Microbiology

Introduction

The science of microbiology encompasses a wide range of different life forms. The one unifying factor is that microbes can only be observed easily using some visual enhancement device, such as a microscope. Literally, microbiology means the study of very small life forms, those that carry out all life processes within a single cell.

After reading this chapter, you should have an understanding of the problems associated with microbiological fouling in cooling water systems. You should also have an understanding of how to best treat those systems so that microbiological problems are minimized.

Species

Bacteria (singular: bacterium) are single-celled organisms that have a simple structure. All life processes are carried out within a single cell, which is generally only 1-5 m m in length (1 m m = approximately 1/25,000 inch). The *fungi* (singular: fungus) are filamentous organisms, much larger than bacteria. They produce a network of interlacing filaments. *Algae* (singular: alga) are photosynthetic organisms; like green plants, they require sunlight to provide the energy for growth, using specialized pigments called *chlorophylls* to trap the light energy. All *Protozoa* (singular: protozoan) are single-celled organisms. They prey on bacterial and algal cells and generally thrive where large populations of these organisms exist. Protozoa are generally not a problem in industrial water systems, although their presence indicates a thriving bacterial and/or algal population

Microbial Metabolism

All living organisms require three elements-carbon, nitrogen, and phosphorus-to build cells and generate energy. In addition, all organisms need to be surrounded by water in order to take up soluble nutrients across the cell membrane and dispose of waste materials. Without some form of water layer, the microorganisms would be unable to perform this function and would be unable to grow. For example, microbes cannot reproduce in water-free diesel oils or in dry environments. Microbes might survive in such environments for many years, but they will be unable to grow.

The presence of carbon, nitrogen and phosphorus compounds in an aqueous environment causes an immediate increase in microbial growth, if other environmental factors are suitable. Phosphorus is the limiting nutrient in many environments; an increase in this element causes a rapid increase in microbial growth.

Certain other elements (for example, iron, sulfur and zinc) are needed only in trace amounts as part of enzyme systems responsible for the breakdown of carbon compounds. However, many bacteria can also use these as a source of energy. For instance, iron bacteria use iron in this way.

Environmental Growth Factors

The factors that determine the extent of microbial growth are:

1. Water

2. Oxygen

3. pH

4. Temperature

5. Pressure

6. Dissolved solids

Water

Water must be present for all living organisms to grow. The nutrients necessary for growth are transported through the cell membranes of microorganisms in aqueous solution. Similarly, the waste products are transported out of the cells in solution. Water is the most convenient way of transporting chemicals around the cells. Microorganisms can survive in the absence of water (in the case of spores, for many years), but water is essential for growth and metabolism.

Oxygen

Most microorganisms grow in the presence of oxygen, using it as an electron acceptor for the oxidation of organic and inorganic compounds. This is known as respiration, and the organisms that use oxygen this way are called *aerobic.* Some species of microorganisms can grow in the absence of oxygen, using fermentation of organic compounds as a source of energy. These organisms are described as *facultatively anaerobic.* The small number of microorganisms that are able to break down organic compounds or carbon dioxide only in the absence of oxygen are described as *anaerobic.* Oxygen is actually toxic to many of these organisms. Anaerobic metabolism is commonly found in many bacterial and fungal species.

Respiration generates more energy for the organism than anaerobic fermentation and results in greater microbial growth. In most water systems this means an increase in the breakdown of organic compounds and the accumulation of extensive products of growth, such as slimes.

pH

The pH level has a pronounced effect on the type and extent of microbial growth. The range for microbial growth extends from pH 1.0 (extremely acid) to pH 11.0 (extremely alkaline), with the majority of species found between pH 4.0 and pH 8.0. This is the pH range in which the majority of microbial enzymes (responsible for energy conversions) are most active.

Microorganisms, because of their quick growth and reproductive rates, can readily adapt to changes in the pH environment. Many bacteria growing at neutral pH values (7.0) can readily adapt to growing in acidic or alkaline environments. The same is true of the fungi and algae, although the range of pH for effective growth is somewhat different. Table 4.1 illustrates the range of pH for active microbial growth and the types of organisms involved.

TABLE 4.1

Range of pH for Microbial Growth

Bacteria	Fungi	Algae
pH 1 to 4 Few species (e.g., sulfur-oxidizing Bacteria)	pH 1 to 5 Many species (e.g., molds)	pH 1 to 5 Very few species
pH 4 to 8 Majority of species	pH 4 to 7 Majority of species (e.g., molds and yeasts)	pH 5 to 9 Majority of species
pH 8 to 11 Few species (spore-formers)	PH 7 to 8 Few species, (e.g., molds)	pH 9 to 11 Few species

Temperature

Microorganisms have a tremendous capacity for adapting to extremes of temperature. They can be roughly divided into three groups by their temperature tolerances.

TABLE 4.2

Range of Temperature for Microbial Growth

Psychophilic	**Mesophilic**	**Thermophilic**
50-68 ° F (10-20 ° C) Few species, mainly bacteria and algae	68-104 ° F (20-40 ° C) Majority of species	104-167 ° F (40-75 ° C) Few species of bacteria and algae; Very few fungi

Pressure

Most microorganisms are adapted to living under atmospheric pressure; a large increase or decrease in pressure can result in death of the cells. The effects of high temperature and pressure are related; at higher pressures many bacteria are able to survive and grow at high temperatures that would normally inhibit their growth.

Dissolved Solids

High levels of dissolved solids cause an osmotic effect on microbial cells. Water is drawn from inside the cells through the semipermeable cell membrane towards the outer salt solution, setting up an osmotic pressure. The greater the difference between the concentration of salts inside the microbial cell and those outside the cell, the greater the osmotic pressure. Eventually, water is drawn from inside the cells, causing death of the cells by shrinkage, or lysis.

Microorganisms in Cooling Systems

Biofouling

The term biofouling refers to organic debris that accumulates as a result of the growth of various organisms. Inorganic and organic particulate matter, such as iron oxides and hydrocarbon oil droplets, may become trapped within this biofouling layer, increasing its volume. Once this combined-fouling layer has been formed, conditions are favorable for corrosion processes.

Biofouling Development

The development of a biofouling layer on a surface depends upon the initial attachment of bacteria [o this surface, their eventual increase in number and the development of a protective slime layer, or glycocalyx. The attachment of microorganisms to surfaces has a survival function. Microorganisms such as bacteria, floating free in an aquatic environment, are surrounded by a layer of water which supplies them with all their nutrients and carries away their waste products. These free-floating bacteria are known as *planktonic bacteria* and, in this state, they are in contact with only a finite amount of nutrients dissolved in the water that surrounds them.

If the water itself had very few dissolved organic nutrients in it, the growth and reproduction of bacteria would be a very slow process. Attachment of bacteria to surfaces shows a great advantage; attached bacteria, known as *sessile or adherent bacteria,* become exposed to a plentiful supply of fresh water containing fresh nutrients that they can extract continuously.

Another advantage that sessile bacteria have is their ability to adsorb dissolved organic molecules (food sources for many bacteria) from the water layer. This attachment or adsorption is facilitated by weak ionic forces. For example, positively charged amine molecules readily adsorb to a negatively charged surface (most surfaces tend to build up a negative charge).

The sessile bacteria thus have a plentiful supply of nutrients awaiting them if they become attached to the surface. Initially, this is a difficult task. Bacteria, being very small particles, usually carry negative charge, so they tend to be repelled by the negative charges on the surfaces. However, most surfaces are rough, with small hidden crevices and pits that can trap small organisms such as bacteria. The rate at which the bacteria contact these surfaces is dependent on physical properties such as size, shape and density. Some bacteria can actively migrate by swimming, using small hairs called flagella, until they find a suitable area of the surface.

Water velocity also determines how many organisms become attached to the surface. Higher water velocities lead to removal of attached bacteria. Settling of bacteria by gravity is negligible in agitated waters. This microbial slime buildup tends to be much more pronounced in stagnant or slowly moving waters.

Having established themselves in a nutrient-rich environment, the bacteria can grow and reproduce so that large numbers of them ultimately become attached to the surface. Then, to protect themselves and their environments, they produce a gelatinous capsule that surrounds each cell that binds one cell with another. These gelatinous capsules, called slime layers, are produced by the bacteria as a protection against predators, such as viruses and protozoa. The slime layer may also act as a supply of added nutrients in times of stress, since it commonly consists of long-chain polysaccharides or protein/polysaccharide complexes.

The stickiness of the slime layer allows the bacteria to attach to each other, so the slime layer increases in size and the bacteria become firmly attached to the surface. Eventually, shear forces from the flowing water limit the thickness of the slime layer by breaking off large clumps. This slime film acts as an attachment or trap for other particulates in the flowing water.

Eventually, the biofilm may also incorporate other larger organisms, such as fungal spores, filaments (the fungi growing within the biofilm) and algal cells trapped by the sticky film. The biofilm may also increase and attract protozoa that feed on the bacteria, fungi and algae. The biofilm may grow to become as much as 1-2 mm thick, depending upon water velocity.

Biofouling may lead to oxygen depletion (anaerobiosis) in the lower layers of the film, because respiring organisms use oxygen faster than it can diffuse into the film from the surrounding water. Under these conditions, bacteria that require oxygen-free conditions to grow can start to develop. These include certain bacteria that can grow in both oxygenated and deoxygenated conditions (known as facultative anaerobic bacteria). Examples are the coliform bacteria, such as *E coli,* and certain species of *Pseudomonas.*

Other bacteria are strictly anaerobic, growing well only in the absence of oxygen. These include certain species of sulfate reducing bacteria (S.R.B.).

When a fouling problem with a cooling water system is being investigated, a value judgment must be made as to whether microorganisms are the initiators of the deposit, because inorganic and organic contaminants may also precipitate from the flowing water and lead to fouling problems. A chemical and microbiological analysis of the deposits will help decide this point.

Problems in Cooling Tower Systems

Growth of algae primarily occurs in the tower deck area because of ample sunlight; algae require sunlight to grow, and many types contain the green pigment chlorophyll, which traps the energy from sunlight. Algae are generally introduced from the atmosphere as spores. They readily become established in areas where there is plenty of sunlight, such as the top of the deck and at the sides of the tower. Or they may be introduced in the makeup water, if it is untreated river or lake water.

Algae produce mainly green or blue-green gelatinous slimes, which may be long and filamentous or loose and flocculent. The slime species most commonly found are the green algae, the cyanobacteria (originally the blue-green algae but now classified as bacteria) and the diatoms, which have a skeleton of silica. Prolific algal growth may produce green or blue-green scums in some cooling tow7er sumps.

Algae slimes can plug distribution nozzles and troughs in the cooling tower deck, causing poor water distribution across the tower and hence reduce cooling efficiency. Water intake screens may also become plugged by algal slimes sloughed off from the tower. The growth of algae may provide a food source that encourages the growth of other organisms, such as bacteria and fungi.

Another group of aerobic bacteria that may cause problems in cooling systems are iron bacteria. These are commonly found in well waters that contain soluble iron and larger amounts of carbon dioxide. The bacteria oxidize the soluble iron (producing a precipitate of brown iron oxide) as a source of energy, and they use carbon dioxide as a source of carbon for cell growth. They generally produce slimy red-brown flocs of hydrated ferric oxide that can cause plugging of filters and heat exchangers.

Brown slimes produced by iron bacteria should not be confused with brown precipitates of iron oxide from corrosion products. One important point to remember is that iron bacteria are mainly found in well waters.

Yellow stringy slimes may also be caused by sulfur-oxidizing bacteria. These bacteria oxidize sulfur or reduced sulfur compounds, such as H2S, to produce precipitates of sulfur and eventually sulfuric acid, which reduces the pH of the water. The sulfuric acid produced by the sulfur bacteria may cause slime production and corrosion of concrete basins. This group of bacteria is mainly found in refinery cooling towers contaminated with reduced sulfur compounds.

The most insidious problem caused by the growth of fungi is the attack on cooling tower wood, which can go unnoticed for many years. Molds and yeasts can break down the cellulose in the wood, leading to structural weakening. This delignification process can be detected by laboratory analysis. A simple field test is to use a sharp object, such as an ice pick. to poke into tower lumber. If the lumber is easily pierced, structural weakening is probably present.

Methods of Control

Problems associated with biofouling have necessitated the creation of slime control programs. A slime control program generally involves the use of chemicals known as biocides and certain surfactant chemicals known as biodispersants.

Biocides are chemicals that kill living organisms. A biostat is a chemical that inhibits the life processes of living organisms without actually killing them. It is possible for a chemical to be a biostat to certain organisms at a low concentration and a biocide to the same organisms at a higher concentration. Chemicals that are added to cooling systems as biocides are generally added at high levels, over a definite time period, e.g., 6-12 hours. This is known as slug addition or shock dose. Chemicals designed to act as biostats are generally added continuously to the system at very low levels.

Organic biocides are classified as non-oxidizing biocides because they do not oxidize other chemicals or oxidize them only very slightly. The inorganic biocides are classified as oxidizing biocides (e.g., chlorine) since they very strongly oxidize many organic and inorganic molecules, becoming reduced themselves. The biocides and biostats work by affecting various parts of the microbial cell.

Another class of chemicals that can be used to remove microbial slimes are surfactants commonly known as biodispersants. These chemicals interfere with the surface tension of water. They thus allow other chemicals to contact surfaces and deposits to some extent. It has been found that biodispersants increase the effectiveness of biocides by allowing the biocides to penetrate the microbial slime, killing the microbial cells and eventually causing sloughing of the slime. Ouaternary ammonium salts and amine salts, being cationic, attach to the negatively charged molecules in the slime layer and reduce the surface tension, thus allowing water to contact the slime layers to a greater extent.

Eventually, the slime layer breaks loose and the water flow takes the slime layers away. The addition of an oxidizing biocide helps break them up further by oxidizing the exposed charged atoms on the slime layers.

The oxidizing inorganic biocides, such as chlorine, may be fed on a continuous basis to maintain a free residual level of 0.1 -0.5 ppm or a total residual level of 0.5-1.0 ppm. Free residual chlorine, present as hypochlorous acid, is a much more effective and faster-acting biocide than combined chlorine, present as chloroamines, e.g., NH2CI. Because microorganisms find it difficult to adapt to chlorine, it is possible to feed chlorine on a continuous basis.

The main drawback with the oxidizing biocides is their high level of reactivity to reduce compounds such as iron and H2S and organic matter (there is little residual left to control microorganisms). They do not effectively penetrate and disperse microbial slimes. These facts generally mean that chlorine is supplemented by the addition of nonoxidizing biocides and dispersants in many systems.

Examples of useful oxidizing biocides are halogens such as chlorine (CI_2), chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), ozone and chlorine, bromine donors such as chlorinated isocyanurates and hydantoins.

Oxidizing Biocides

Chlorine initially dissolves in water to form hypochlorous acid (HOCI) and hydrochloric acid (HCI).

Cl2 + H2O = HOCl + HCl

The production of acids leads to a drop in the pH of the water. The oxidizing biocides can react with many minerals, such as ferrous iron, as well as organic matter, producing precipitates of iron oxide and organic matter. The reaction with organic matter is much slower than with inorganic ions. This explains why the chlorine demand of waters containing organic acids (e.g., humic and fulvic acids) gradually increases over time. Chlorine, supplied as a liquid in pressurized cylinders or as a 10-15% solution in water (sodium hypochlorite NaOCI) stabilized with sodium hydroxide, is the most commonly used oxidizing biocide because of its low cost and biocidal activity at very low levels.

When chlorine is added from cylinders it forms a gas that combines with water to form hypochlorous and hydrochloric acids. This is an equilibrium reaction, which means that chlorine gas may be formed by the addition of excess HCI. The formation of Cl_2 generally occurs at pH levels below 4.0.

At pH levels around neutral, chlorine is present mainly as HOCI; and as the pH increases, the proportion of HOCI to Cl_2 increases. At pH values above 7.0 HOCI dissociates as follows:

$HOCl = OCL^- + H^+$

This is also an equilibrium reaction; it may be forced to the left to produce more HOCI by adding excess H^+ ions (in the form of acid), which will also decrease the pH. At pH 4.0 most of the chlorine is present as HOCI, whereas at

pH 10.0 most of the chlorine is present as OCI- ions.

At pH values above 7.0 most of the biocidal activity of chlorine is provided by the HOCI molecule. The hypochlorite ion evidences little biocidal activity; it acts more as a biostat in high pH waters. This explains why chlorine is much less effective at pH values above 8.0 in cooling systems, although continuous chlorination may provide enough OCI- and chloroamines at these pH levels so that microbial growth is inhibited.

Chlorine may also be supplied in convenient forms such as sodium hypochlorite (a 10-1 5% solution of NAOCI in water) and calcium hypochlorite (a solid containing 70% $Ca(Ocl)_2$)

It should be kept in mind that the addition of hypochlorites may increase the pH of the cooling water (due to the formation of NAOH), whereas the addition of chlorine gas may reduce the pH (due to the formation of HCI).

Chlorine Dioxide (ClO_2)

Chlorine dioxide has more oxidizing power than chlorine (about 2.5x) and is generally more effective against microorganisms at pH levels above 8.0. The main advantage of using ClO_2 in cooling water treatments is its nonreaction with phenols and ammonia and its much lower tendency to form the potentially toxic trihalomethanes when it is in contact with soluble organic matter.

Halogen-Release Biocides

The halogen-release biocides are organic biocides that release chlorine and/or bromine in contact with water. For example, chlorinated isocyanurates release chlorine and hydantoins release chlorine and bromine. These products are solid forms of halogens, and they have the advantage of added stability. The hydantoins have particular advantages, because the addition of chlorine and bromine means that lower residuals are generally effective (bromine being more effective at high pH than chlorine). Bromine chloride is a liquid that releases bromine on contact with chlorine, again allowing lower residuals to be used.

Hydrogen Peroxide (H_2O_2)

H_2O_2 is a very strong oxidizing agent and an effective biocide at very low levels over a wide pH range. The product actively generates oxygen from solution when reacted with certain organic matter. H_2O_2 is generally not used for the treatment of cooling waters because of its high cost and reactivity. It reacts with most materials in cooling systems (such as wood and metals), so that very little residual is left in large systems.

Nonoxidizing Biocides

Nonoxidizing biocides are organic molecules that react with various parts of the microbial cell, generally at a specific site. They do not oxidize cell components. The most common groups of nonoxidizing biocides are aidehydes, organo-sulfur biocides, organo-nitrogen biocides, amine salts, and heavy metals.

Aldehydes

The most common forms of these biocides are formaldehyde and glutaraldehyde. Formaldehyde is generally not used in cooling systems, because many bacterial species readily adapt to its presence (some bacteria grow well in the presence of up to 3000 ppm of formaldehyde) as well as using it as a source of carbon for cell growth. This is the reason it is used as a preservative at high levels (1 0%). Glutaraldehyde, a much more effective biocide than formaldehyde, can be used effectively in small systems. It is especially effective against sulfate-reducing bacteria and algae, but less effective against aerobic bacteria and fungi.

Organo-Sulfur Biocides

This large group of biocides includes isothiazolones, methylene bisthiocyanate, bistrichlorosulfones, and carbamates.

lsothiazolones are very effective against aerobic bacteria, fungi and algae. They are less effective against sulfate-reducing bacteria (due to reaction with sulfides). It has been found that the isothiazolones are much more effective in cooling systems when used in conjunction with chlorine.

Methylene bisthiocyanate is generally effective against aerobic bacteria and fungi at pH levels below 7.5. It is much less effective against algae and sulfate reducing bacteria.

Bistrichlorosulfones are not very effective biocides in cooling water treatments. They have some effect against aerobic bacteria, but are less effective against S.R.B., fungi and algae.

Carbamates are very effective fungicides, but less effective against aerobic bacteria, S.R.B. and algae. Carbamates become much more effective against aerobic bacteria when used in water containing high levels of iron and zinc and heavy metals such as chromium. This is due to hydrolysis of biocidal products, which are catalyzed by metal ions. These products are more effective at high pH levels and are slow-acting.

Organo-Nitrogen Biocides

This is a large group of biocides and contains bromonitrostyrene and guanidines. Bromonitrostyrene is an effective bactericide, but it is less effective against other organisms. Its main disadvantage is its high toxicity (creating toxic fumes and handling problems) and its low water solubility.

The guanidine biocides, which include such products as dodecylguanidine salts, are effective bactericides and algaecides but are less effective fungicides. Many of these products are cationic (positively charged) molecules that may cause severe foaming problems. They become adsorbed to many surfaces and may be lost to the system. They are good surfactants and have many properties in common with quats and amines.

Amine Salts

The amine salts are cationic biocides and are good surfactants. Examples include quaternary ammonium salts and diamine acetate salts. The quats are effective against aerobic bacteria, S.R.B. and algae, but less effective against fungi. Polyamine salts are effective against aerobic bacteria and S.R.B., but less effective against algae and fungi. Both quats and amines are fast-acting biocides: they are generally more effective in combination with chlorine.

Heavy Metals

Heavy metals include copper salts and tin salts. Copper salts are good algaecides but very poor fungicides and bactericides. They may cause corrosion if used in cooling systems. They are generally added to ponds and lakes to control algae.

Tin salts are good general-purpose biocides. Formulated with quats to increase their dispersability, they have been found effective against S.R.B., fungi and algae. They are less effective against aerobic bacteria. These biocides have proven useful for the control of fungal wood rot.

Methods for the Detection and Enumeration of Microorganisms

The most common field methods for the detection and enumeration of microorganisms are as follows:

1. *Plate Counts,* using a solid agar nutrient medium as the growth substrate in Petri dishes, have been used for many years for identifying and enumerating bacteria and fungi. More recently the introduction of Petrifilm Plates has made on-site plate counts much easier.

2. *Dipslides.* These use an agar nutrient medium placed on a plastic tab or slide. Counts are obtained by dipping the slide in the water sample and then incubating the slide for 24 or 48 hours. Numbers of microorganisms are estimated by comparing the slide to a standard chart. This method is only semi-quantitative.

3. *Adenosine Triphosphate (ATP) Analysis.* A measurement of A.T.P. levels provides some indication of the total biomass contained within a water mass. Measurement of A.T.P. will not distinguish between the types of cells contributing to the biomass, e.g., bacteria, fungi or algae.

Biocide Kill Test Procedure

The aim of a kill test procedure is to select a biocide that will produce good microbial control within an industrial water system at levels that are cost-effective.

The residence time or Holding Time Index (HTI.) within the cooling system should be determined beforehand. Various biocides should be tested within the system at various concentrations, using an exposure time equivalent to the residence time of the system. Thereafter, the number of surviving microorganisms can be determined using plate counts, dipslides or A.T.P. analysis.

The selected biocide should also be compatible with other chemical treatments such as scale inhibitors, corrosion inhibitors and dispersants