biodeteriorative actions. The fermentation of rotten fruits to produce alcohol, disposal of surplus or redundant materials by degradative processes using biological agents etc are examples of biodegradaion.

FURTHER READINGS/REFERENCES

Eggins, H.O.W; and Oxley, T.A. 1980. Biodeterioration and Biodegradation. International Biodeterioration Bulletin. ISSN 0020-6164 16(2).

Gross R.A. and Kalra, B. 2002. Biodegradable polymers for the environment. *Sciences 297*: 803-807.

Hurst C.J.; Crawford, R.L.; Knudsen, G.R; McLnerney M.J., and Stetzenbach, L.D. 2002. *Manual of Environmental Microbiology*. 2nd ed. Washigton, D.C ASM press.

Nester E.W; Roberts, C.E; Pearsall, N.N.; Anderson, D.G; and Nester M.T 1998. *Microbiology. A human perspective*. McGrawHill Co. Inc New York, Boston, Califonia

Unit 5. MICROBIAL INTERACTIONS

Introduction

Our discussion of microbial ecology has so far considered microbial communities in complex ecosystems. However, the ecology of microorganisms also involves the metabolism, physiology and behavior of microbes as they interact with one another and with higher organisms. In this unit, we shall defined and describe various types of microbial interactions and present a number of illustrative examples.

Objectives

The objective of this unit is to show how microorganisms interact and co-exist in common habitats. This will hopefully enable us to appreciate how nature balances up populations of organisms *in vivo* or *in vitro.*

Main contents

Neutralism (00). This is simply a case of non interaction between component species of an environment. Lewis (1967) reported that the mixed growth of a *Lactobacillus* species and a *Streptococcus* species in a chemostat culture produced individual population sizes which were the same as those in separate monocultures under the same growth conditions.

Mutualism (++). This type of interaction occurs when both members of the mixed culture derive some advantage from each other's presence in terms of increased growth rates or increased population sizes. Yeoh, Bungay and Krieg (1968) described a two membered mixed culture of *Bacillus polymyxa* and *Proteus vulgaris* grown in a carbon-limited chemostat in a simply growth medium which could not sustain the growth of either population on its own. This indicates that each organism was in some way completely dependent on the other population to complement its minimum growth requirements. The *Proteus* species produces nicotinic acid which is essential for the growth of *B. polymyxa*, while the *Bacillus* species reciprocated by excreting the vitamin biotin which promotes *Proteus vulgaris* growth. However, in this case regular oscillations were established and ascribed to the effect of a third interaction between the two populations. *P. vulgaris* produces a proteinaceous compound which inhibited the growth of *B. polymyxa* and caused a decrease in its population size. This in turn reduced the rate of biotin addition to environment and as its concentration declined, it could not maintain the original proteus population size which also went into decline. At a point, the concentration of the inhibiting protein was lowered sufficiently to cause a resurgence of the *B. polymyxa* population and the whole cycle was repeated.

Commensalism (+0). This is the situation where one member of a community benefits from the presence of a second population which itself does not derive any advantage or disadvantage from the activity of the first organism. Commensalism is an extremely common interaction in nature and the process of organism succession can be thought of as a chain of commensal relationships; the growth of one population generating a particular set of conditions thereby enabling a second population to develop.

Commensalism can alternatively be called metabiosis, which is the relationship when an organism causes a change in the conditions which then favours the development of other species associated with it. Metabiosis may be caused in any of the following ways:

(i) **Supply of Nutrients.** One organism may produce a substance which becomes a nutrient for another organism e.g the breakdown of large molecules into easily available smaller molecules like the breakdown of starch by *Micrococcus rouxianus* to maltose for use by other micrococci or the breakdown of starch by *Aspergillus oryzae* to glucose which are used by yeasts. Supply of nutrients can also be by synthesis of an essential nutrient which is not supplied by the substrate e.g yeasts and molds produce vitamin B which is used by *Lactobalillus* species. The yeast *Saccharomyces cerevisiae* produces riboflavin (vitamin B2) which is needed by *Lactobacillus casei* for growth. The stability of this community was verified to be due to a second interaction, namely competition for growth limiting quantities of carbon and other sources of energy, glucose. The bacterium has a greater affinity for glucose than the yeast but if the *Lactobacillus* species used too much of the carbon source, it would cause a decrease in the size of the yeast population and hence a decrease in the rate of the riboflavin supply and this ensured that the populations equilibrated to constant sizes under constant growth conditions.

(ii) **Changes in pH**. One organism may alter the pH of an environment to favour the growth of others e.g *Leuconostoc mesenteriodes* produces acid which lowers the pH for *Lactobacillus* species to grow (in the production of *yoghurt*).

(iii) **Changes in Electron Potential or Oxygen Tension (Eh).** In cheese manufacture, the Eh is reduced to about-119mV to encourage the growth of microaerophiles. *Streptococcus* species grow on whale meat and reduces the Eh to a level that enables the anaerobic clostridia to grow.

(iv) **Elimination of antimicrobial factors.** One organism may neutralize or destroy an antimicrobial factor which has been inhibiting the growth of a second organism e.g nisin which is synthesized by some strains of *Streptococcus lactis* is active against Gram positive bacteria including spores of *Clostridium* and *Bacillus* species. However, some strains of *Lactobacillus* species are capable of inactivating nisin against these other organisms.

(v) **Alternation of water activity (^{a}w)**. by one organism may encourage the growth of other organisms e.g Osmophilic yeasts can grow in the presence of high sugar concentrations, breakdown the sugar and lower the ^{a}w so that less tolerant organisms can grow in the solution.

(vi) **Alteration of Cellutar Structures.** Biological structures of some foods constitute a physical barrier. However, if this barrier is breached in some way, contamination and growth by invading organisms are encouraged e.g fruit rotting fungi destroy the skin of the fruits thereby laying it open for the entry and growth of other organisms mainly yeasts.

Amensalism (0-). This is an interaction in which the growth of one population is restricted by the presence of a second population which is unaffected by the metabolism of the inhibited population. Amensalism occurs when organisms produce antimicrobial compounds such as antibiotics or colicins or through nonspecific effects such as the elevation of the dissolved oxygen tension or changes in P^H.

Amensalism can be alternatively referred to as antagonism, in which case one organisms makes the growth conditions less favourable for other organisms. Antagonism can be achieved in several ways which include:

(i) **Consumption of essential nutrients**. Here, one organism with a more rapid growth rate quickly consumes the essential nutrients in the medium thereby discouraging the others.

(ii) **Accumulation of major metabolites to toxic levels.** There are many metabolites that are produced up to toxic levels as end products of metabolism e.g acids which are inhibitory both to other organism as well as the producer. Growth slows down as acidity increases until it stop eventually. This is clearly indicated in the case of alcohol and cheese production.

(iii) **Production of antibiotics.** Nisin is produced by *Streptococcus lactis* that are found naturally in many products. Nisin is active against Gram positive bacteria, and inhibits undesirable organism like *Clostridium* species.

Prey-predator relationship (+-). Here one organism, the predator gains directly at the expense of the second organism, the prey since the prey forms the complete nutritional requirements of the predator. In extreme cases, the prey may go into extinction; but typically in open growth systems, continuous oscillations of the two populations can be established with the increasing phase of the predator population lagging behind the increase in the prey population. This has been observed in the case of *Dictyostelium discoidium* (amoebae) feeding on *Escherichia coli* and a number of models have been proposed to describe the population fluctuations.

Competition (- -). In this case both populations are limited either in terms of growth rate or final population size by a common dependence on an external factor required for growth. Competition is very important because it provides a selective mechanism for evolution. Competition as Gause observed is the basis of the struggle for existence.

CONCLUSION

Many associations of two or more microorganisms have been described in mechanistic terms and these are normally based on aspect of nutrition. All these associations however, may be described by a few basic types of interactions which can be illustrated in the simplest interacting system, namely a two remembered mixed population containing organisms A and B. There are only three possible responses a growing population in this case let us say organism A can make to the presence of the second population organism B.

a. The growth and metabolic activities of population A may have a beneficial or positive effect on the growth of population B. For example, this could occur if population A excreted a compound which stimulated the rate of growth of population B, compared with its rate in the absence of population A. Alternatively the same positive effect might be achieved if population A utilized a compound initially present in the common habitat which was toxic to population B and hence restricted its development.

b. The presence of organism A could have a detrimental or negative influence on the growth of organism B. This situation could arise if organism A excreted a metabolite which was toxic to organism B.

c. It is also possible that the growth of population A, the effect of its metabolic activities and its demands upon the resources of the common environment have no effect on the growth of population B. A neutral response of this kind would be shown by similar growth patterns for population B whether or not population A was present.

SUMMARY

The various combinations between any two populations interacting in a common environment may be summarized in a simple matrix as shown below:

	Effect on the growth of organism B by the activity of organism A		
	+	0	-
Effect on the growth of organism B by the activity of organism A	++	0+	-+
0	+0	00	-0
-	+-	0-	--

For a two-membered mixture, nine responses could theoretically be established, illustrating a maximum of six fundamental types of interactions.

TUTOR MARKED QUESTION

Question: Describe with elaborate examples, mutualism as a microbial interaction.

Answer: Mutualism occurs when members of a mixed culture derive some advantage from each other's presence in terms of increased growth rates or increased population sizes. Yeoh, Bungay and Krieg (1968) described a two membered mixed culture of *Bacillus polymyxa* and *Proteus vulgaris* grown in a carbon-limited chemostat in a simply growth medium which could not sustain the growth of either population on its own. This indicates that each organism was in some way completely dependent on the other population to complement its minimum growth requirements. The *Proteus* species produces nicotinic acid which is essential for the growth of *B. polymyxa*, while the *Bacillus* species reciprocated by excreting the vitamin biotin which promotes *Proteus vulgaris* growth. However, in this case regular oscillations were established and ascribed to the effect of a third interaction between the two populations. *P. vulgaris* produces a proteinaceous compound which inhibited the growth of *B. polymyxa* and caused a decrease in its population size. This in turn reduced the rate of biotin addition to environment and as its concentration declined, it could not maintain the original proteus population size which also went into decline. At a point, the concentration of the inhibiting protein was lowered sufficiently to cause a resurgence of the *B.* *polymyxa* population and the whole cycle was repeated.

FURTHER READINGS/REFERENCES

Backhed, F.; Ley, R.E.; Sonnenbury, J.L.; Peterson, D.A.; and Gordon, J.I. 2005. Host-bacterial mutualism in the human intestine. *Science. 307:* 1915 – 1920.

Mentschel, U.; and Steinert, M. 2001. Symbiosis and pathogenesis: common themes, differences, outcomes. *Trends in Microbiol. 9*(12): 585.

Velicer, G.J. 2003. Social life in Microbial world. *Trends in Microbiol. 11*(7): 330-337

Willey, J.M.; Sherwood, L.M.; and Woolverton, C.J. 2008. *Microbiology*. McGrawHill Co. Inc. New York.