



Freshwater Microbiology Course

For 2nd Microbiology & chemistry

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Prepared by

Dr. Asmaa Sabry Yassein

Assistant Professor of Microbiology

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Microbiology

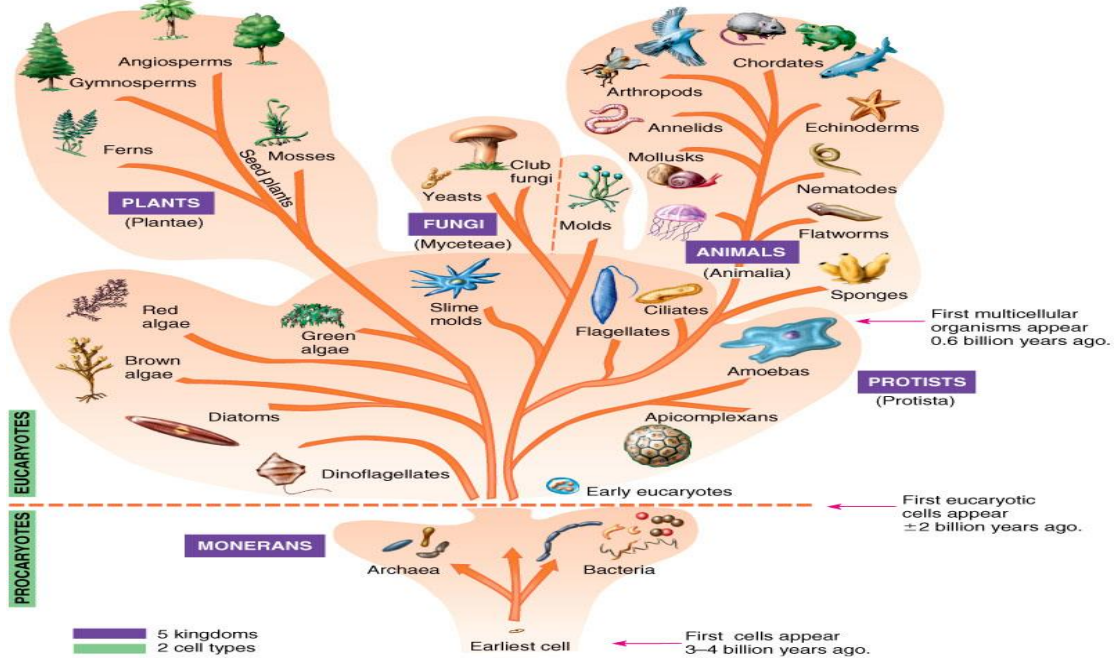
- Study of organisms too small to be seen by the naked eye.

Microbes or Microorganisms

- commonly referred to as “germs” or “bugs”
- Include bacteria, viruses, fungi, algae, protozoa and helminths.
- Prions (“infectious proteins”) are recent addition. A protein particle that is believed to be the cause of brain diseases such as BSE, scrapie, and Creutzfeldt–Jakob disease. Prions are not visible microscopically, contain no nucleic acid, and are highly resistant to destruction.

Branches of Microbiology

- **Bacteriology** study of bacteria
- **Mycology** study of fungi and yeast
- **Virology** study of viruses
- **Parasitology** study of parasitic protozoans and helminths
- **Immunology** study of the humoral and cellular immune response to disease agents and allergens



Types of kingdoms

Fresh water microbiology

The aquatic existence

It is now generally accepted that life originated between 3.5 and 4 billion years ago in the aquatic environment, initially as self-replicating molecules (Alberts et al., 1962). The subsequent evolution of prokaryotes, followed by eukaryotes, led to the existence of microorganisms which are highly adapted to aquatic systems.

Life in the aquatic environment (freshwater and marine) has numerous potential advantages over terrestrial existence. These include physical support (buoyancy), accessibility of three-dimensional space, passive movement by water currents, dispersal of motile gametes in a liquid medium, minimal loss of water (freshwater systems), lower extremes of temperature and solar radiation, and ready availability of soluble organic and inorganic nutrients.

Potential disadvantages of aquatic environments include osmotic differences between the organism and the surrounding aquatic medium (leading to endosmosis or exosmosis) and a high degree of physical disturbance in many aquatic systems. In undisturbed aquatic systems such as lakes, photosynthetic organisms have to maintain their position at the top of the water column for light availability. In many water bodies (e.g., lake water column), physical and chemical parameters show a continuum – with few distinct microhabitats. In these situations, species compete in relation to different growth and reproductive strategies rather than specific adaptations to localized environmental conditions.

The global water supply – limnology and oceanography

Water covers seven tenths of the Earth's surface and occupies an estimated total volume of $1.38 \times 10^9 \text{ km}^3$. Most of this water occurs between continents, where it is present as oceans (96.1 per cent of global water) plus a major part of the atmospheric water. The remaining 3.9 per cent of water present within continental boundaries (including polar ice-caps).

The distinction between oceans and continental water bodies leads to the two main disciplines of aquatic biology – oceanography and limnology.

- Oceanography is the study of aquatic systems between continents. It mainly involves saltwater, with major impact on global parameters such as temperature change, the carbon cycle and water circulation.
- Limnology is the study of aquatic systems contained within continental boundaries, including freshwater and saltwater sites.

The study of freshwater biology is thus part of limnology. Although freshwater systems do not have the global impact of oceans, they are of major importance to biology. They are important ecological features within continental boundaries, have distinctive groups of organisms, and show close links with terrestrial ecosystems.

Table: Global distribution of water (adapted from Horne and Goldman, 1994)

Site	Volume (km ³)	% of water within continents
Oceans	1 322 000 000	
Polar ice caps and glaciers	29 200 000	54.57
Exchangeable ground water	24 000 000	44.85
Freshwater lakes	125 000	0.23
Saline lakes and inland seas	104 000	0.19
Soil and subsoil water	65 000	0.12
Atmospheric vapour	14 000	0.026

Freshwater systems: some terms and definitions

Freshwater microorganisms

Microorganisms may be defined as those organisms that are not readily visible to the naked eye, requiring a microscope for detailed observation. These biota have a size range (maximum linear dimension) up to 200 µm, and vary from viruses, through bacteria and archaea, to micro-algae, fungi and protozoa. Higher plants, macro-algae, invertebrates and vertebrates do not fall in this category and are not considered in detail, except where they relate to microbial activities. These include photosynthetic competition between higher plants and micro-algae and the role of zooplankton as grazers of algae and bacteria.

The five major groups of microbial organisms are

- 1- Algae
- 2- Bacteria
- 3- Viruses
- 4- Fungi
- 5- Protozoa

The requirements of freshwater microorganisms are light and inorganic nutrients

Lentic and lotic freshwater systems

Freshwater environments show wide variations in terms of their physical and chemical characteristics, and the influence these parameters have on the microbial communities they contain. But one important distinction needs to be made at this stage – between lentic and lotic systems. Freshwater environments can be grouped into standing waters (lentic systems – including ponds, lakes, marshes and other enclosed water bodies) and flowing waters (lotic systems – rivers, estuaries and canals). The distinction between lentic and lotic systems is not absolute, and almost all water bodies have some element of through-flow. Key differences between lentic and lotic systems in terms of carbon availability and food webs.

Biodiversity of microorganisms

- Domains of life

With the exception of viruses (which constitute a distinct group of non-free living organisms) the most fundamental element of taxonomic diversity within the freshwater environment lies in the separation of biota into three major domains – the Bacteria, Archaea, and Eukarya. Organisms within these domains can be distinguished in terms of a number of key fine-structural, biochemical, and physiological characteristics.

- Size range

Size is an important parameter for all freshwater microorganisms, affecting their location within the freshwater environment, their biological activities, and their removal by predators. In the case of free-floating (planktonic) organisms, the maximum linear dimension ranges from <0.2 mm –

>200 mm, with separation of the biota into five major size categories from femtoplankton to macroplankton.

Femtoplankton (<0.2 mm)

The distinction between particulate (insoluble) and non-particulate (soluble) material in freshwater systems is usually defined in terms of retention by a 0.2 mm pore-size filter membrane. On this basis, the smallest size group, the Femtoplankton (<0.2 mm) falls within the non-particulate category and the constituent viruses and small bacteria are strictly part of the dissolved organic material (DOM) or dissolved organic carbon (DOC) of the freshwater environment.

Picoplankton (0.2–2 mm)

This group is almost entirely composed of prokaryotes (bacteria and blue-green algae) with potentially rapid growth rates and the ability to carry out rapid colonization of freshwater environments. These organisms have negligible sinking rates, and are subject to significant predation by small rotifers, protozoa, and filter-feeding crustaceans. Some large linear viruses (family Inoviridae) also fit into this size category and have been linked to the infection of bacterial populations.

Nanoplankton (2–20 mm)

Typically eukaryote flagellated unicellular organisms, this group include fungal zoospores, algae, and protozoa. These organisms are the principal food of micro- and macro-zooplankton, and have low sinking rates and high potential growth rates.

Microplankton (20–200 mm)

Larger microplankton are retained by traditional 70 mm mesh size phytoplankton nets, and are highly prone to sinking in the absence of buoyancy aids. These organisms are consumed by larger crustacea, and are also the principal food of pelagic and benthic omnivorous fish. Growth rates are moderate to low.

Macroplankton (>200 mm)

These have similar biological features to the larger microplankton, and are characterized by the colonial blue-green algae and by the multicellular zooplankton (rotifers and crustacea). The

biology of meso-trophic and eutrophic lakes in temperate climates are typically dominated by these size categories over the summer growth period, with separate population peaks of colonial algae (diatoms, blue- greens) and zooplankton (crustacea) at different times of year. Although macroplanktonic organisms are characteristically slow-growing, they typically make the greatest contribution to biomass under conditions of adequate nutrient supply. Differences between picoplankton and macroplankton in terms of growth rate, short-term colonization and long- term domination of freshwater environments reflect fundamental differences in evolutionary selection strategy and the way these biota are adapted to different environmental conditions.

Autotrophs and heterotrophs

Freshwater microorganisms may be divided according to their feeding activity (trophic status) into two major groups:

- Autotrophs – synthesize their complex carbon compounds from external CO₂. Most also obtain their supplies of nutrient (e.g., nitrogen and phosphorus) from simple inorganic compounds. These phototrophic microorganisms include microalgae and photosynthetic bacteria, and are the main creators of biomass (primary producers) in many freshwater ecosystems. This is not always the case, however, since photosynthetic microorganisms are outcompeted by larger algae and macrophytes in some aquatic systems, particularly wetland communities.
- Heterotrophs – use complex organic compounds as a source of carbon. By far the majority of freshwater microorganisms (most bacteria, protozoa, fungi) are heterotrophic. Even within the algae, various groups have evolved the capacity for heterotrophic nutrition and many organisms currently included in the protozoon assemblage have probably evolved from photosynthetic ancestors.

Heterotrophic nutrition involves a wide diversity of activities with microorganisms obtaining their organic material in three main ways – saprotrophy, predation, and in association with living organisms. Saprotrophic organisms obtain their nutrients from non-living material. This may be assimilated in three main ways: direct uptake as soluble compounds (chiefly bacteria), indirect

uptake by secretion of external enzymes (exo- enzymes) followed by absorption of the hydrolytic products (bacteria and fungi), and ingestion of particulate matter by phagocytosis (protozoa). Predation is carried out by protozoa, and involves capture, ingestion, and internal digestion of other living organisms such as bacteria and algae. Protozoa can capture their prey either by active motility or, as sedentary organisms, by the use of filter feeding processes. The third major aspect of hetero- trophy involves associations with living organisms and includes parasitism and symbiosis. Parasitism almost invariably involves a strict dependence of the parasite on the host organism as part of the parasitic life cycle, though in some cases the benefits to the parasite are not entirely clear.

Planktonic and benthic microorganisms

Freshwater organisms can be divided into two main groups, according to where they spend the major part of their growth phase – pelagic organisms (present in the main body of water) and benthic organisms (associated with the sediments).

Planktonic microorganisms

These biota frequently have specialized mechanisms to migrate or maintain their position vertically within the water column, and are particularly well represented by micro-algae and bacteria. Planktonic organisms can be further divided into holoplanktonic forms (where the organism is present in the water column for a major part of the annual cycle) and meroplanktonic organisms (where the planktonic phase is restricted over time). The distinction between holoplanktonic and meroplanktonic species is particularly well shown by the algae, where the meroplanktonic state can be viewed as an adaptation for short-term competition.

Benthic microorganisms

Present at the surface and within sediments, these are dominated by biota such as fungi, protozoa, and bacteria that are able to break down sedimented organic debris. The diversity of benthic life forms is particularly well represented by the protozoa, which include both attached and freely-motile organisms, with a variety of feeding mechanisms.

ECOSYSTEMS

Ecosystems vary in size and composition, and contain a wide range of organisms which interact with each other and with the environment. Individual ecosystems have a number of important properties (McNaughton, 1993; Berendse, 1993):

- A distinct pattern of interactions between organisms.
- Defined routes of biomass formation and transfer.
- Maintenance of the internal environment.
- Interactions with the external environment.

The range of organisms present in aquatic communities defines and characterizes the system concerned, and are involved in the generation and transfer of biomass. They have distinct roles and interactions within the ecosystem, occupying particular trophic levels and forming an interconnected system of feeding relationships (the food web). The balance of individual species within the food web is determined primarily by resource (light, nutrient availability) and competition. This in turn affects variety in the range and proportions of the different organisms (biodiversity), with important implications for the overall functioning of the system. Community structure is closely related to ecosystem stability and physical stress levels in the environment (see Section 1.6). Interactions between ecosystems and their surrounding environment occur in various ways. One example of this is the net exchange of carbon between the aquatic ecosystem and the adjacent atmosphere, which can be quantified in relation to net ecosystem production (NEP).

The biofilm community: a small-scale freshwater ecosystem

Microbial biofilms provide a useful model system for considering fundamental aspects of community interactions and ecosystem function (Allison et al., 2000). Their small scale makes them amenable to laboratory as well as environmental experimentation, and the close proximity of organisms within the biofilm leads to high levels of biological interaction. Microbial biofilms occur as discrete communities within a gelatinous matrix, and are present as a surface layer on rocks and stones in lakes and rivers. The biological composition of biofilms varies with environmental conditions, including factors such as ambient light intensity, water flow rate and prior colonization history. In some cases they are entirely bacterial.

1- Quorum sensing

The physiological activities of bacteria vary considerably in relation to population density (number of cells per unit volume of medium). The pattern of gene activity, in particular differs markedly within a single bacterial species between the sparse planktonic populations that occur in the general water medium and the dense community of cells present in the microbial biofilm. In Gram-negative bacteria depends on releasing signal molecule acyl homoserine lactone (AHL) into the water medium.

2- Gene transfer

Microbial biofilms are particularly important in relation to gene transfer between bacterial cells, since they are a part of the aquatic environment where the transfer process is optimized due to the close proximity of the organisms concerned.

Interactions between microorganisms

- 1- Competition for resources: competition between phytoplankton species
- 2- Antagonism: Nutrient competition between algae and bacteria- repression of eukaryote algae by blue-greens under bloom conditions- Destruction of blue-green algae by antagonistic bacteria.
- 3- Trophic interactions: Association between bacteria and blue-green algae. Coupling of productivity between phytoplankton and bacterioplankton. Ingestion of bacteria by HNF protozoa. Infection of phytoplankton by cyanophage and phycovirus viruses. Infection of phytoplankton populations by chytrid fungi. Infection of bacterial populations by bacteriophages.
- 4- Epiphytic associations: Associations between protozoa, bacteria, and algae with colonial blue greens (phycosphere ecosystem).

The influence of physic-chemical conditions on microbial communities

At the micro level, the aquatic medium surrounding freshwater biota is a heterogeneous mixture of three main constituents – particulate material, soluble components, and water matrix. Soluble constituents (inorganic compounds and dissolved organic carbon-DOC).

Physical properties of water

Water is unusual in comparison with other molecules of a similar structure (H_2S , NH_3 , HF) in that it exists as a liquid rather than a vapour at the earth's surface, and is one of only two inorganic liquids (together with mercury) that can exist in this state under ambient temperature and pressure. Many of the unique properties of water result from its unusual molecular structure, with the separation of charges between electronegative oxygen and electro-positive hydrogen atoms. This charge asymmetry leads to weak hydrogen bonding between molecules, resulting in molecular complexes – $(\text{H}_2\text{O})_n$ – with a semi-crystalline structure. It is these chemical properties that determine the liquid state of water at room temperature, along with other physical properties.

Algae: the major microbial biomass in freshwater systems

In the freshwater environment, light energy conversion and related synthesis of carbon compounds is carried out by three major groups of organisms (primary producers) – higher plants (macrophytes), algae, and photosynthetic bacteria. Algae are the main microorganisms involved in this process and may be defined as simple plants (lacking roots, stems, and leaves) that have chlorophyll-a as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells. Although algae include prokaryotic organisms (blue-green algae), the closely-related photosynthetic bacteria differ in terms of cell size, pigmentation, and physiology (strict anaerobes, not evolving oxygen) and are generally placed in a distinct category.

Algae as freshwater microorganisms. Freshwater algae range in size from microscopic organisms (unicellular and colonial) to macroscopic forms which are visible to the naked eye and appear plant-like. Planktonic algae are typically microscopic (micro-algae), and are part of the microbial community which is the main theme of this book. In contrast, various benthic or attached algae are macroscopic, and do not fit into the broad area of aquatic

microorganisms. The green algae in particular include large filamentous forms such as *Cladophora* and *Chara*, and description of these is limited to their ecological role as attached algae or periphyton.

Freshwater algae constitute a diverse group of biota and occupy a wide range of aquatic habitats.

Major taxonomic divisions of freshwater algae

Freshwater algae do not occur as a formal taxonomic group of organisms, but represent a loose and diverse collection of divisions with members that are united by possession of the general features outlined previously. The heterogeneity of freshwater algae is emphasized by the fact that they are split between two of the major domains of living organisms. The Bacteria (a prokaryote group that includes blue-green algae) and the Eukarya (including all eukaryote algae). Although there is little consensus among phycologists in terms of exact groupings, they are separated here into 10 principle divisions or classes. Microscopical appearance, motility and ecological features. Examination of environmental samples under the light microscope reveals a wide range in algal morphology and size, with variation from unicellular to colonial forms. Motility occurs both on solid surfaces (benthic algae) and within the water column (planktonic forms) and involves both active (mucilage extrusion, cilia and flagella) and inactive (buoyancy mechanisms) processes. Planktonic diatoms are non-motile, depending on water movement to maintain their position within the water column. Within the eukaryote algae, three groups – the euglenoids, dinoflagellates, and cryptomonads are entirely unicellular and are actively motile by flagella.

Algae are present in all freshwater environments including lotic and lentic systems, plus snowfields, aerosols, and a range of extreme aquatic situations. Within lotic and lentic systems, certain algal groups (euglenoids, dinoflagellates, cryptomonads, and chrysophytes) show a preference for planktonic conditions, while others (blue-green algae, green algae, and diatoms) are equally planktonic or benthic. Although the great majority of freshwater algal species have a widespread geographic distribution (cosmopolitan), there are some species of chrysophytes, green algae, red algae, and diatoms which are restricted (endemic) to certain geographic regions or particular water bodies.

Biodiversity in freshwater systems Biodiversity within the major algal divisions in freshwater systems is indicated by the range of habitats that are colonized and by the diversity of genetic, physiological, biochemical, and structural characteristics that occur within the group. The number of genera within divisions provides an index of phenotypic biodiversity and in the freshwater algae of North America (a well-characterized group occurring over a wide geographic area) ranges from >300 (Chlorophyta) to <5 (Phaeophyta).

Biochemical and cytological characteristics

Biochemical features

Major biochemical features include pigmentation, storage products, external covering (cell wall) composition, and identity of osmotically-active low MW organic solutes.

1- Pigmentation: is derived from three main groups of molecules; chlorophylls, carotenoids, and phycobilins.

Algal pigments are localized within the algal cell in association with the photosynthetic or thylakoid membranes. Chlorophylls are composed of a porphyrin ring system with a central magnesium atom, and occur as four main types – chlorophyll-a, -b, -c (c1, c2, and c3) and -d. Chlorophyll-a (Figure 4.10) occurs in all photosynthetic algae as the primary photosynthetic pigment (the light receptor of photosystem I of the light reaction) and varies from 0.3– 3 percent of algal dry weight. The other chlorophylls function as accessory pigments and have a limited but distinctive distribution within the different algal groups.

Carotenoids are long-chain molecules that can be divided into two main groups: carotenes – oxygen-free hydrocarbons, and xanthophylls, their oxygenated derivatives. Of the four carotenes present in algae, β -carotene, occurs in all the algal groups while α -, γ - and ϵ -carotenes have a more restricted occurrence (Table 3.3). Xanthophylls occur as a wide range of molecules, with approximately 30 different types being recognized and forming a distinctive pattern of distribution within the different algal groups.

Phycobilins are water-soluble red or blue pigments located on (blue-green algae, red algae) or inside (cryptophytes) the photosynthetic membranes. The pigment molecule or chromophore is a tetrapyrrole and occurs in a combination with non-pigmented protein (the apoprotein) to form

the phycobiliprotein. The blue chromophore is phycocyanobilin and the red chromophore phycoerythrobilin.

2- Storage products: Algae contain a range of high and low molecular weight carbohydrate storage products. The high MW starch-like compounds. Low MW storage carbohydrates include sucrose (important reserve in green algae and euglenoids) and trehalose (blue-green algae).

3- Cell wall composition:

In general, algal cell walls are composed of two constituents – a skeletal or fibrillar component plus an amorphous matrix. The most common skeletal component is cellulose (a polymer of 1,4 linked -D-glucose), but other macromolecules – including pectin, peptidoglycan (mucoprotein), and protein – may also be involved (Table 3.3). Amorphous mucilaginous components are an important part of cell wall structure in red and brown algae, and form a separate (mucilage) layer in many algal groups. Diatoms are unique in having a cell wall made of amorphous, hydrated silica that is associated with proteins, polysaccharides, and lipids (Fischer et al., 1999).

Cytological characteristics

Cytological features are of fundamental importance in distinguishing the different algal groups.

The most fundamental division within the algal assemblage, into prokaryotes (blue-green algae) and flagella, and the fine structure of nuclear chromatin.

General Summary of the different groups:

General features of the different algal groups.

Blue-green algae (Cyanophyta)

Also referred to as cyanobacteria, this is the only prokaryote group of algae. Cyanophyta are important constituents of periphyton and phytoplankton communities, where they are present both as unicellular (picoplankton) and colonial forms. Blue-green algae are thought to have evolved during the early Precambrian era, when they were exposed to a reducing atmosphere and high levels of irradiation (particularly UV). During their long existence, they have colonized nearly all freshwater, marine, and terrestrial habitats, including such extreme environments as hot springs (up to 70 C), hyper-saline lakes, high arctic and alpine lakes, and hot and cold deserts. The general success of blue-green algae in aquatic environments has been attributed to the following

Efficient light harvesting mechanisms, with ability to adjust to spectral differences by variations in accessory pigments. Continued photosynthesis at low concentrations of CO₂ and high pH. Resistance to damaging radiation, by producing a range of compounds that act as photoprotectants by absorbing short wavelengths. Temperature adaptations – different species can grow in extreme hot or cold environments. In temperate and tropical lakes, blue-green algae are able to maintain growth rates at high summer temperatures where other algae show temperature inhibition.

Efficiency in nutrient uptake. Blue-green algae show a whole range of features related to nutrient uptake:

- they are able to dominate both oligotrophic (as picoplankton) and eutrophic (large colonial greens) environments; in eutrophic conditions, dense algal blooms out-compete eutrophic algae; - some blue-greens can fix gaseous nitrogen, allowing them to grow at low N/P ratios.
- blue-greens produce siderophores under conditions of iron stress, allowing them to scavenge Fe(III) at limiting environmental levels.
- blue-greens do not require exogenous sources for vitamin requirements.

They have chemical (toxins) and physical (large colonies) mechanisms to resist removal by filter-feeding zooplankton.

They have evolved specialized symbiotic bacterial associations, which are particularly important in relation to heterocyst functions.

Some of these characteristics have specific importance in the ability of blue-green algae to form dominant blooms in eutrophic waters.

Green algae (Chlorophyta)

These comprise the most diverse class of algae. They are important as both planktonic and attached organisms, with morphologies ranging from simple unicells to complex colonial forms. Some macroscopic members of the green algae (Chara, Cladophora) have a higher-plant like appearance and are important members of the periphyton. Certain groups within the green algae have specific ecological requirements, including flagellated chlorophytes (nutrient-rich standing waters) and coccoid unicells and colonies (high light, nutrient, and temperature, standing waters). Desmids are more common in ponds and ditches that have low conductance and low to moderate nutrient levels.

Euglenoids (Euglenophyta)

In terms of general abundance and species diversity, this is a relatively minor group of freshwater algae. These organisms may become particularly abundant, however, in the phytoplankton of standing waters rich in nutrients where they may be readily identified under the microscope by their unicellular spindle-shaped morphology and active motility.

Yellow-green algae (Eustigmatophyta, Raphidophyta and Tribophyta)

Yellow-green algae are a diverse group of freshwater organisms, occurring in a wide range of habitats, and with a large number of reported genera (at least 90 in North America). Although widespread, these algae are not very prominent members of the freshwater flora, since many are small coccoid forms that occur only in small numbers.

Dinoflagellates (Dinophyta)

Dinoflagellates are often relatively minor components of lake phytoplankton in relation to species counts. The large size of these organisms, however, means that their bio volume contribution to phytoplankton is much more significant – and they often make a major contribution to overall algal biomass. Along with colonial blue-greens, these algae are prime examples of K-selected organisms and tend to form blooms in temperate lakes towards the end of summer when stratification is stable and epilimnion nutrients are in decline. Under such conditions, these highly motile organisms are able to migrate into the nutrient-rich hypolimnion to obtain their supplies of nitrate and phosphate.

Cryptomonads (Cryptophyta)

These unicellular organisms are particularly diverse in temperate regions, where they typically occur as phytoplankton in lakes and ponds. The short cell cycle and ability for active growth of cryptomonads means that they are particularly common during the clear-water phase of the annual cycle in temperate lakes, along with other r-selected organisms.

Algal responses to limiting nutrients: The induction of genes which promote inorganic nutrient uptake is a key aspect in the ability of algae to respond to fluctuations in nutrient availability. Exposure to conditions in which nutrients are limiting may also trigger a molecular response. Limitation in Fe availability, for example, leads to the replacement (in both eukaryote and blue-

green algae) of the electron transfer catalyst ferredoxin by the iron-free but functionally-equivalent protein flavodoxin (Porta et al., 2003). Environmental populations of planktonic micro-algae exhibit flavodoxin accumulation as a biochemical marker for conditions of iron depletion. In blue-green algae, flavodoxin is encoded by the gene *isiB*, which combines with *isiA* to form an operon under the control of the Fe-dependent repressor Fur. Decrease in external Fe concentration to sub-critical levels inactivates Fur and leads to expression of the *isiAB* operon. This operon is widely spread throughout blue-green algae, and in most cases is only responsive to the environmental parameter of Fe deficiency. The occurrence of flavodoxin accumulation and *isiAB* transcription as diagnostic markers for iron deficiency has led to the construction of a blue-green algal (*Synechococcus* sp. PCC 7942) reporter strain for freshwater environments (Porta et al., 2003). This has *luxAB* reporter genes fused to the *isiAB* promoter, and provides a means of assessing low Fe availability, as perceived by the test organism.

Responses to stress: Stress factors include all those environmental changes which have an adverse effect on biological function, and have been considered previously in relation to ecosystems. They also operate at the cellular and molecular level, and freshwater algae are able to adapt to sudden but moderate changes such as salt stress, heat shock, acute nutrient starvation, and high light levels by a range of adaptive molecular processes. Processes of microorganism acclimation to environmental stress are mainly regulated at the level of transcriptional activation or repression, where the change is acting on single genes. An example of this is the light induction of the DNA repair enzyme photolyase as a response to damaging light levels. In addition to stress responses involving single genes, groups of genes may also be regulated in stressed cells by the activity of alternative sigma factors which replace the primary sigma factors that normally operate under favourable conditions (Huckauf et al., 2000). In blue-green algae, these alternative sigma factors have been identified mainly in group 3 of the σ^{70} family. The role of alternative sigma factors in stress responses has been studied in the single-celled blue-green alga *Synechocystis* sp. by examining the effect of mutagenesis on specific sigma genes (Huckauf et al., 2000). These studies identified three gene products (group 3 factors) as important in stress responses – regulatory protein RsbU (important in regenerating growth after nitrogen- and sulphur-starvation), SigF protein (required for the induction of salt stress proteins)

Chrysophytes (Chrysophyta)

The majority of chrysophytes are unicellular or simple colonial forms, and are typically planktonic although attached forms do exist. They are typically associated with standing waters which have low to moderate nutrients, alkalinity, and conductance (pH slightly acid to neutral).

Diatoms (Bacillariophyta)

Diatoms initially appeared in the fossil record about 185 million years ago, and have been abundant in surface waters for the past 115–110 million years. The major biomass of diatoms occurs in marine systems, where they are the most important microbial primary producers and the major contributors to global carbon fixation. These micro-algae are also of major importance in freshwaters, where they occur as both planktonic and attached (biofilm) organisms in lakes, streams, and estuaries. Diatoms may be unicellular or colonial, and constitute one of the largest classes of freshwater micro-algae (Fischer et al., 1999). The cell wall (frustule) is unique among living organisms in being almost entirely composed of silica (Section 5.10), and these organisms are well known for the intriguing species-specific design and ornamentation of this rigid and very dense structure. Diatoms are divided into two main groups – centric diatoms (radial symmetry, typically planktonic) and pennate (bilateral symmetry, many benthic species), with further taxonomic subdivisions in relation to frustule morphology (Lee, 1997).

Red algae (Rhodophyta)

Red algae are predominantly marine in distribution, with only 3 per cent of over 5000 species worldwide occurring in true freshwater habitats (Wehr and Sheath, 2003). Although freshwater red algae (such as the large filamentous alga *Batrachospermum*) are largely found in streams and rivers, these organisms may also occur as marine invaders of lakes and brackish environments.

Certain freshwater red algae in the littoral zones of the Great lakes Basin (USA), for example, appear to be originally marine and to have lost the capacity for sexual reproduction. These include the filamentous red alga *Bangia atropurpurea* (Lin and Blum,

1977), which reproduces only by asexual mono-spores – in contrast to marine species which undergo alternation of generations and carry out sexual reproduction. Attached red algae (e.g., *Chroodactylon ramosum*) also contribute to the epiphytic flora of lake periphyton.

Brown algae (Phaeophyta)

As with red algae, brown algae are almost entirely marine – with less than 1 per cent of species present in freshwater habitats (Wehr and Sheath, 2003). These species are entirely benthic, either in lakes or rivers, and have a very scattered distribution.

Freshwater brown algae include genera such *Pleurocladia* and *Heribaudiella*, and are the least diverse of all freshwater algae. Their morphologies are based on a relatively simple filamentous structure, and they lack the complex macro-morphology typical of the brown seaweeds.

Phytoplankton size and shape

Freshwater phytoplankton, composed of photo-synthetic bacteria and algae, shows considerable variation in the size and shape of individual organisms (cells or colonies). Phytoplankton dimensions are important in relation to enumeration and assessment of biovolume, designation of size category (picoplankton to macro-plankton), and biological activity.

Mucilaginous and non-mucilaginous algae

In addition to differences in size and shape, freshwater algae also vary considerably in the presence of extracellular mucilage. This is seen particularly clearly in the lake environment, where some species of planktonic algae have cells embedded in a large volume of mucilage – while others have no apparently detectable mucilage at all. The presence of mucilage is not readily observed by bright-field light microscopy, but can be seen under phase contrast (Figure 3.9) and by special preparation techniques such as negative staining and fluorescence microscopy of lectin-stained preparations. Atomic force microscopy (AFM) can also be used to provide high-resolution information on the topography and material properties of the mucilage layer of living algae. Recent AFM studies on three diatoms, for example, have revealed differences in mucilage surface nanostructure and in the dynamic properties (adhesion and stretching) of surface polymer chains (Higgins et al., 2003).

An outer layer of mucilage increases overall size and confers a distinctive surface chemistry to the algal cells. Mucilage has an important role in the biology of these organisms and has ecological implications in terms of biogeochemical cycles. Even algae that are regarded as ‘non-mucilaginous’ typically have a surface layer of polysaccharide material. Combined carbohydrate analysis and atomic force microscopy demonstrated a thin sugar-rich layer of surface mucilage in the diatom *Pinnularia* (Chiovitti et al., 2003), for example, which would be difficult to see under the light microscope. This thin mucilage layer in diatoms has particular importance in separating the cell wall from the external aquatic medium and reducing silica solubilization in living organisms. Mucilage is composed of a complex macromolecular network enclosing a water matrix. Although water is the major component (>95 per cent) by weight, the chemistry of mucilage is dominated by the macromolecular component and the exposed sugar and charged groups which are associated with this. Relatively little is known about the detailed chemistry of algal mucilage, although chemical analyses have been carried out on the surface slime of a number of blue-green algae. In *Microcystis flos-aquae*, for example, where individual cells are embedded in a globular mass of surface slime, the macromolecular component is almost entirely polysaccharide, with no detectable protein (Plude et al., 1991). Gas chromatographic analysis of the polysaccharide indicates a composition closely similar to higher plant pectin, with galacturonic acid as the main sugar plus minor quantities of neutral sugars (galactose, glucose, xylose, mannose, and rhamnose). The surface mucilage of other blue-green algae is also polysaccharide-based, but differences occur in sugar composition and detectable levels of protein are present in some cases. Variations in chemical composition may account for differences in mucilage appearance and consistency in different algae.

Role of mucilage in phytoplankton activities

The presence of a layer of surface mucilage can be seen as an ecological strategy that has evolved in all the major algal groups and affects a number of characteristics – including an increase in unit (single cell or colony) size, approximation to a spherical shape, decrease in overall density, and the acquisition of a characteristic surface chemistry. This mucilage is important for both planktonic and benthic micro-algae.

Heterotrophic nutrition in freshwater algae

Although the majority of freshwater algae are photoautotrophic, carrying out photosynthesis and forming complex organic compounds from inorganic precursors, some also have the facility for heterotrophic nutrition. These organisms are able to use external organic compounds for energy, metabolism, and growth (Sanders, 1991; Tuchman, 1996). The ability of some algae to supplement their autotrophic life style by the uptake of complex organic carbon from the environment occurs in two separate ways: organotrophy: direct uptake of soluble organic molecules by absorption over the cell surface, and phagotrophy: ingestion of particulate organic matter.

Table: Transition from autotrophy to heterotrophy in flagellate algae

Mode of nutrition	Characteristics
Autotrophy	Synthesis of all required organic compounds from inorganic sources. Algae use light energy (phototrophy).
Heterotrophy	Requirement for external organic compounds- either as a specific metabolites or as a general carbon source
Organotrophy	Uptake of soluble organic molecules at cell surface
1- Auxotrophy	Metabolic requirement for specific external metabolites (Vitamins, growth factors)
2- Photo-Organotrophy	Facultative organotrophs, able to obtain carbon from CO ₂ or soluble organic compounds
3- Chemo-Organotrophy	Facultative or obligate organotrophs obtain carbon from organic compounds
Phagotrophy	Uptake of particulate matter by phagocytosis
1- Mixotrophy	Facultative phagotrophs, able to carry out photosynthesis and phagotrophy
2- Phagotrophy	Obligate phagotrophs, feeding via food vacuoles for uptake of particulate matter

Table: Auxotrophic requirements for vitamin B12 (Cyanocobalamin)

Algal group	General requirement	Specific requirement
Euglenoids	All require B12 (photoauxotrophic)	Absolute requirement for <i>Euglena gracilis</i> – 4900 to 22 000 molecules of B12 needed
Chrysophytes	Many phagotrophs, also require B12	<i>Oochromonas danica</i> can synthesize all vitamins except B12 and several fat-soluble vitamins ^b
Prymnesiophytes	Generally require either thiamine or vitamin B12	<i>Coccolithus huxleyi</i> needs only thiamine ^c

Major groups of cryophilic algae

Cryophilic (snow and ice) algae

Table 3: Taxonomic diversity and habitats of snow and ice algae

Algal Group	Representative species	Typical snow and ice habitat
Blue-green algae	<i>Phormidium frigidum</i>	Aerobic or anaerobic mats under ice
	<i>Lyngbya martensiana</i>	
	<i>Anabaena cylindrica</i>	Ponds and ice surface
	<i>Nostoc commune</i>	
Green algae	<i>Chlamydomonas nivalis</i>	Melting snow-packs and ponds
	<i>Chloromonas brevispina</i> <i>Chloromonas nivalis</i>	Can form red blooms, with populations of 10^5 – 10^6 cells ml ⁻¹
Euglenoids	<i>Notosolenus sp.</i> <i>Euglena sp.</i>	Melting snow-packs and ponds
Dinoflagellates	Gyrodinium sp. Gymnodinium pascheri	Snow surfaces, occasionally forming red blooms
Cryptomonads	Cryptomonas frigoris	Melting snow-packs and ponds
Chrysophytes	Chromulina chionophila Ochromonas sp.	Melting snow-packs and ponds
Diatoms	Navicula sp. Nitzschia sp. Pinnularia sp.	Widely present in aerobic conditions of polar lakes, particularly in algal mats

Physiological adaptations of snow algae

Survival and active growth in the snow environment requires the ability to adapt to a relatively brief growth period and to withstand high levels of solar irradiation, low nutrients and temperature, high acidity, and extended periods of desiccation.

1- Brief growth period

Many snow algae are motile and single-celled. These characteristics reflect the need for free water (for movement and growth) and the relatively brief time period (snow melt) when this may be available. During this brief growth phase, motility, unicellularity, and reproductive strategy are all important in making optimal use of the environment.

2- Rapid colonization and motility

Motility is important for colonization of the snowpack when free water (melt water) becomes available. Having flagella allows the cells to move within the water film that surrounds snow crystals at the time of snow melt. Other snow microbes that are not flagellates (non-motile algae, fungi, and bacteria) are passively moved within the snow, melt water.

3- Rapid population growth: r-selected species Snow algae are typically good examples of r-selected species, single-celled with small size and short cell cycle. Such organisms show maximum increase in population under appropriate (i.e., free water) conditions, and are able to dominate the new environment within a short period of time.

4- Reproductive strategy

The production of resting spores is an essential part of the life cycle of snow algae, ensuring survival between the brief periods of growth. The fastest route to producing these resting spores would be by asexual reproduction, since more time is needed to complete the sexual phase of the snow algal life cycle. Field observations suggest that in the most severe environments, where the snowpack is inconsistent from year to year, asexual reproduction may be favoured by natural selection (Hoham and Duval, 2001). This ecological strategy, however, would lead to a

decrease in genetic diversity and algae living in such environments may be headed for evolutionary extinction.

5- Protection from harmful irradiation

The exposed, often high-altitude habitats of snow algae means that these organisms frequently have to tolerate excessive levels of irradiation. Shortwave radiation values as high as 86 000 lux, for example, have been measured in exposed alpine snow containing *Chlamydomonas nivalis* (Mosser et al.,1977). Ultraviolet radiation is particularly high in snow environments due to reduced atmospheric shielding (alpine regions) or where the stratospheric ozone layer is relatively thin (polar regions).

Influence of light and nutrients on pigmentation of snow algae

*High nutrient and high light

1- green cells

Active growth

High chlorophyll level

Photo-protection

*Low nutrient (particularly nitrogen depletion) and high light

Red cells

Poor growth- chlorophyll breakdown- photo-protection

* In case of low light

Green cells

Poor growth- chlorophyll retained

Nutrient limitation

Nutrient levels are frequently very limited in snow environments. Adaptations of snow algae to low-nutrient conditions include the requirement for mineral but not organic molecules, their ability to remain inert over long periods as resistant zygotes, and the ability of motile stages to migrate towards nutrient sources (chemotaxis). Snow algae rapidly colonize habitats where nutrients are high, including snow surrounding sea bird colonies in Antarctica.

The small size of (unicellular) snow algae, with high surface area/volume ratios, also optimizes nutrient absorption under limiting conditions.

Temperature

Various workers have queried whether snow algae are adapted specifically to low temperatures or are simply surviving at the edge of their temperature range. In order to answer this question, algae have been isolated and their growth characteristics studied under controlled laboratory conditions. Hoham (1975) has suggested that true snow algae are obligate cryophiles (or psychrophiles), growing optimally below 5 °C, abnormally at 10 °C, and not surviving over this limit. Obligate cryophiles include species of *Chloromonas* and *Chlainomonas* and strains of *Chlamydomonas nivalis*. Other snow algae, such as *Raphidonema nivale*, are able to grow at temperatures up to 15 °C in the laboratory, and qualify as facultative cryophiles.

Specific adaptations to life in the cold include the ability to photosynthesize at low temperatures and the occurrence of a variable fatty acid ratio.

Fatty acid ratio

Various studies have indicated higher ratios of monounsaturated/saturated fatty acids in snow algae compared with their temperate relatives. Such ratios are considered to have a cryoprotective function since high levels of unsaturated fatty acids cause increased membrane fluidity, which is important in maintaining membrane transport at low temperatures. It has been suggested that the growth temperature range of an organism depends primarily on the ability to regulate its membrane fluidity, and the ability to

maintain this fluidity at low temperature. In cold-adapted organisms, the lower temperature limit is thus not determined by membrane constraints, but by the freezing properties of aqueous solutions inside and outside the cell.

Photosynthesis

Photosynthetic optima for snow algae show some variation between species. Many snow algae show maximum photosynthetic activity at 3 to 4 °C (Hoham, 1975; Mosser et al., 1977) while others, such as *Chlamydomonas nivalis*, photosynthesize optimally at 10–20 °C, but retain substantial activity at 3 to 4 °C. The ability of snow algae to carry out photosynthesis at freezing temperatures is a key factor in their survival.

Acidity

The pH of snow which contains algae has been widely sampled in different continents and has indicated universally acidic conditions, with pH values typically in the range 4–6.2. Laboratory studies on *Chloromonas pichincae*, which normally grows at pH 4.9–5.2 in nature, have demonstrated a growth optimum at pH 6. Other isolates of *Chloromonas* indicate adaptation to more acid conditions. Little is known about the biochemical and physiological mechanisms which underlie this low pH tolerance.

Desiccation

Desiccation presents the final challenge for many snow algae, which have to survive long periods of potential water loss. This is particularly the case for resistant spores. In many of these cells, the presence of thick complex walls, with primary and secondary layers, may reduce desiccation.

Bacteria: the main heterotrophic microorganisms in freshwater systems

Bacteria occur as one of three major groups of prokaryotes in the freshwater environment, differing from blue-green algae in their heterotrophic mode of nutrition but showing many physiological similarities to the actinomycetes (Section 8.2). The majority of bacterial cells have a maximum linear dimension in the picoplankton (0.2–2 μm) range, though some freshwater bacteria fall into the femtoplankton (<0.2 μm) and nanoplankton (2–20 μm) categories. These organisms can be readily observed in water samples by light (dark field, phase-contrast) microscopy or by transmission and scanning electron microscopy.

The environmental scanning electron microscope (ESEM) image visualizes the preparation in the wet state, showing the cells in their native state and revealing the presence of copious mucilage. This is secreted by the bacteria and accumulates in the culture medium (Figure 6.1(a)). Chemical processing removes mucilage, giving a much 'cleaner' preparation, which may be more useful for making bacterial counts. The relatively uniform appearance of the laboratory monoculture differs markedly from environmental samples (Figure 6.11), where considerable variation occurs in relation to size and morphology.

General diversity, habitat preferences, and ecological significance of freshwater bacteria

Freshwater bacteria are a very diverse assemblage of prokaryote organisms, varying in their morphology, physiology, and ecological preferences. Bacteria may be conveniently grouped into a number of natural assemblages based on characteristics such as cell shape, spore-forming capabilities, and whether they are aerobic/anaerobic or Gram-positive/ Gram-negative.

Habitat preferences

Bacteria are widespread throughout the freshwater environment, forming extensive pelagic and benthic populations in a wide range of habitats including mudflats, bogs, sulphur springs, lakes, and rivers (Table 6.1). Although some bacteria, such as *Escherichia coli*, are present as accidental contaminants, most freshwater bacteria show close physiological adaptations to their environment. Strict anaerobes, for example, are confined to anoxic sediments and hypolimnia. In some cases, particular organisms (e.g., *Bacillus pituitans*) have a very restricted habitat range, while others such as *Pseudomonas aeruginosa* are very widespread – being routinely found in freshwater, soil, and aerial samples.

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Environmental significance of freshwater bacteria

With extensive benthic populations in lakes and rivers, and pelagic population levels of 10^2 – 10^8 cells ml⁻¹, bacteria are by far the most abundant of all the free-living freshwater biota, and are ecologically important in a number of ways. Within the freshwater environment, bacteria are:

- taxonomically very varied, and make a major contribution to the phenotypic, genetic and molecular biodiversity.
- the main heterotrophic microorganisms in different aquatic systems,
- a very diverse assemblage in terms of metabolic activities, occupying key roles in geochemical cycles.
- particularly important in anaerobic environments, where algae and other free-living biota are much less metabolically active.
- a key component of the microbial food web, within which they are particularly important in recycling algal secretory products via the microbial loop.
- able to outcompete algae for nitrates and phosphates under nutrient-limiting conditions.
- able to form key associations with other biota, particularly algae, and
- the primary colonizers of many benthic habitats, forming permanent bacterial biofilms on many different types of substrate.

Bacteria are the most opportunistic of all the free-living freshwater biota and are the ultimate r-strategists (see Section 1.2.6). In this respect they surpass almost all algal groups in terms of small size and short cell-cycle duration, and in terms of high rates of growth and nutrient

absorption. Within the water column, bacteria differ from pelagic algae in showing one further adaptation towards r-selection – the majority of organisms within the population are metabolically inactive, awaiting transient favourable environmental conditions to trigger growth and division. These inactive forms appear as tiny micro-cells within environmental samples.

Metabolic diversity of freshwater bacteria

Variations in the metabolic activities of bacteria are an important aspect of their diversity within the freshwater environment, and reflect their different roles and locations within the ecosystem. In this section metabolic diversity is considered in relation to key metabolic parameters, variations in CO₂ fixation, aerobic and anaerobic decomposition of organic substrates and the metabolic transition which occurs from high- to low-nutrient availability.

Key metabolic parameters

Four key features define the metabolic status of individual freshwater bacteria.

The source of energy. Either from light (photo-trophs) by photosynthesis or from chemical energy (chemotrophs). Chemotrophs use energy obtained from energy-yielding (exergonic) reactions to oxidize organic matter.

The source of electrons for growth (electron donor). These are obtained either from organic (organotrophs), or from chemical compounds (lithotrophs) such as sulphide, hydrogen, and water.

The source of carbon, required for synthesis of bacterial biomass. This is obtained either by reducing CO₂ during photosynthesis (auto-trophs), or from complex organic compounds (heterotrophs).

The terminal electron acceptor. The final electron acceptor in the process of respiration involves either oxygen (aerobic respiration) or other molecules (e.g., sulphate, nitrate) in anaerobic respiration.

The main role of bacteria in the freshwater environment is the breakdown of organic biomass and the recycling of various key elements (nitrogen, phosphorus, sulphur) which are present within the various organic compounds. In line with this, the majority of freshwater bacteria are heterotrophic, living on organic carbon compounds present in the aquatic medium or in

the sediments. Using the above terminology, the typical bacterium in the water column or substratum of a freshwater system is a chemo-organo-heterotroph, while the typical algal cell is a photo-litho-autotroph.

CO₂ fixation

Although CO₂ fixation in freshwater environments is usually associated with the photosynthetic requirements of algae and macrophytes, some bacteria also have this activity. Bacteria which are able to assimilate CO₂ directly are of three types, as follows.

- Photosynthetic bacteria (photoautotrophs). Using light energy to mediate CO₂ uptake.
- Chemosynthetic bacteria (chemoautotrophs). These organisms typically occur within the water column at the boundary layer between aerobic and anaerobic zones, using reduced inorganic compounds as energy substrates. The reduced compounds are largely derived by decomposition of organic matter within the anaerobic hypolimnion. Chemosynthetic fixation of CO₂ is particularly high in conditions of steep redox potential gradient which occur at the top of the anaerobic zone, but is low in other parts of the water column. The requirement for an anaerobic hypolimnion means that chemosynthesis tends to be prominent in eutrophic rather than oligotrophic lakes, and in the stable long-term redox gradients that develop in meromictic lakes.
- Heterotrophic bacteria. Most non-photosynthetic fixation of CO₂ is carried out by heterotrophic bacteria. This dark CO₂ fixation provides a useful parameter for measuring heterotrophic productivity.

Breakdown of organic matter in aerobic and anaerobic environments

The breakdown of biological material by bacteria ultimately involves an oxidation/reduction reaction, with the transfer of electrons from the organic substrate (oxidation) to an electron acceptor (reduction).

- Aerobic conditions

Aerobic conditions may be defined as environments where oxygen is freely available, and is used in the oxidation of organic (and some inorganic) substrates. Aerobic environments contain

obligate aerobic microorganisms (which are restricted to oxygen as the secondary electron acceptor) and facultative anaerobes (which are able to use other secondary acceptors in addition to oxygen).

In a lake, the concentration of dissolved oxygen (DO) ranges from supersaturation (lake surface) to very low levels in sediments. This range of oxygen concentration correlates with a range of oxidizing ability or oxidation/reduction potential (redox potential). Redox potential (Eh) can be measured in reference to a standard electrode, and is normally expressed as millivolts (mV). In well-oxygenated environments, the redox potential is normally in excess of +360mV, falling to -500 mV in highly reducing conditions.

- Anaerobic decomposition of organic matter

Anaerobic environments are those where the concentration of oxygen is too low for it to be used as an electron acceptor. These environments may be divided into 2 main groups 1- Low oxygen environments. 2- Anoxic environments, where oxygen is completely absent.

Low oxygen environments. Although oxygen is the electron acceptor in fully-oxygenated environments, removal of DO by metabolic processes may reduce the availability to such an extent that other electron acceptors (Mn^{4+} , NO_3^- , Fe^{3+} , and SO_4^{2-}) become used instead.

The free oxidation energy released per molecule of organic matter via each of these acceptors varies considerably, from 380 to

3050 kJmol⁻¹. Since oxidation

of organic matter follows a sequence in which acceptors generating most energy take precedence, each of these acceptors will be used in turn until depleted levels lead on to the next one. This process continues until all oxidizable substrates or all electron acceptors are removed from the system. This chemical sequence is paralleled by an ecological succession, in which whole communities of bacteria change with the chemical environment. Bacteria that use Mn^{4+} , NO_3^- , Fe^{3+} are facultative anaerobes, able to use either oxygen or an inorganic electron acceptor – depending on prevailing conditions

Anoxic environments. These environments, where oxygen is completely absent, contain populations of obligate anaerobic bacteria. Oxidation/reduction reactions using inorganic electron acceptors are severely restricted, though sulphate-reducing bacteria are able to use SO_4^{2-} when oxygen is completely exhausted ($E_h < 200 \text{ mV}$).

breakdown of organic material occurs by fermentation processes.

In anaerobic conditions, organic matter is metabolized by a variety of heterotrophic bacteria, which obtain energy by substrate phosphorylations. In this situation, where oxygen no longer acts as the universal hydrogen acceptor, the situation becomes complex, with a range of organic and inorganic compounds taking over this role. In some cases, the same compound can act as hydrogen acceptor or donor, depending on environmental conditions. Large quantities of organic detritus are degraded under the anaerobic conditions which occur in the hypolimnion and sediments of eutrophic lakes, and in the sediments of ponds, rivers, and waste-treatment plants (septic tanks, anaerobic lagoons). The process of anaerobic degradation converts biomass to CO_2 , CH_4 , and NH_3 .

Comparison with the oxidation process (using oxygen as electron acceptor) shows that bacteria in the oxygenated part of the water column obtain much more energy from aerobic breakdown of organic material compared with those carrying out anaerobic fermentation on the sediments.

The process of fermentation occurs as 2 distinct stages:

1- Initial hydrolysis and fermentation

Hydrolytic and fermentative conversion of proteins, carbohydrates, and fats to a range of breakdown products (primarily fatty acids) is carried out by a heterogeneous group of facultative and obligate anaerobic bacteria. These bacteria generate large amounts of organic acids and are collectively referred to as acid formers. The degradative activity of these organisms results in the formation of CO_2 and various reduced end products, including H_2 , H_2S , acetic, propionic, lactic, and butyric acids, ethanol, and amines. These compounds would accumulate in the anaerobic environment if they were not metabolized in various ways.

2- Removal of breakdown products.

Removal of oxidizable intermediate and end products is carried out by obligate anaerobes which are able to use a range of hydrogen acceptors such as sulphates, nitrates, and CO_2 . The different groups of organisms that carry out this terminal oxidation include the following.

Denitrifying and sulphate-reducing bacteria which use nitrate and sulphate as the ultimate electron acceptor. The activity of these organisms is limited by sulphate and nitrate availability, which has to diffuse from the epilimnion into the hypolimnion in eutrophic lakes. In aerobic sediments, bacteria such as *Thioploca* obtain their nitrate at the surface, then migrate into anaerobic regions to use the oxygen in nitrate for sulphur oxidation.

Methane-producing bacteria, operate under strict anaerobic conditions, and include two rod-shaped (*Methanobacterium*, *Methanobacillus*) and two coccoid (*Methanococcus*, *Methanosarcina*) genera. Methane is generated by one of two processes.

In the first situation, bacteria use CO_2 as the acceptor of hydrogen derived from the organic acids:

In the second process, acetic acid is directly converted to CO_2 and methane

The methane generated by reduction of CO_2 escapes to aerobic regions where it is readily oxidized.

Photosynthetic bacteria. Reduced substrates such as H_2S are released from decaying biomass as part of the fermentation process. Subsequent oxidation of reduced substrates by photosynthetic bacteria involves the removal of hydrogen and electrons to drive the reduction of CO_2 in the process of photophosphorylation. The uptake of CO_2 by these organisms as part of an assimilatory sulphate-reduction system is thus important for both carbon compound formation and the disposal of excess hydrogen and electrons from the reduced substrates.

Other end products of anaerobic fermentations, including H_2 , CH_4 , H_2S , and N_2 can also be metabolized by photosynthetic bacteria, which are able to use hydrogen as an electron acceptor simultaneously with the sulphate assimilatory reduction system. Unlike the other two major groups of anaerobic bacteria involved in the terminal degradation of biomass, photosynthetic

bacteria are located at the top of the anaerobic hypolimnion rather than in the lower regions and sediments.

Bacterial adaptations to low-nutrient environments

Gram-positive bacteria tend to form dormant spores, while Gram-negative bacteria have molecular and physiological mechanisms which enable them to persist at low metabolic (but not dormant) activity until adequate nutrient levels return; they then exploit the improved growth conditions and undergo a burst of synthetic activity and population increase (Zambrano and Kolter, 1996). The ability of bacteria to live through conditions of acute nutrient deprivation is referred to as 'starvation-survival' and may be defined as 'the process of survival in the absence of energy-yielding substrates' (Morita,

1982).

Low-nutrient aquatic environments

Starvation survival has been investigated particularly in relation to the planktonic bacteria of marine environments, where they may be carried passively within water masses for many years (Menzel and Ryther, 1970) under conditions of extremely low organic carbon availability (Morita, 1997). Although many of the environmental starvation adaptations shown by aquatic bacteria (Table 6.4) have been studied specifically in marine organisms, the low-nutrient responses of freshwater organisms are expected to be closely similar.

Organic nutrients are normally available to freshwater bacteria from exogenous sources or from algae via the microbial loop (Section 1.5.2). Carbon limitation of bacterial populations become particularly acute where there is limited exogenous supply and where algal populations are low due to a lack of available inorganic nutrients (e.g., oligotrophic standing waters) or absence of light

The starvation response

The starvation response in aquatic bacteria is a classic example of environmentally-induced molecular activity, and involves the activation of a cohort of 'starvation genes' followed by associated changes in biochemical activity, cell size and shape, and bacterial populations

Molecular activation

Transition from high- to low-nutrient environment promotes a fundamental change in cell physiology, with a switch from growth to maintenance. Energy reserves need to be mobilized and the cell has to survive in the absence of multiplication (Reeve et al., 1984). These changes result from the activities of a large set of genes which are induced at the onset of starvation. This induction is the result of the initial activation of the *rpoS* gene and the formation of a starvation-specific transcription factor (RpoS or σ^S) which confers new promoter recognition sites on the RNA polymerase (Loewen and Hengge-Aronis, 1994).

The role of RpoS in the induction of starvation-induced dormancy (Figure 6.8) parallels its role in the transition of bacterial populations to a stationary phase during batch culture and the induction of stationary phase characteristics during quorum sensing in biofilms

Regulation of RpoS (starvation-specific transcription factor) by environmental changes in nutrient concentration is mediated by changes in the concentration of internal metabolites (Zambrano and Kolter, 1996), including cAMP and ppGpp (which promote transcription) and UDP-glucose (which represses translation). Factor RpoS is also regulated by post-transcriptional control of its molecular stability

Activity of RpoS-controlled genes

About 30–50 proteins are thought to be induced via the RpoS-controlled genes, including enzymes which are involved in hydrolysis, protein and carbohydrate synthesis, and protection of DNA. The phenotypic effects and biological implications of this genetic activity are summarized in Table 6.4. Biochemical changes include a decrease in surface membrane (with changes in biochemical composition), decreases in ATP content, utilization of internal storage compounds and also conversion of internal non-storage molecules for energy formation. Decrease in the surface membrane, with utilization of cell contents for energy formation, results in a decrease in size and a change to spherical shape (for elongate bacteria). The small size of bacteria in the starvation state is one of the most obvious features of this metabolic condition, and may result in the formation of very tiny ‘ultramicrocells’.

Reduction in biomass formation and cell division leads to a marked decrease in the bacterial population. Amy and Morita (1983) noted four distinct patterns of change during the starvation survival process, the most common of which involved an initial increase in total count followed by a prolonged decline. Viable counts also showed sharp decline, with reductions of over 99 per cent over a 4-week starvation period being recorded for some aquatic bacteria (Kurath and Morita, 1983). Resulting populations include a high proportion of dead cells, cells with minimal metabolic activity (surviving but unable to divide in the short-term), and cells with low metabolic activity that are able to respond rapidly to high nutrient and form colonies on nutrient agar.

Photosynthetic bacteria

As noted earlier, photosynthetic bacteria can be divided into three major groups – the green sulphur bacteria (Chlorobacteriaceae), purple sulphur bacteria (Thiorhodaceae), and the purple non-sulphur bacteria (Athiorhodaceae).

General characteristics

Although these groups are defined primarily in terms of colour and metabolic substrate, they also show differences in terms of cell size, fine structure, photosynthetic activities (Section 4.5), involvement in the sulphur cycle (Section 6.8), general ecology, and cell motility. The main characteristics of these groups within each group, cell shape varies greatly between species, including spherical, elliptical, rod-shaped, and vibrioid forms. Differences in size occur between species and also between major groups. The overall size of green sulphur bacteria tends to be less than purple sulphur bacteria, which reach maximum diameters of 5–10 μm . The location of photosynthetic pigments in green sulphur bacteria within distinctive ‘chlorobium vesicles’ distinguishes the fine structure of these organisms from the other two major groups. Gas vacuoles are found in those organisms (purple and green sulphur bacteria) which form discrete layers in stratified lakes, but not the less ecologically-defined purple non-sulphur bacteria (Walsby, 1994). This distinction highlights the importance of gas vacuoles in the depth-regulation of photosynthetic bacteria.

Motility

Motility of photosynthetic bacteria has been investigated particularly in laboratory cultures, where there is considerable variation within species and within major taxonomic groups. Individual photo-synthetic bacteria, like algae, come in one of three categories – non-motile, actively motile (by flagella), and passively motile (by gas vacuoles). Unlike algae, these characteristics are to some extent interchangeable, with environmental factors having an important effect. Cultures of Thiorethodaceae exposed to high sulphide concentrations and light intensity undergo a conversion of all motile to non-motile cells, which sink to the bottom of the vessel and produce copious slime. Differences in motility within this group are shown in Table 6.6, where particular genera may fit into one of four categories depending on their capabilities.

The ecological significance of active and passive motility is particularly apparent in situations such as the lake water column, where both processes are important in the exact positioning that occurs at the top of the hypolimnion. Many of these organisms are killed by exposure to oxygen in the presence of light, so it is important that they do not stray up into the aerated waters of the epilimnion. Migration of purple sulphur and green sulphur bacteria has been observed during summer stratification in holomictic lakes, where upward movement of bacterial populations follows the extension of the anaerobic high-sulphide zone that accompanies the rise in the thermocline. Flagellate forms are able to migrate by their own flagellar activity. In vitro studies have suggested that vertical movements of these organisms occur in response to gravity (negatively geotactic) and oxygen (negatively aerotactic). The response to light is non-directional in terms of the stimulus and involves a reversal in direction of flagellar movements followed by a reversal in the direction of cell movement.

Table. Active motility and gas vesicles in purple sulphur bacteria

	Genera with gas vacuoles	Genera without gas vacuoles
Genera with motile stage	Lamprocystis	Thiospirillum Chromatium
Genera with no motile stage	Rhodotheca Thiodictyon	Thiocystis Thiococcus Thiocapsa

Ecology

The metabolic activities and requirements of photo-synthetic bacteria determine their ecological niche in the aquatic environment. These organisms are often found in narrowly-defined micro-environments within relatively heterogeneous aquatic systems including ponds, ditches, marshes, estuarine mud flats, rivers, and lakes. In contrast to the Athiorhodaceae, which occur widely but never at high population density, the Thiorhodaceae and Chlorobiaceae are often present at high abundance – appearing as red or greenish layers on mud or forming substantial blooms in lakes.

Hidden blooms of photosynthetic sulphur bacteria are often found below the surface of well-stratified productive lakes, where they are restricted to the top of the hypolimnion (immediately below the thermocline) as a discrete layer. Within this layer, green sulphur bacteria are often localized below an overlying population of purple sulphur bacteria, in accordance with the higher H₂S tolerance of the Chlorobacteriaceae.

Bacteria and inorganic cycles

Freshwater bacteria have a key role in geochemical transitions within the aquatic environment, and are important in the cycling of metabolically-important elements such as nitrogen, Fe and

sulphur. The cycling of other elements, such as silicon (Section 5.9, Figure 5.24), may also involve bacterial activity. Studies by Patrick and Holding (1985) have shown that solubilization of diatom frustules (regenerating silicic acid) is increased in the presence of natural populations of freshwater bacteria.

Bacterial metabolism and the sulphur cycle

The cycling of sulphur within the freshwater environment involves alternate phases of anabolic and catabolic activity. Sulphate ions are taken up by all lake biota and converted to sulphydryl (SH) groups in the synthesis of proteins. Breakdown of proteins on death of the organism results in the release and conversion of simple sulphur compounds, leading ultimately to the regeneration of sulphate ions. Four main types of microbial metabolic activity are involved in the sulphur cycle – protein decomposition, sulphate reduction, aerobic and anaerobic sulphide oxidation

Microbial interactions involved in the cycling of sulphur within the aquatic environment are clearly localised in eutrophic lakes, where the occurrence of distinct aerobic and anaerobic zones within the water column leads to clear separation of microbial activities. The aerobic epilimnion is the main region of incorporation of inorganic sulphur compounds into lake biomass (trophogenic zone), while the anaerobic hypolimnion and sediments are the primary sites of conversion from organic to inorganic sulphur (tropholytic zones). Dissolved inorganic sulphur occurs primarily as sulphate ions (SO_4^{2-}) within the oxygenated lake water of the epilimnion. These ions are taken up by algae and other biota and are subsequently reduced to sulphydryl (SH) groups during protein synthesis. Death and sedimentation of lake biota leads to cell breakdown in the hypolimnion and lake sediment, with further reduction of sulphydryl groups to H_2S during the process of protein decomposition. This anaerobic process is carried out by a wide range of bacteria, including *Pseudomonas liquefaciens* and *Bacterium delicatum* (Kuznetsov, 1970). Protein decomposition occurs mainly in the surface sediments, where bacterial population counts are up to three times greater than in lake water. Reduction of sulphate generated from mineral sources also occurs in the sediment. In these anaerobic conditions, sulphate provides a source of oxygen for the oxidation of molecular hydrogen or carbon

Bacterial biofilms

A biofilm is essentially a community of microorganisms (typically algae or bacteria) which is attached to an exposed surface. In the case of bacterial biofilms, where individual cells are typically non-motile and closely associated within an extracellular matrix, the microenvironment is very different from that of the planktonic phase. Direct interactions between organisms become particularly important in biofilms, ranging from relatively simple adhesion contacts to exchange of genetic information.

Biofilms occur in association with a wide range of physical interfaces in the freshwater environment (Brisou, 1995) including: air/water boundaries, present at the top of the water column and at the interface of submerged pockets of air and bubbles, water/biomass boundaries, such as the surface of algae, zooplankton, and submerged higher plants, water/solid-inorganic surfaces, such as suspended particulate material, particulate sediments, and large rocks and stones.

Biofilms are particularly important in association with the stones and sediments of lotic communities, which are dominated by benthic organisms. They are also well-adapted to running-water environments, since the dense microbial community that develops at the solid surface is able to resist large-scale detachment by the intense shearing forces generated by water flow. The biofilm development described below is typical of the sequence that occurs on exposed rock surfaces at the bottom of a fast-flowing stream.

Bacterial Interactions With Phytoplankton

In the water column of lakes and other standing waters, phytoplankton interact with both planktonic (free-living) and attached (epiphytic) bacteria. In the first case, the interactions are to some extent remote, while epiphytic associations involve a much closer interchange. In some cases, epiphytic associations develop into a clear symbiotic relationship, with close metabolic coupling – to the mutual benefit of both organisms.

Antagonistic interactions between bacteria and algae

Antagonistic interactions between bacteria and algae include all those activities that directly reduce

the population or growth rate of one or the other organism. In the case of algae, this involves the production of antibiotics, which may be defined as low molecular weight compounds which have a specific inhibitory effect. Bacteria act against algae via a range of mechanisms, including production of antimicrobial compounds, parasitism, predation, and epiphytic associations (Figure 6.19). Bacterial antagonists to blue-green algae have particular potential for use as biological agents in the control of nuisance algal blooms

Anti-microbial compounds Antimicrobial compounds that cause lysis of algae can be divided into three main groups – enzymes, antibiotics, and very low MW volatiles.

Viruses: major parasites in the freshwater environment

Although freshwater viruses have probably been the least researched of all aquatic microorganisms, their widespread occurrence and general role as parasites gives them an ecological significance equal to the other, more extensively studied, microbial groups

Viruses as freshwater biota

Various ecological studies have been carried out on the role of viruses in freshwater planktonic systems (see, for example, Weinbauer and Höfle, 1998), but there is little information on viruses in benthic systems such as biofilm communities. The relative lack of ecological information on freshwater compared with marine viruses also means that information obtained from marine environments may fill useful gaps in our understanding of freshwater systems.

Viruses are small in size and are the only group of freshwater organisms which are non-cellular and belong exclusively to the femtoplankton (Section 1.2.2, Table 1.3). Although most viruses are <70 nm in diameter, a small fraction are larger than 100 nm, with some filamentous forms exceeding 1 mm in length (Middelboe et al., 2003).

General role in the freshwater environment

Viruses are potentially important in planktonic and benthic freshwater ecosystems in a number of ways. They have a significant influence on the growth and productivity of the major biomass (algae) and the greatest population (bacteria) of freshwater biota. They cause death and lysis of freshwater micro-organisms, liberating dissolved organic carbon (DOC), nitrogen, and phosphorus into the water and generating nutrients for the microbial loop. Viruses thus have a significant impact on carbon and nutrient flow in microbial food webs. Although viruses occur as discrete particles within the aquatic medium, their small size places them within the normally defined limits of 'soluble' organic material (<0.2 mm diameter). They thus directly contribute to the DOC as macromolecular complexes containing proteins, nucleic acid, and lipid components.

Major groups and taxonomy of freshwater viruses

Within a particular water body, viruses may be divided into two main groups according to whether they are naturally present (endogenous) or introduced (exogenous).

Endogenous viruses

Endogenous viruses occur as parasites of all freshwater biota, and thus influence the aquatic food web at all levels. Within the endogenous viruses, particular attention will be given in this chapter to three major groups:

- cyanophages – viruses of prokaryote (blue-green)

- algae,

- phycoviruses – infecting eukaryote algae,

- bacteriophages – parasites of freshwater bacteria.

Exogenous viruses

Exogenous viruses are introduced into the aquatic system from an external source, such as sewage. These viruses are not a natural part of the freshwater ecosystem and do not infect the naturally-occurring freshwater biota. Examples include human enteric viruses such as coliphages, which are infective agents of gut (coliform) bacteria, and may enter the environment with their host bacteria via untreated domestic effluent. Coliphages may be used as viral indicators of the

general level of faecal contamination in natural waters, and may also provide information on the source of impact.

Natural waters may also be contaminated by viruses that directly infect humans (rather than human gut bacteria) – including hepatitis A virus, human immunodeficiency virus (Chen and Suttle, 1995), and noroviruses (Lamothe et al., 2003).

Fungi and fungal-like organisms: aquatic biota with a mycelial growth form

Fungi and fungal-like organisms lack chlorophyll and have a major saprophytic (or saprotrophic) role in aquatic environments, where they are important decomposers of both plant and animal detritus. The breakdown of biomass by these organisms is important in the regeneration of soluble materials, and they play a substantial role in the carbon, nitrogen, and phosphorus cycles of lakes, rivers, and other freshwater habitats. As heterotrophic organisms, fungi are a key component of many aquatic food webs, and are in direct competition for organic material with bacteria and protozoa. Each of these groups has adopted a particular strategy to promote their saprophytic existence, which in the case of fungi involves the development of a filamentous branching growth form – the mycelial growth habit.

Fungi have been viewed traditionally as a broad group of organisms that are united in their mycelial growth form and in the associated mode of heterotrophic nutrition. This chapter considers the aquatic biology of fungi and fungal-like organisms in terms of their taxonomic and trophic diversity (Part A) and their ecological role as saprophytes and parasites (Part B). Particular emphasis is given to the role of fungi in the control of phytoplankton populations. As with other groups of microorganisms there is increasing use of molecular techniques in the analysis of biodiversity (Eggar, 1992) and functional activities.

Fungi and fungal-like organisms: the mycelial growth habit.

The mycelium is a highly successful vegetative structure which involves the production of a mass of branching tubular hyphae. These penetrate the organic substrate, secrete extracellular enzymes, and absorb soluble nutrients over their surface. The mycelial habit promotes optimal exploitation of the food source – with rapid penetration by hyphal tip extension, extensive colonization of bulk substrate and large-scale digestion within a short period of time.

The ecological success of the mycelial growth form can be gauged by the fact that it has evolved separately in at least three quite different groups of organisms – actinomycetes, oomycetes, and true fungi. These three groups have distinctive cell walls, are non-motile (though may have motile reproductive cells), and reproduce by means of spores.

Table: Three major groups of aquatic microorganisms showing mycelial growth form

Group	Status	Possible ancestry	Key features
Actinomycetes	Prokaryote	Gram-positive	Mucopeptide cell wall
Oomycetes ^b	Eukaryote	Eubacteria ^a Heterokont Algae ^b	Cell wall: glucan/cellulose Main sterol: fucosterol
True fungi	Eukaryote	Choanoflagellate	Cell wall: chitin Main sterol: ergosterol

Actinomycetes:

prokaryote organisms occurring in a multiplicity of natural and man-made environments, and are best known for their economic value as producers of antibiotics, vitamins, and enzymes. The variety and ecological importance of actinomycetes in aquatic systems have often been overlooked due to problems of isolation and laboratory culture. The filamentous nature of many actinomycetes typically results in strong adherence to the substratum, making them difficult to remove from organic material. Even where actinomycetes are being cultured from spores, the long incubation times that the slowly-extending colonies of these organisms require often result in overgrowth of the culture plate by bacteria and fungi. This results in culture plates being discarded before the actinomycetes even appear. In spite of these problems, actinomycetes have been widely detected (Wipat et al., 1992) and isolated from aquatic systems, where they have a distinctive ecological role. The aquatic biology of these organisms will be considered in relation to their taxonomic characteristics, habitat preferences, nutritional activities, and their ability to out-compete and antagonize other microorganisms.

Competition with other microorganisms

Although actinomycetes tend to follow fungi as part of a predictable temporal decomposition sequence, their interactions with fungi and other microorganisms are more dynamic than this procession might suggest. Interplay between the two groups of organisms involves both synergistic and antagonistic activities, including the production of antimicrobial compounds such as antibiotics and degrading enzymes.

Actinomycetes produce a wide range of antibiotics, including aminoglycosides, macrolides, and macquarimicides (Zenova and Zvyaginstevev, 2002), allowing them to inhibit the growth of bacteria, fungi, viruses, and protozoa. This competitive ability may be an important factor in allowing them to enter the decomposition sequence as secondary colonizers.

Actinomycetes are also able to lyse green and blue-green algae by the production of lytic enzymes, using the algal breakdown products as substrate for their growth. Extensive lysis of blue-green algae has been demonstrated in relation to naturally occurring algal blooms (Yamamoto et al., 1998) and also in laboratory experiments (Sigeo et al., 1999b) and these organisms have considerable potential as blue-green algal biological control agents.

Oomycetes

Oomycetes are a common group of organisms found in both aquatic and terrestrial environments all over the world. Most of the aquatic forms are freshwater rather than marine and are commonly referred to as water moulds. These freshwater species grow mainly in well-aerated streams, rivers, ponds, and lakes where they occur most commonly in shallow waters near to the bank or shoreline.

Table: Major taxonomic groups within the oomycetes

Phylum	Characteristics	Order	Life-style
Oomycota (water moulds)	Wide range- Zoospores biflagellate- anterior tinsel, posterior whiplash	Saprolegniales: Saprolegnia- Aphanomyces	Mostly saprophytes, but some important parasites of fish and crayfish
		Lagenidiales: Olpidiopsis	Parasites of algae, water moulds and small animals
		Leptomitales: Apodachlya	Saprophytes in clear, unpolluted waters
		Rhizipidiales: Aqualinderella	Saprophytes in anaerobic, polluted waters
		Peronosporales: Pythium	A few aquatic parasites of algae, fungi, and mosquito larvae

Oomycetes and true fungi

Oomycetes have traditionally been grouped with the ‘true fungi’ on the basis of similarities in gross morphology (Figure 8.2). These eukaryotic organisms, however, have no close phylogenetic relationship with fungi, and appear to be more closely related to heterokont algae with chloro- phyll-a and -c (Alexopoulos et al., 1996). Oomy- cetes are placed in the phylum Oomycota (Alexopoulos et al., 1996), and differ from true fungi by the possession of:

- a zoospore with distinctive flagellation and fine structure,

- the production of a diploid vegetative body (thallus) in which meiosis occurs in the developing gametangia,
- oogamous reproduction, leading to the formation of a thick-walled oospore, mitochondria with tubular cristae,
- cell walls that are composed mainly of β -1,3- and β -1,6-glucans and cellulose, rather than chitin, as is the case for true fungi.

Other biochemical characteristics also separate the Oomycota from the true fungi. Synthesis of the amino-acid lysine differs in the two groups, being produced in the oomycetes via the alpha aminoadipic pathway rather than via the diamino-pimelic pathway (used by true fungi and also higher plants). Sterol metabolism also differs between the two groups. Oomycetes fall into two main groups, one of which is unable to synthesize sterols while the other can synthesize these compounds *de novo* from mevalonic acid. The most commonly formed sterol is fucosterol rather than ergosterol, which is typical of the true fungi. Other differences include the chemical identity of storage compounds (water soluble β -1,3 glucans in oomycetes, glycogen in fungi) and the presence of acyclic polyols (sugar alcohols) which are widely distributed in true fungi but appear to be absent from oomycetes.

True fungi

True fungi are a distinctive group of eukaryotic organisms that typically resemble oomycetes in the development of the mycelial habit, but differ from them in the key features noted previously. Some true fungi, such as chytrids, have lost the mycelial habit – and have adopted a unicellular morphology with a simple rhizoid system.

As with the oomycetes, they are widespread, heterotrophic organisms which obtain their nutrients by:

- saprophytic decomposition of dead material (detritus), which occurs mainly on sediments,
- parasitic interactions with the whole range of biota present in freshwater systems.

In addition to these two main modes of nutrition, some fungi are also symbiotic. Trichomycete fungi (phylum Zygomycota), for example, are widely present in the gut of aquatic arthropods, where there appears to be mutual benefit to host and fungus. Other fungi are predacious, capturing

their prey by a range of entrapment mechanisms, then ingesting break-down products released from the digested remains. Various zygomycete fungi, for example, attack protozoa, rotifers, and nematodes.

Old and new terminology

Traditional taxonomy considered fungi as a very broad assemblage of organisms, dividing them into four major groups – phycomycetes, ascomycetes, fungi imperfecti, and basidiomycetes, all of which have representatives in the freshwater environment. Of these, phycomycetes are the only fungal group with motile spores (zoospores) and are the commonest group of fungi in freshwater systems. The fungi imperfecti are asexual forms derived mainly from ascomycetes and basidiomycetes, and are well represented as saprophytes and predatory fungi in freshwater situations.

The above groups are based primarily on comparative morphology and developmental patterns of the sexual reproductive structures. These relationships are now being re-examined by fine-structural, biochemical, and molecular studies, including the sequence analysis of rDNA noted above. With the emergence of new information and resulting changes in the understanding of phylogenetic relationships, there have been alterations in the arrangement of taxonomic groups and in nomenclature.

Table: Classification of aquatic fungi: comparison of past and current names that have been applied to the major fungal groups (table adapted from Deacon, 1997, and Alexopoulos et al., 1996)

	Past names	Current name
		Phylum
Phycomycetes	Chytridiomycetes	Chytridiomycota*
	Hyphochytridiomycetes	Hyphochytriomycotata
	Oomycetes	Oomycota
	Plasmodiophoromycetes	Plasmodiophoromycot
	Zygomycetes	Zygomycota*
	Trichomycetes	
Ascomycetes		Ascomycota*
Fungi imperfecti		Deuteromycota*
Basidiomycetes		Basidiomycota*

Table: Taxonomic and trophic diversity of true fungi

Phylum	Characteristics	Order	Life-style
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Chytridiomycota	<p>Coenocytic thallus, ranges from simple to well-developed mycelium</p> <p>Only fungi that have true motile cells (zoospores and gametes); these have single posterior whiplash flagellum</p>	<p>Chytridiales</p> <ul style="list-style-type: none"> –Rhizophydium –Nowakowskiella <p>Blastocladales</p> <ul style="list-style-type: none"> –Allomyces –Coelomomyces <p>Monoblepharidales</p> <ul style="list-style-type: none"> –Monoblepharis <p>Spizellomycetales</p> <ul style="list-style-type: none"> – Rozella 	<p>Mainly parasites of algae, water moulds, animal eggs and protozoa; some saprophytes</p> <p>Animal and plant parasites, freeliving saprophytes</p> <p>Mainly saprophytes</p> <p>Includes the endoparasite Rozella</p>
Zygomycota	<p>Coenocytic thallus, typically well-developed mycelium</p> <p>Sexual resting spores (zygospores) formed in all zygomycetes and some trichomycetes</p>	<p>Zygomycetes</p> <ul style="list-style-type: none"> –Zoophagus <p>Trichomycetes</p> <ul style="list-style-type: none"> – Smittium 	<p>A few predatory aquatic species of amoebae, rotifers, and nematodes</p> <p>All are obligate associates of arthropods; mostly symbiotic</p>
Ascomycota	<p>Septate mycelium</p> <p>Production of ascospores in ascocarps</p>	Saccharomycetales	Some saprophytic yeasts
Basidiomycota	<p>Septate hyphae with clamp connections and dolipore septal</p>	<p>Aquatic members typically placed in Deuteromycota (have</p>	

	<p>pore structures</p> <p>Produce basidiospores on basidia</p>	lost sexual stages)	
<p>Deuteromycota (Fungi imperfecti)</p>	<p>No clear sexual stages</p> <p>A taxonomically mixed assemblage</p>	Hyphomycetes	<p>Saprophytes present in both clear and stagnant water</p>

Practical Part

Introduction to Microbiology

Microbiology is the study of microscopic organisms (microbes), which are defined as any living organism that is either a single cell (unicellular), a cell cluster, or has no cells at all (acellular). This includes eukaryotes, such as fungi and protists, and prokaryotes. Viruses and prions, though not strictly classed as living organisms, are also studied.

Microbiology is a broad term which includes virology, mycology, parasitology, bacteriology, immunology, and other branches. A microbiologist is a specialist in microbiology and these related topics. Microbiological procedures usually must be aseptic and use a variety of tools such as light microscopes with a combination of stains and dyes. As microbes are absolutely required for most facets of human life (including the air we breathe and the food we eat) and are potential causes of many human diseases, microbiology is paramount for human society.

Microbiology is the study of microbes, which affect almost every aspect of life on the earth. In addition, there are huge commercial and medicinal benefits in understanding microbes. The application of this understanding is known as applied microbiology. There are many different types of applied microbiology which can be briefly defined as follows:

Medical Microbiology

Medical microbiology is the study of the pathogenic microbes and the role of microbes in human illness. This includes the study of microbial pathogenesis and epidemiology and is related to the study of disease pathology and immunology.

Pharmaceutical Microbiology

The study of microorganisms that are related to the production of antibiotics, enzymes, vitamins, vaccines, and other pharmaceutical products. Pharmaceutical microbiology also studies the causes of pharmaceutical contamination and spoil.

Industrial Microbiology

The exploitation of microbes for use in industrial processes. Examples include industrial fermentation and waste-water treatment. Closely linked to the biotechnology industry. This field also includes brewing, an important application of microbiology.

Microbial Biotechnology

The manipulation of microorganisms at the genetic and molecular level to generate useful products.

Food Microbiology and Dairy Microbiology

The study of microorganisms causing food spoilage and food-borne illness. Microorganisms can produce foods, for example by fermentation.

Agricultural Microbiology

The study of agriculturally relevant microorganisms. This field can be further classified into the following subfields:

- Plant microbiology and plant pathology – The study of the interactions between microorganisms and plants and plant pathogens.
- Soil microbiology – The study of those microorganisms that are found in soil.
- Veterinary microbiology – The study of the role in microbes in veterinary medicine or animal taxonomy.
- Environmental microbiology – The study of the function and diversity of microbes in their natural environments. This involves the characterization of key bacterial habitats such as the rhizosphere and phyllosphere, soil and groundwater ecosystems, open oceans or

extreme environments (extremophiles). This field includes other branches of microbiology such as: microbial ecology (microbially-mediated nutrient cycling), geomicrobiology, (microbial diversity), water microbiology (the study of those microorganisms that are found in water), aeromicrobiology (the study of airborne microorganisms) and epidemiology (the study of the incidence, spread, and control of disease).

A microbe, or microorganism, is a microscopic organism that comprises either a single cell (unicellular); cell clusters; or multicellular, relatively complex organisms. The study of microorganisms is called microbiology, a subject that began with Anton van Leeuwenhoek's discovery of microorganisms in 1675, using a microscope of his own design.

Microorganisms are very diverse; they include bacteria, fungi, algae, and protozoa; microscopic plants (green algae); and animals such as rotifers and planarians. Some microbiologists also include viruses, but others consider these as nonliving. Most microorganisms are unicellular, but this is not universal, since some multicellular organisms are microscopic. Some unicellular protists and bacteria, like *Thiomargarita namibiensis*, are macroscopic and visible to the naked eye.

Microorganisms live in all parts of the biosphere where there is liquid water, including soil, hot springs, on the ocean floor, high in the atmosphere, and deep inside rocks within the Earth's crust. Most importantly, these organisms are vital to humans and the environment, as they participate in the Earth's element cycles, such as the carbon cycle and the nitrogen cycle.

Microorganisms also fulfill other vital roles in virtually all ecosystems, such as recycling other organisms' dead remains and waste products through decomposition. Microbes have an important place in most higher-order multicellular organisms as symbionts, and they are also exploited by people in biotechnology, both in traditional food and beverage preparation, and in modern technologies based on genetic engineering. Pathogenic microbes are harmful, however, since they invade and grow within other organisms, causing diseases that kill humans, animals, and plants.

The Pathogenic Ecology of Microbes

Although many microorganisms are beneficial, many others are the cause of infectious diseases. The organisms involved include pathogenic bacteria, which cause diseases such as plague,

tuberculosis, and anthrax. Biofilms —microbial communities that are very difficult to destroy—are considered responsible for diseases like bacterial infections in patients with cystic fibrosis, Legionnaires’ disease, and otitis media (middle ear infection). They produce dental plaque; colonize catheters, prostheses, transcutaneous, and orthopedic devices; and infect contact lenses, open wounds, and burned tissue.

Biofilms also produce foodborne diseases because they colonize the surfaces of food and food-processing equipment. Biofilms are a large threat because they are resistant to most of the methods used to control microbial growth. Moreover, the excessive use of antibiotics has resulted in a major global problem since resistant forms of bacteria have been selected over time. A very dangerous strain, methicillin-resistant *Staphylococcus aureus* (MRSA), has wreaked havoc recently.

In addition, protozoans are known to cause diseases such as malaria, sleeping sickness, and toxoplasmosis, while fungi can cause diseases such as ringworm, candidiasis, or histoplasmosis. Other diseases such as influenza, yellow fever, and AIDS are caused by viruses.

Food-borne diseases result from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food. ” Hygiene” is the avoidance of infection or food spoiling by eliminating microorganisms from the surroundings. As microorganisms (bacteria, in particular) are found virtually everywhere, the levels of harmful microorganisms can be reduced to acceptable levels with proper hygiene techniques. In some cases, however, it is required that an object or substance be completely sterile (i.e., devoid of all living entities and viruses). A good example of this is a hypodermic needle.

Types of microorganisms

Bacteria

Bacteria are unicellular organisms. The cells are described as prokaryotic because they lack a nucleus. They exist in four major shapes: bacillus (rod shape), coccus (spherical shape), spirilla (spiral shape), and vibrio (curved shape). Most bacteria have a peptidoglycan cell wall; they divide by binary fission; and they may possess flagella for motility. The difference in their cell wall structure is a major feature used in classifying these organisms.

According to the way their cell wall structure stains, bacteria can be classified as either Gram-positive or Gram-negative when using the Gram staining. Bacteria can be further divided based on their response to gaseous oxygen into the following groups: aerobic (living in the presence of oxygen), anaerobic (living without oxygen), and facultative anaerobes (can live in both environments).

According to the way they obtain energy, bacteria are classified as heterotrophs or autotrophs. Autotrophs make their own food by using the energy of sunlight or chemical reactions, in which case they are called chemoautotrophs. Heterotrophs obtain their energy by consuming other organisms. Bacteria that use decaying life forms as a source of energy are called saprophytes.

Archaea

Archaea or Archaeobacteria differ from true bacteria in their cell wall structure and lack peptidoglycans. They are prokaryotic cells with avidity to extreme environmental conditions. Based on their habitat, all Archaeans can be divided into the following groups: methanogens (methane-producing organisms), halophiles (archaeans that live in salty environments), thermophiles (archaeans that live at extremely hot temperatures), and psychrophiles (cold-temperature Archaeans). Archaeans use different energy sources like hydrogen gas, carbon dioxide, and sulphur. Some of them use sunlight to make energy, but not the same way plants do. They absorb sunlight using their membrane pigment, bacteriorhodopsin. This reacts with light, leading to the formation of the energy molecule adenosine triphosphate (ATP).

Fungi

Fungi (mushroom, molds, and yeasts) are eukaryotic cells (with a true nucleus). Most fungi are multicellular and their cell wall is composed of chitin. They obtain nutrients by absorbing organic material from their environment (decomposers), through symbiotic relationships with plants (symbionts), or harmful relationships with a host (parasites). They form characteristic filamentous

tubes called hyphae that help absorb material. The collection of hyphae is called mycelium. Fungi reproduce by releasing spores.

Fungi share some characteristics with other eukaryotes, plants, and animals; these include:

- **Characteristics similar to plants:** Fungi have a cell wall and vacuole similar to plants, and like moss and ferns, they also produce spores. Furthermore, they can also reproduce by both sexual and asexual means.
- **Characteristics similar to animals:** Animals and fungi both lack chloroplasts and are heterotrophic, thus requiring organic compounds as an energy source.
- **Characteristic similar to other eukaryotes:** Fungi, like other eukaryotes, possess membrane-bound nuclei (containing genetic material) and other organelles, such as mitochondria. Furthermore, they have an extensive range of soluble carbohydrates and storage compounds, like sugar alcohols and polysaccharides.

Most fungal species cause disease in plants and animals, but some also have practical applications in different areas, for example:

- Fungi are used in food processing, preparation, and preservation. They are also used for the fermentation of food items; for example, *Saccharomyces cerevisiae*, also known as Baker's yeast, is used to make bread and other wheat-based products.
- Some fungal species have applications in modern chemotherapeutics and traditional medicines. For example, penicillin G is an antibiotic obtained from *Penicillium chrysogenum*; griseofulvin is isolated from *Penicillium griseofulvum* (used to treat fungal infections) polysaccharide-K compound isolated from *Trametes versicolor* is used as an adjuvant for cancer therapy in Europe and Japan.
- Some fungi, like white-rot fungi, work well in the bioremediation processes.

Protozoa

Protozoa are unicellular aerobic eukaryotes. They have a nucleus, complex organelles, and obtain nourishment by absorption or ingestion through specialized structures. They make up the largest group of organisms in the world in terms of numbers, biomass, and diversity. Their cell walls are made up of cellulose. Protozoa have been traditionally divided based on their mode of locomotion: flagellates produce their own food and use their whip-like structure to propel forward, ciliates have tiny hair that beat to produce movement, amoeboids have false feet or pseudopodia used for feeding and locomotion, and sporozoans are non-motile. They also have different means of nutrition, which groups them as autotrophs or heterotrophs.

Algae

Algae is a large group of predominantly aquatic photosynthetic organisms. They range from unicellular microalgae, like chlorella and diatoms, to multicellular algae, such as giant kelp and brown algae.^[11] Most algae are autotrophic like plants but lack many significant cells and tissues present in plants, such as stomata, xylem, and phloem.

Algae are classified in several ways based on a variant of characteristics. And depending on the pigments they contain, algae are classified into three groups:^[11]

- Green algae (Chlorophytes)
- Red algae (Rhodophytes)
- Brown algae (Phaeophytes)

Some algal species live in symbiotic relationships with other organisms. The host derives its nutritional requirements from algae and provides them protection in return. Some of them are:^[11]

- **Lichens:** It is a complex life form formed by two organisms, a fungus, and algae, symbiotically linked together. Here, fungi are the dominant partners that impart more physical characteristics to lichens, and algae predominantly associated with forming lichens are either green algae or blue-green algae. A few examples of lichens are *Graphis scripta* and *Ochrolechia parella*, and *macrolichen*.
- **Coral Reefs:** They are large underwater structures made up of the skeletons of colonial marine invertebrates called coral. Some of the corals build reefs by extracting calcium

carbonate from seawater. They are called hard corals.^[13] They often harbor dinoflagellates algae in their cells, which help them live healthy.

- **Sea Sponges:** A few species of algae live on the surface of sponges, where they provide sugar and oxygen for their growth and get protection from predators in return.

Some practical benefits of algae in human life include:

- Algae are used to derive agar used in food and labs to grow plants and microorganisms.
- Alginate extracted from brown algae is used in some food and medical dressing.
- Some algae species are used as fertilizers, soil conditioners, and livestock feed.
- *Chlorella* and *Spirulina* are used in some countries as nutritional food items.

Viruses

Viruses are noncellular entities that consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. Although viruses are classified as microorganisms, they are not considered living organisms. Viruses cannot reproduce outside a host cell and cannot metabolize on their own. Viruses often infest prokaryotic and eukaryotic cells causing diseases.

Multicellular Animal Parasites

A group of eukaryotic organisms consisting of the flatworms and roundworms, which are collectively referred to as the helminths. Although they are not microorganisms by definition, since they are large enough to be easily seen with the naked eye, they live a part of their life cycle in microscopic form. Since the parasitic helminths are of clinical importance, they are often discussed along with the other groups of microbes.

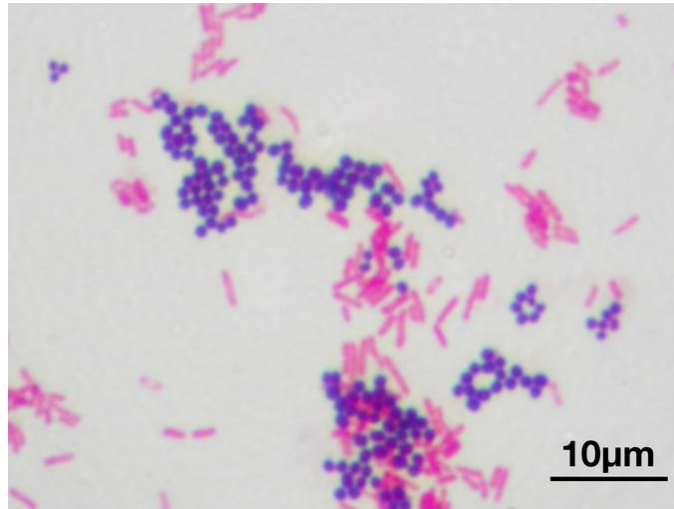


Figure: **Gram Stain:** This is a microscopic image of a Gram stain of mixed Gram-positive cocci (*Staphylococcus aureus*, purple) and Gram-negative bacilli (*Escherichia coli*, red).

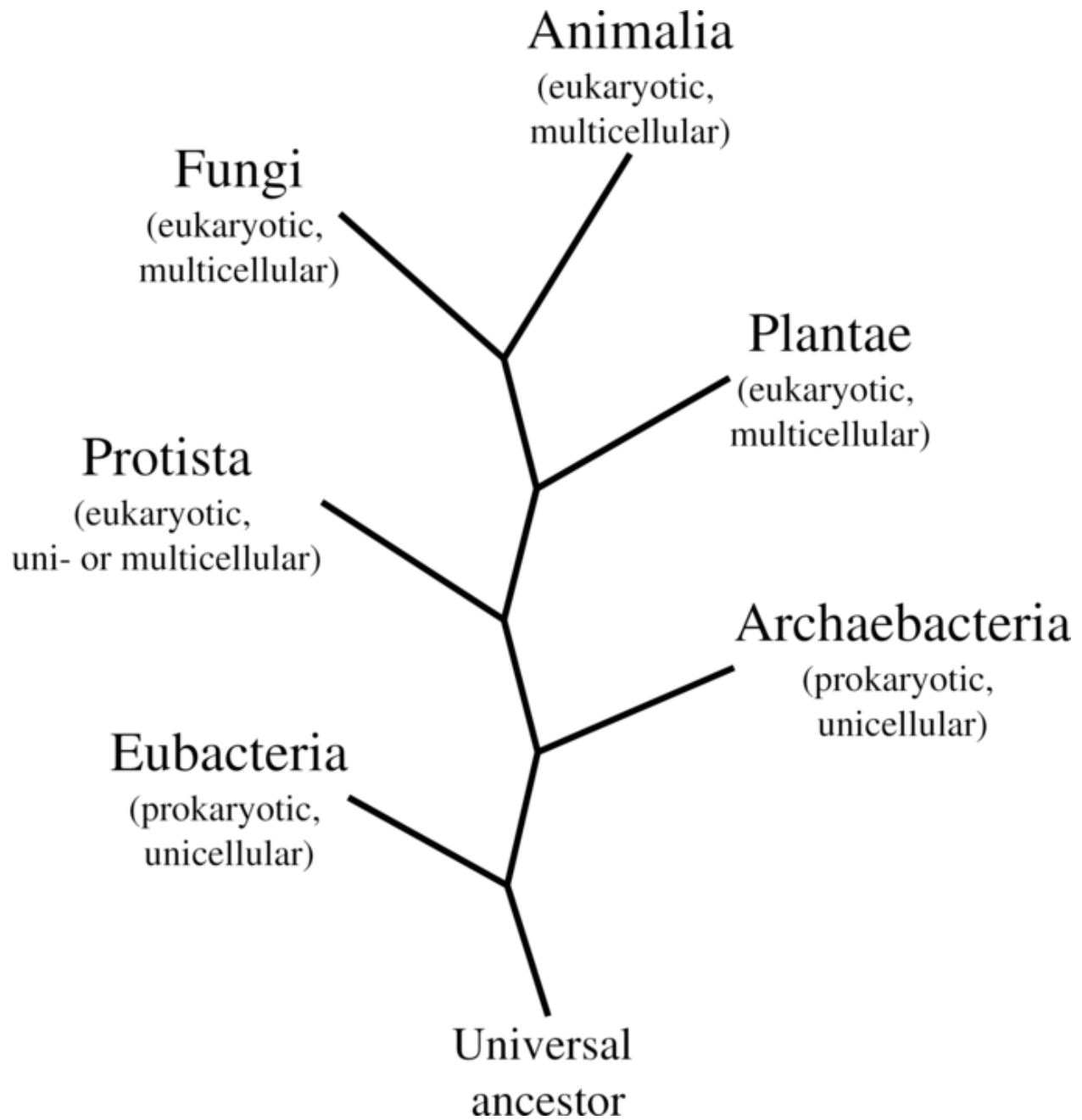


Figure: **Types of microorganisms:** This tree of life shows the different types of microorganisms.

Aquatic Microbiology

Definition

Aquatic microbiology is the study of microorganisms and their activities in fresh and marine water including rivers, lakes, springs and sea.

It is the study of microorganism's viruses, bacteria, algae, protozoa and microscopic fungi which inhabit these natural waters. Some of these microorganisms are indigenous to natural bodies of water other are entering to water from soil or from industrial or domestic wastes.

Importance

Aquatic microorganisms and their activities have great importance in many ways.

1. They may affect human health and other animal life. They are responsible for many diseases like typhoid, cholera, dysentery, campylobacter enteritis.
2. They occupy a high position in food chain by providing rich nourishment for the next higher level of aquatic life..
3. They are instrumental in the chain of biochemical reactions which involve recycling of element. e.g. mineralization of organic compounds i.e. the dissimilation of organic compounds to carbon dioxide , water and various inorganic salts
4. The natural water is major food source.

Bacteriological evidences of pollution

Analysis of water sample on a routine basis is not possible. Each pathogen required detection .Therefore water is examined to detect E. coli the bacterium which indicate fecal pollution.

The coliform group of bacteria includes all aerobic and facultative anaerobic, gram negative, non spore forming bacilli that produce acid and gases from fermentation of lactose. These are normal inhabitants of large intestine of humans and other animals and are present in feces.

Thus presence of any of these bacterial species in water is evidence of fecal pollution of human and animal region. Both qualitative and quantitative methods are used for checking quality of water.

Qualitative analysis of water

Three basic tests to detect coliform bacteria in water are

- Presumptive test
- Confirmed test
- Completed test

- **Presumptive Test**

Presumptive test is screening test for detection of coliform bacteria. Measured portion of water to be tested are added to lactose fermentation broth containing an inverted glass vial (durum tube). Tubes of lactose media are inoculated with 10ml, 1ml and 0.1ml portions of water sample. Development of gas in any of tube in presumptive test is evidence of presence of coliform bacteria in sample.

If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe.

- **Confirmed Test**

It requires selective and differential media such as Brilliant green lactose bile media (BGLB) and Eosin methylene blue.

Streak form a lacto positive broth tube obtained from presumptive test on Eosin methylene blue. E. coli produced dark small colonies almost black centered with greenish metallic shine. Enterobacter produce large pinkish colonies.

Brilliant green lactose bile media (BGLB)

This medium inhibits the growth of lactose fermenters other than coliforms. Thus gas fermentation in BGLB confirms that coliforms are present.

- **Completed test**

It is final analysis of water sample and isolated colony is picked from plate and inoculated in tube of lactose broth and streaked on nutrient agar slant to perform a gram strain.

RESULT

Fermentation of lactose broth and demonstration of gram negative bacilli shows a positive confirmatory test.

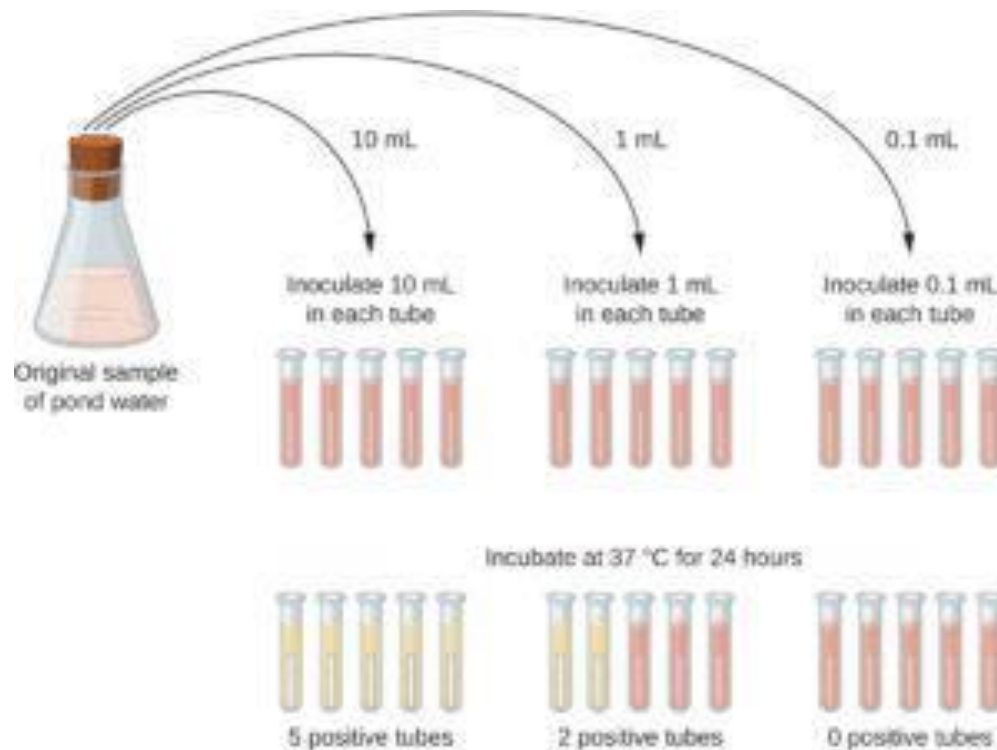
Quantitative analysis of water

Most Probable Number (MPN) is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in soils, waters, agricultural products and is particularly useful with samples that contain particulate material that interferes with **plate count enumeration methods**.

Principle

Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilize the lactose present in the medium to produce acid and gas. The presence of acid is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction (i.e both color change and gas production) and comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical tables.

Procedure of the test

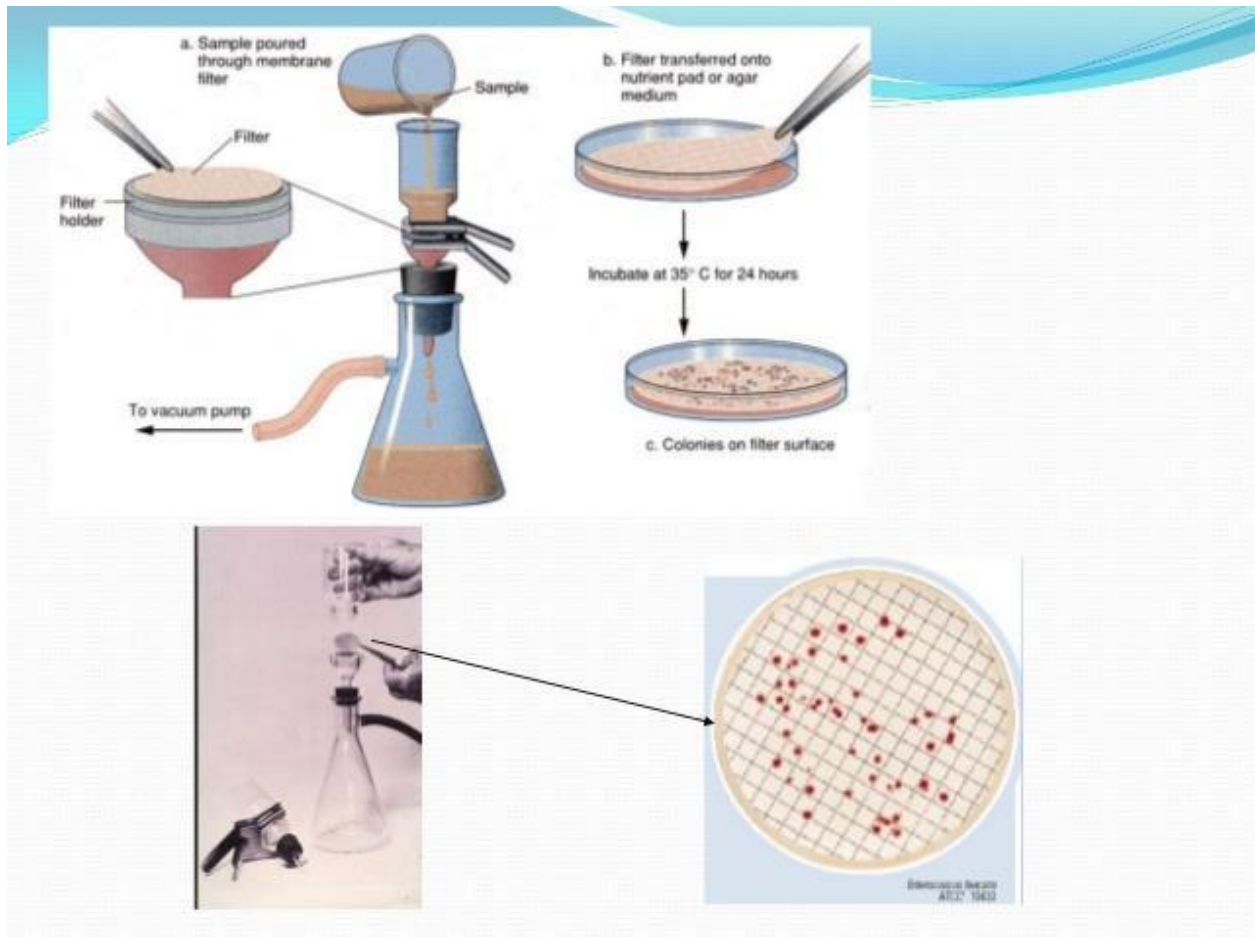


MPN values per 100 ml of sample and 95% confidence limits for various combinations of positive and negative results (when five 10-ml, five 1-ml and five 0.1 ml test portions are used)

No. of tubes giving a positive reaction :			MPN (per 100 ml)	95% confidence limits	
5 of 10 ml	5 of 1 ml	5 of 0.1 ml		Lower	Upper
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46

- **Membrane Filter Method**

A measured volume of water sample to be tested is drawn through the filter disc. The bacteria are retained on the surface of membrane. The filter containing trapped microorganism transferred to a sterile Petri dish containing media after incubation colonies will developed, count them. The number of colonies shows the number of bacteria in water sample.



Distribution of aquatic microorganisms

Microorganisms in an aquatic environment may occur at all depths ranging from surface region to very bottom of ocean. The surface layer and bottom sediments have the highest concentration of microorganisms.

- **Plankton** The aggregation of floating microbial life in surface region of aquatic ecosystem is called plankton. Plankton may be composed primarily of algae called phytoplankton or it may be predominantly protozoan's and another minute animal life called zooplankton.
- **Benthic microorganism**
Microbial inhabitants of bottom region of body of water are referred as benthic microorganisms. It is the richest of an aquatic ecosystem in terms of number and kind of microorganisms.

Problems of unsafe drinking water

Drinking water of most communities is obtained from the surface source such as rivers streams and lakes. Such natural water suppliers particularly streams, lakes and rivers are likely to be polluted with industrial and domestic water. Polluted water contains vast amount of organic matter that serves as excellent nutritional source for growth and multiplication of microorganisms. These pathogens are responsible for intestinal infections such as dysentery, cholera, typhoid, fever and parathyroid fever.

WHO (World health organization) estimates that 1.7 million deaths per year results from unsaved water supply. Most diarrheal diseases and 90% of these deaths occur in children living in under developing countries where potable water at minimum .In addition to bacterial infection unsaved water suppliers are responsible for numerous parasitological infections. These parasitic protozoa are *Entamoeba histolytica*, *Blantidium coli*. Some bacterial pathogens are *Salmonella typhi*, *Vibrio cholerae*.

These are enteric pathogens. They cause infection in intestine and they live in water or aquatic ecosystem.

Microbiology of Water

The Essentialness of Water

- Blood in our veins approximates composition of sea water
- Concept of *hydrophilic* and *hydrophobic* nature of biological molecules
- These molecules determine shape of biological molecules and thus decide the specificity of all living processes

Essential for All living organisms

Water covers 70% of the world



All Microbes Live in an Aqueous Environment

- Ecology of aquatic environments is complex
- Most aquatic environments are teeming with life
- Microbes have evolved to live in:
 - Saturated salt solutions
 - Below freezing to $>110^{\circ}\text{C}$

Waters full of toxic substance , i.e. copper, cyanide, lead, silver, gasoline, oil, benzene, and many others



Terminology

- **Potable** - (clean) water – free of all **objectionable** material, including pathogens, tastes, odors, colors, toxins, radioactive material, organisms, oils, gases, etc.
- **Fresh** – non-salt or sea water
- **Pollution** – anything that makes it Non-Potable
- **Sewage** – the community waste or garbage that mother nature and we dump onto sewers or land
- **Drinking Water**
 - No coliforms contamination acceptable
- **Recreational water**
 - 200 fecal coliforms /100 ml
- **Fish and wildlife habitat**
 - 5000 fecal coliforms/100 ml
- **Shellfish**
 - 14 fecal coliforms/100 ml

Table: Bacteria Found In Surface Water

Bacteria	Disease/ infection	Symptoms
<i>Aeromonas</i>	Enteritis	Very thin, blood- and mucus-containing diarrhea
<i>Campylobacter jejuni</i>	Campylobacteriose	Flue, diarrhea, head- and stomachaches, fever, cramps and nausea
<i>Escherichia coli</i>	Urinary tract infections, neonatal meningitis, intestinal disease	Watery diarrhea, headaches, fever, homiletic uremia, kidney damage
<i>Plesiomonas shigelloides</i>	Plesiomonas-infection	Nausea, stomachaches and watery diarrhea, sometimes fevers, headaches and vomiting
<i>Typhus</i>	Typhoid fever	Fevers
<i>Salmonella</i>	Salmonellosis	Sickness, intestinal cramps, vomiting, diarrhea and sometimes light fevers
<i>Streptococcus</i>	(Gastro) intestinal disease	Stomach aches, diarrhea and fevers, sometimes vomiting
<i>Vibrio El Tor</i> (freshwater)	(Light form of) Cholera	Heavy diarrhea

Pathogens of Most Concern on Fresh Produce

- *Salmonella*
- *Shigella*
- *Escherichia coli*
- *Campylobacter*
- *Yersinia enterocolitica*
- *Staphylococcus aureus*
- *Clostridium* species
- *Bacillus cereus*
- *Vibrio* species
- Viruses (Hepatitis A, Norwalk)
- Parasities/protozoa

Waterborne Infectious Disease (U.S. 1997-1998)

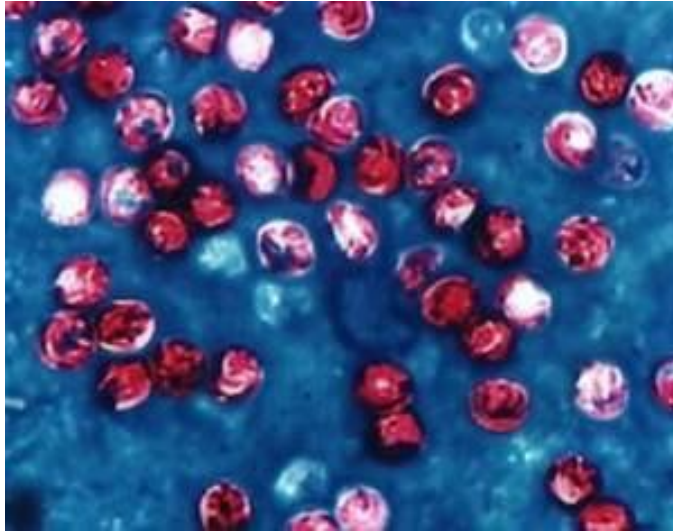
<u>Disease</u>	<u>Agent</u>	<u>Outbreaks</u>	<u>Cases</u>
• Shigellosis	<i>Shigella sonnei</i>	1	183
• Giardiasis	<i>Giardia lamblia</i>	4	159
• Cryptosporidiosis	<i>Cryptosporidium parvum</i>	2	1432
• Gastroenteritis	<i>E. Coli 0157:H7</i>	3	164
• Acute gastrointestinal illness	Unknown	5	163

Table: Protozoa Found in Surface Water

Microorganism	Disease	Symptoms
<i>Amoeba</i>	Amoebic dysentery	Severe diarrhea, headache, abdominal pain, chills, fever; if not treated can cause liver abscess, bowel perforation and death
<i>Cryptosporidium parvum</i>	Cryptosporidiosis	Feeling of sickness, watery diarrhea, vomiting, lack of appetite
<i>Giardia</i>	Giardiasis	Diarrhea, abdominal cramps, flatulence, belching, fatigue
<i>Toxoplasma gondii</i>	Toxoplasmosis	Flu, swelling of lymph glands With pregnant women subtle abortion and brain infections

Giardiasis and Cryptosporidiosis

- Both are protozoans
- Transmission through water (97% of all surface water carry cysts)
- Resistant to chlorine, but can be filtered



Bacterial-Indicator Organisms Common Groups

- Coliforms
 - Total coliforms
 - Fecal coliforms
 - *Escherichia coli*
- Streptococci
 - *fecal streptococci*
 - *enterococci*
- Spore Formers
 - *Clostridium perfringens*

Indicator Organisms

- **General coliforms** – indicate water in contact with plant or animal life (universally present)
- **Fecal coliforms** – mammal or bird feces in water
- **Enterococcus bacteria** (type of fecal streptococci)– feces from warm blooded animals in water

Some Factors Affecting Ratio of Indicator Organisms to Pathogens

- Feces from human populations with higher infection rates are of greater concern
- All treatment methods and environmental conditions affect pathogens and indicators differently
- Chlorinated water may have zero indicators and pathogens, but loaded with viruses.
- Pathogens can “hide” from treatment inside suspended solids.

The ratio of indicators to actual pathogens is not fixed

Direct Tests For Pathogens

- Involves selective cultivation to large numbers
 - Time consuming
 - Expensive
 - Potentially dangerous to lab personnel
- Molecular tests

- Require testing for each pathogen
- Expensive
- Require expertise

Viral Sources of Waterborne Disease

- Hepatitis A: inflammation and necrosis of liver
- Norwalk-type virus: acute gastroenteritis
- Rotaviruses: acute gastroenteritis, especially in children
- Enteroviruses: many types affect intestines and upper respiratory tract
- Reoviruses: infects intestines and upper respiratory tract



Virus Detection

Very difficult and costly

- Electron microscopy
- Immunoassays
- Cell cultures
- Reverse transcription-polymerase chain reaction (RT-PCR)

Chlorination of Water



The most commonly used sanitizer!

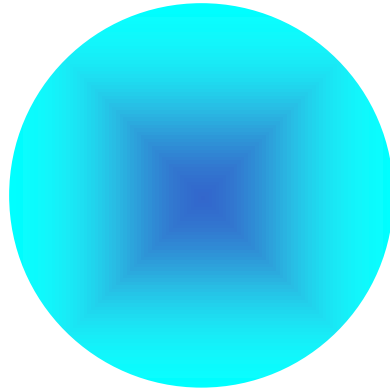
Methods of Treatment

- Shock Chlorination (50-100 ppm, contact of at least 6 hours)
- Continuous Chlorination – for recurring bacterial contamination problems – a measurable amount of free residual chlorine

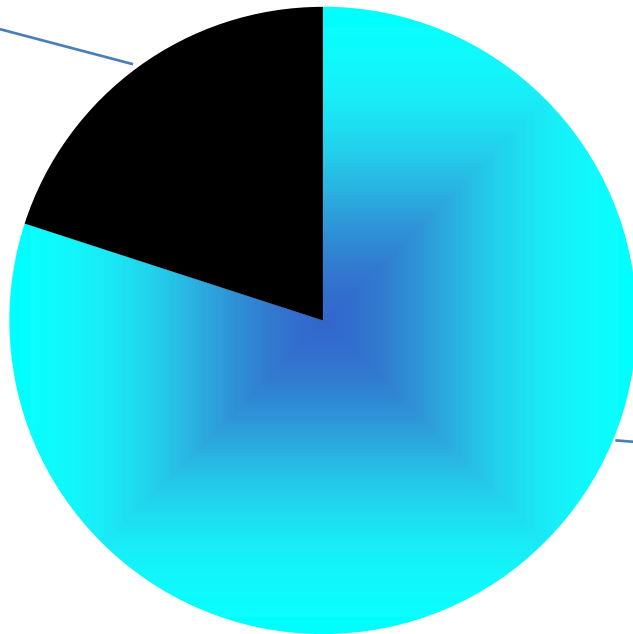
Chlorine Terms

- Chlorine Dosage – total added
- Chlorine Demand - inorganic
- Combined Residual Chlorine - organic
- Free Residual Chlorine

Chlorine dosage



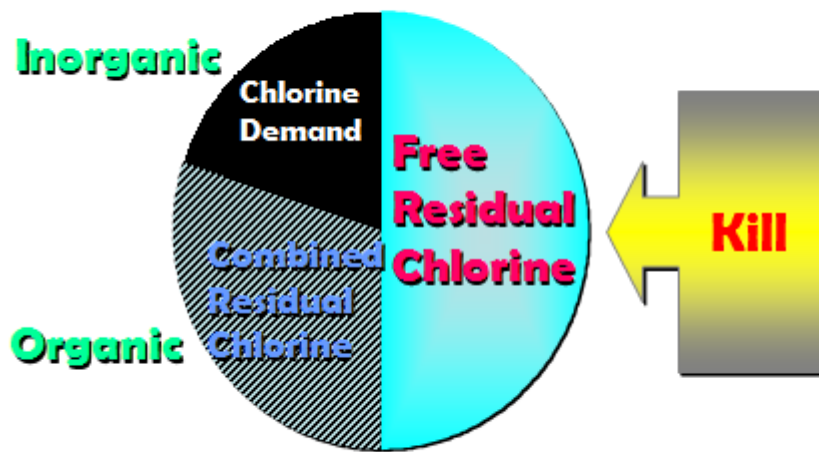
chlorine demand



residual chlorine



Chlorine Dosage



Free Residual Chlorine

- Chlorine remaining after combining with organic matter
- Bacteria kill rate proportional to concentration of free residual



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