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CHAPTER ONE

Water Chemistry and Microbiology

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1. WATER CHEMISTRY

1.1 Water Molecule

Water is a simple molecule that has only three atoms, but its unique properties make it the most important molecule in life. This extraordinary importance of water molecule came basically from the arrangement of atoms in the molecule and the bonds involved.

All life processes involve water. Water is an excellent solvent for many ionic compounds, as well as for other substances capable of forming hydrogen bonds with water.

Water molecule with molecular formula H₂O has two hydrogen atoms and one oxygen atom; the oxygen atom is connected to the two hydrogen atoms by two covalent bonds (Fig. 1). This bond is a polar covalent bond with concentration of the electron density around oxygen which is one of the most electronegative atoms. This arrangement gives water molecule a dipolar character where oxygen atom is the negative pole and hydrogen atoms are the positive poles. This dipole character enables water molecules to be connected among themselves by intermolecular forces called hydrogen bonds. Hydrogen bonds are a relatively strong type of intermolecular interaction in which hydrogen atom from a certain molecule is connected to an electronegative atom (such as oxygen, nitrogen, or fluorine) of another molecule.

Although many compounds can form hydrogen bonds (such as NH₃ and HF), the difference between H₂O and other polar molecules is that each oxygen atom can form two hydrogen bonds, the same as the number of lone electron pairs on the oxygen atom. Thus, water molecules are joined together in an extensive three-dimensional network in which each oxygen

![Fig. 1 Water molecule and hydrogen bonds shown by the dashed lines.](image)
atom is approximately tetrahedrally bonded to four hydrogen atoms, two by covalent bonds and two by hydrogen bonds. This confers on water its unique physical and chemical properties that will be discussed in the next sections (Chang, 2010; Ebbing and Gammon, 2009; Manahan, 2000).

1.2 Physical Properties of Water

Table 1 summarizes some of the basic physical properties of water. Most of these properties are highly affected by temperature. Therefore, some values are given at different temperatures. Also the effect of these properties on

| Property                        | Value     | Temperature (°C) | Effect and Significance                                      |
|---------------------------------|-----------|------------------|-------------------------------------------------------------|
| Density                         | 0.9999    | 0                | Solid form is less dense than its liquid form; ice floats at the surface of liquid water |
|                                 | 1.000 g/mL| 3.98             |                                                             |
|                                 | 0.9971 g/mL| 25              |                                                             |
|                                 | 0.9584    | 100              |                                                             |
| Viscosity                       | 1.79 cP   | 0                | Hot molasses flows much faster than cold molasses            |
|                                 | 0.89 cP   | 25               |                                                             |
|                                 | 0.28 cP   | 100              |                                                             |
| Surface tension                 | 75.6 dyn/cm| 0                | Water bugs (striders) seem to skitter across this skin as if ice-skating. You can actually float a pin on water, if you carefully lay it across the surface |
|                                 | 72.5 dyn/cm| 25              |                                                             |
|                                 | 58.9 dyn/cm| 100              |                                                             |
| Dipole moment                   | 1.8546 D  |                  | Reflects the polarity of water molecule                      |
| Specific heat                   | 1 cal/g/°C|                  | Water can absorb a substantial amount of heat while its temperature rises only slightly |
| Latent heat of fusion           | 80 cal    | 0                |                                                             |
| Latent heat of vaporization     | 540 cal   | 100              |                                                             |
| Boiling point                   | 100°C at 1 atm |        |                                                             |
water behavior is highlighted briefly (Chang, 2010; Ebbing and Gammon, 2009; Manahan, 2000; Venkateswariu, 2003).

1.3 Chemical Properties of Water

Before discussing the chemical properties of water we shall introduce some terms that will be encountered a lot in the coming sections.

1.3.1 Solutions, Units, and Concentration

A solution is a homogeneous mixture of two or more substances, consisting of ions or molecules. Solute is the component present in smaller amount and solvent is the component in greater amount.

The general term concentration refers to the quantity of solute in a standard quantity of solution.

Concentration might have different expressions depending on the unit of the amount of solute and the unit the amount of solution or solvent. When the solvent is water, it is called aqueous solution.

The molar concentration or molarity (abbreviated M) of a solution is the number of moles of the solute species that is contained in 1 L of the solution. It has the dimensions of mol/L. Percent concentration is another way of expressing concentration. Percent composition can be expressed as mass percent (w/w %) which is the percentage of the mass of solute over the mass of solution. Or simply concentration could be expressed in units of mass per volume (i.e., g/L, mg/L).

We can divide the substances that dissolve in water into two broad classes, electrolytes and nonelectrolytes. An electrolyte is a substance that dissolves in water to give ions that make the solution an electrically conducting solution such as sodium chloride. A nonelectrolyte is a substance that dissolves in water to give a nonconducting solution because it does not form ions such as sucrose, table sugar (Chang, 2010; Ebbing and Gammon, 2009).

1.3.2 Major Chemical Reactions in Water

There are principally three types of chemical reactions that could take place in water: precipitation, acid/base, and oxidation–reduction reaction.

In precipitation reaction, dissolved ions react with each other and form a solid compound or precipitate. A typical precipitation reaction that occurs in water is the formation of calcium carbonate solid when solution of calcium is mixed with solution of carbonate (Davis and Cornwell, 2012).

\[
\text{Ca}^{2+} \quad _{\text{aq}} \quad + \quad \text{CO}_3^{2-} \quad _{\text{aq}} \quad \rightarrow \quad \text{CaCO}_3(s)
\]
Many definitions are available for acids and bases. However, for the purpose of simplicity here, acids can be defined as proton donor and bases as proton acceptor. A neutralization reaction is a reaction of an acid and a base that results in an ionic compound and possibly water. When a base is added to an acid solution, the acid is said to be neutralized. The ionic compound that is produced in a neutralization reaction is called a salt (Chang, 2010; Ebbing and Gammon, 2009). The net equation for most acid base reaction is:

\[ H^+_{(aq)} + OH^-_{(aq)} \rightarrow H_2O_{(l)} \]

Oxidation–reduction reaction is a reaction in which electrons are transferred between species or in which atoms change oxidation number. The substance that loses the electron is said to be oxidized and it is called a reducing agent. While the substance that accept the electron is said to be reduced and is called oxidizing agent. When one species release electron, another one should be available to accept the electron. Iron pipe corrosion is an example of oxidation–reduction reaction in which iron metal oxidizes and loses two electrons, while hydrogen ions accepts those electrons and reduces to hydrogen gas (Chang, 2010; Davis and Cornwell, 2012; Ebbing and Gammon, 2009)

\[ Fe \rightarrow Fe^{2+} + 2e^- \]
\[ 2H^+ + 2e^- \rightarrow H_2(g) \]

1.3.3 pH
The pH of a solution is a measure of hydronium ion (H₃O⁺) concentration, which is, in turn, a measure of acidity. In acidic solutions the pH is smaller than 7, while it is greater than 7 in basic solutions. The pH range in water samples is rarely below 4 or above 10. Determining pH of water is essential as it affects many of the chemical and biological processes that take place in water (Davis and Cornwell, 2012; Venkateswariu, 2003).

1.3.4 Alkalinity
Alkalinity refers to the capability of water to neutralize acid. It is defined as the sum total of all titratable bases down to about pH 4.5. It is found experimentally by determining how much acid it takes to lower the pH of water to 4. In most waters the only significant contribution to alkalinity is the
carbonate species and any free H\(^+\) or OH\(^-\). The total H\(^+\) that can be taken up by water containing primarily carbonate species is

\[
\text{alkalinity} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2+}] + [\text{OH}^-] - [\text{H}^+]
\]

In most natural water situations (pH 6–8) the OH\(^-\) and H\(^+\) are negligible; therefore,

\[
\text{alkalinity} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2+}]
\]

Note that \([\text{CO}_3^{2-}]\) is multiplied by 2 because it can accept two protons

\[
\begin{align*}
\text{H}_2\text{CO}_3 & \rightarrow \text{H}^+ + \text{HCO}_3^- \rightarrow pK_{a1} = 6.35 \\
\text{HCO}_3^- & \rightarrow \text{H}^+ + \text{CO}_3^{2-} \rightarrow pK_{a2} = 10.33
\end{align*}
\]

From this we can see that:
- below pH 4.5 all carbonates are H\(_2\)CO\(_3\) and the alkalinity is negative
- pH 7.5–8.3 all carbonates are HCO\(_3^-\) and alkalinity equals [HCO\(_3^-\)]
- above pH 11.5 all carbonates are CO\(_3^{2-}\) and alkalinity equals 2[CO\(_3^{2+}\)] + [OH\(^-\)]

By convention alkalinity is not expressed in molarity units as shown in the above equations, but rather as mg/L as CaCO\(_3\) (Davis and Cornwell, 2012; Manahan, 2000).

1.3.5 Water Hardness

Hardness is a term usually used to characterize water. Technically, it is the sum total of all polyvalent cations. Practically it is the sum of calcium and magnesium ions which are predominant cations in natural waters. Hard water requires more soap and synthetic detergent for laundry and contributes to scaling in boilers and industrial equipment.

Hardness is expressed in mg/L as CaCO\(_3\), as in alkalinity. Both calcium and magnesium have a valence of two when converting to CaCO\(_3\). The sum of calcium and magnesium is the total hardness (TH), which is subdivided to carbonate and noncarbonated hardness.

Carbonate hardness is often called temporary hardness because heating the water will remove it. When water is heated, the insoluble carbonates precipitate and tend to form bottom deposits in hot water heaters. Carbonate hardness is equal to the total hardness or alkalinity, whichever is less.

Noncarbonated hardness is correspondingly called permanent hardness because it is not removed when water is heated. Noncarbonated hardness
is the total hardness in excess of the alkalinity. If the alkalinity is equal to or greater than the total hardness, there is no noncarbonated hardness (Davis and Cornwell, 2012; Manahan, 2000).

### 1.4 Hydrologic Cycle

The cyclic movement of water through the environment is called the hydrologic cycle. The global hydrological cycle is powered by solar energy. It is a closed system, in continual circulation.

The hydrologic cycle, illustrated in Fig. 2, begins as water moves from the ocean’s surface into the air above through **evaporation**. Water evaporates into the atmosphere and forms clouds above the ocean. Additional water is drawn from the soil by plants, and is then evaporated into the atmosphere from leaves and stems in a process called **transpiration**. As the air rises and the temperature drops, the moisture-laden air condenses, forming clouds and eventually resulting in precipitation. **Precipitation** is the term applied to all forms of moisture falling to the ground such as rain, snow, sleet, and hail.

In some cases, the raindrops soak into the earth and move slowly into the groundwater. Sometimes it **runs off** the land surface and moves quickly in a swift-flowing stream. Other times, the raindrop rests in deep river pools or lakes, is taken up by plants and animals, or enters the atmosphere again.

![Fig. 2 The hydrologic cycle.](image-url)
through evaporation. Other water seeps downward into the soil. This process is called **infiltration**. If the rock below the soil is permeable, then the water **percolates** the rock and is stored as groundwater. Ultimately, all water makes its way back to the ocean, which is like a giant reservoir. Water is stored in the ocean until it is delivered to the land as a result of evaporation and precipitation and the cycle continues (Davis and Cornwell, 2012; Manahan, 2000; Vigil, 2003).

### 1.5 Types of Water Bodies (Classification of Water by Source)

According to the United States Geological Survey (Winter et al., 1998), most of the fresh water (84.9%) is locked up as ice in glaciers. Of the balance, 14.16% constitutes groundwater, while that in lakes and reservoirs mounts to 0.55%. Another 0.33% is in form of soil moisture and atmospheric water vapor. Thus, only a very small fraction of fresh water, about 0.004%, flows through rivers and streams. The volume of sea water is 15 times greater than that of fresh water.

Natural waters can be classified into two categories: sea water (inclusive of estuarine water) and fresh water.

Surface waters might possess color, odor, taste, suspended solids, etc. Groundwaters are expected to be free from organic odor and have a relatively less variable composition at the same source.

The United States Geological Survey (Venkateswariu, 2003; Winter et al., 1998) has classified different waters on the basis of their total dissolved solids (TDS) content as given in **Table 2**.

### 1.6 Water Quality

Many diverse factors have to be taken into account before making comments on water quality. For this reason, the concentrations of inorganic

| Water Quality | TDS (mg/L) |
|---------------|------------|
| Fresh         | Less than 1,000 |
| Slightly saline | 1,000–3,000 |
| Moderately saline | 3,000–10,000 |
| Very saline   | 10,000–35,000 |
| Briny         | Greater than 35,000 |
and organic substances dissolved in a body of water and their amount need to be monitored regularly. Agents that alter water quality can be classified under four major categories.

### 1.6.1 Physical Agents

Physical properties of water are related to the appearance of water, namely, the color, temperature, turbidity, taste, and odor. To be suitable for use, water must be free from all impurities that are offensive to the sense of sight, taste, or smell and one very important physical characteristic that should be encountered when discussing water quality is turbidity (Davis and Cornwell, 2012).

The presence of suspended materials such as clay, slit, finely divided organic material, plankton, and other inorganic materials in water is called **turbidity**. Turbidity is a measure of the clarity of water. Low-turbidity water is clear, while high turbidity water is cloudy or murky. The unit of measuring turbidity is turbidity unit (TU). Turbidity larger than 5 TU is easily detected in a glass of water and is objectionable for aesthetic reasons (Davis, 2010; Davis and Cornwell, 2012).

### 1.6.2 Chemical Agents

Those include all the major dissolved constituents in water such as humic substances, in addition to the minor constituents such as **heavy metals**, **detergents**, **pesticides**.

The chemical analysis of a domestic water supply should ordinarily include the determination of water hardness, alkalinity, pH, conductivity, and the presence of chloride, sulfate, and nitrate. Other chemicals of importance are: iron, manganese, fluoride, copper, sodium, and zinc, in addition to some toxic substances such as arsenic, barium, cadmium, chromium, lead, mercury, selenium, silver, and cyanides.

The significant concentrations with respect to several chemicals that might be present in natural waters are given in Table 3. Above these levels, such chemicals can cause undesirable effects (Davis, 2010; Davis and Cornwell, 2012; Venkateswarlu, 2003).

The American Water Works Association (AWWA) has issued its set of goals with respect to major physical and chemical agents. These goals are shown in Table 4.

### 1.6.3 Biological Agents

Biological agents are very important due to their relation to public health and may also be significant in modifying the physical and chemical
characteristics of water. Water for drinking and cooking should be free from disease-causing organisms (pathogens). These organisms include bacteria, protozoa, viruses, fungi, and helminthes (worms) (Davis and Cornwell, 2012).

Table 3 Chemical Constituents of Significance in Natural Waters (Venkateswarlu, 2003; Winter et al., 1998)

| Chemical Constituent | mg/L |
|----------------------|------|
| Bicarbonate          | 150–200 |
| Carbonate            |       |
| Calcium              |       |
| Magnesium            | 25–50 |
| Sodium               | 60 (Irrigation) |
|                      | 20–120 (Health) |
| Iron                 | Less than 3 |
| Manganese            | Less than 0.05 |
| Chloride             | 250 |
| Fluoride             | 0.7–1.2 |
| Sulfate              | 300–400 (Taste) |
|                      | 600–1000 (Laxative action) |

Table 4 Water Quality Goals According to the AWWA

| Agent (Property)       | Goal, mg/L                        |
|------------------------|-----------------------------------|
| Turbidity              | <0.1 turbidity units (TU)         |
| Color                  | <3 color units                    |
| Odor                   | None                              |
| Taste                  | None objectionable                |
| Aluminum               | <0.05                             |
| Copper                 | <0.2                              |
| Iron                   | <0.05                             |
| Manganese              | <0.01                             |
| Total dissolved solids (TDS) | 200.0                         |
| Zinc                   | <1.0                              |
| Hardness               | 80.0                              |
1.6.4 Radiological Factors
This factor must be considered in areas where there is a possibility that water comes in contact with radioactive substances. Radioactive substances could find their way to drinking water from atomic energy power sources and the mining of radioactive materials, as well as naturally occurring radioactive materials (Davis, 2010; Davis and Cornwell, 2012).

Seasonal variations in the quality of some surface waters could be large enough to make the use of such waters more problematic. The self-purification capacity and the water intake structure are also important factors that affect the quality of water. Whatever might be the quality of water available to a user, it can certainly be upgraded by properly designed and executed treatment procedures.

Governments with the aid of organizations such as Environmental Protection Agency (EPA) and World Health Organization (WHO), as well as local water authorities have set specifications for water. These specifications vary according to the intended use of water (for drinking, agriculture, industry, or disposal wastewater into the aquatic system or landfills). Water specifications lay down the maximum contamination levels or maximum permissible level with respect to several chemicals and pathogens that might affect water quality in order to meet minimum requirement for protection of public health (Davis, 2010; Davis and Cornwell, 2012; Weiner and Matthews, 2003).

1.7 Water Quality Management
1.7.1 Water Pollutants and Their Sources
Water pollutants are categorized as point source or nonpoint source. When water pollution arises from a single source, this is called point source pollution (an example would be chemicals from a single factory). Conversely, when pollution affecting a body of water issues from multiple sources (multiple factories), it is called nonpoint source pollution. Point source pollutants are all dry-weather pollutants that enter watercourses through pipes or channels. Point source pollution comes mainly from industrial facilities and municipal wastewater treatment plants. Storm drainage, agricultural runoff, construction sites, and other land disturbances are considered nonpoint source pollution (Weiner and Matthews, 2003).

The range of pollutants is vast. The major pollutants that could affect water quality are overviewed here.

Oxygen-demanding materials might be discharged from municipal wastewater treatment plants, food-processing plants, breweries, as well as
paper mills, compose one of the most important types of pollutants because these materials decompose in the watercourse, and can deplete the water of dissolved oxygen.

**Sediments and suspended solids (SS)** may also be classified as a pollutant. Sediments consist of mostly inorganic material washed into a stream as a result of land cultivation, construction, demolition, and mining operations. Sediments interfere with fish spawning because they can cover gravel beds and block light penetration, making food harder to find. Sediments can also damage gill structures directly, smothering aquatic insects and fishes. Organic sediments can deplete the water of oxygen, creating anaerobic conditions, and may create unsightly conditions and cause unpleasant odors.

**Nutrients**, mainly nitrogen and phosphorus, can promote accelerated eutrophication, or the rapid biological aging of lakes, streams, and estuaries. **Phosphorus and nitrogen** are common pollutants in residential and agricultural runoff, and are usually associated with plant debris, animal wastes, or fertilizer.

**Heat** may be classified as a water pollutant when it is caused by heated industrial effluents or from alterations of stream bank vegetation that increase the stream temperatures due to solar radiation. Heated discharges may drastically change the ecology of a stream or lake. Although localized heating can have beneficial effects like freeing harbors from ice, the ecological effects are generally harmful. Heated effluents lower the solubility of oxygen in water because gas solubility in water is inversely proportional to temperature, thus reducing the amount of dissolved oxygen available to oxygen-dependent species. Heat also increases the metabolic rate of aquatic organisms (unless the water temperature gets too high and kills the organism), which further reduces the amount of dissolved oxygen because respiration increases.

**Synthetic chemicals and pesticides, herbicides, fertilizers, pharmaceutical substances such as antibiotics, cosmetics and personal care products, detergents, toxic chemicals, and heavy metals** can adversely affect aquatic ecosystems as well as making the water unusable for human contact or consumption. These compounds may come from municipal wastewater, industrial effluents, or agricultural and urban runoff.

**Pathogenic microorganisms** are important pollutants that directly affect human health. Water-borne pathogen contamination in ambient water bodies and related diseases are a major water quality concern throughout the world. Water-borne diseases (i.e., diarrhea, gastrointestinal illness) are caused by various bacteria, viruses, protozoa, algae, and fungi.
A major pathogen is fecal coliform bacteria (i.e., *Escherichia coli*) that is the bacteria that normally live in the intestinal tract of warm-blooded animals and indicate contamination by animal wastes. Other bacterial pathogens include *Vibrio cholera* which cause cholera, and *Shigella* and *Salmonella* that cause dysentery. Other types of microorganisms that could contribute to biological water pollution are: protozoa (such as *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica* that cause diseases such as *Cryptosporidiosis*, *Giardiasis*, and *Amoebiasis*); viruses such as Coronavirus, Hepatitis A virus (HAV) that cause Hepatitis A, and Poliovirus which cause Poliomyelitis; algae such as *Desmodesmus armatus* that cause desmodesmus infection; and several fungi such as *Aspergillus* which most frequently affects the lungs. Some higher organisms such as nematodes could be present in water and lead to water-borne disease (Tortora et al., 2010).

Such species can be introduced into water bodies as the result of municipal and industrial wastewater discharges, or as a result of aquaculture activities. In addition to causing diseases, the presence of these organisms in water could alter the original microbial floral community in those water bodies.

**Oil pollution** can result from leak out of oil from huge tanker loaded with crude oil and cause water pollution with petroleum compounds.

**Acids and bases** from industrial and mining activities can alter the water quality in a stream or lake to the extent that it kills the aquatic organisms living there, or prevent them from reproducing. *Sulfur*-laden water leached from mines, including old and abandoned mines as well as active ones, contain compounds that oxidize to sulfuric acid on contact with air (Davis and Cornwell, 2012; Manahan, 2000; Weiner and Matthews, 2003).

Oxygen-demanding materials and nutrients are pollutants of significant importance; they deserve particular focus and will be the subject of discussion in the following sections.

### 1.7.2 Dissolved Oxygen

Dissolved oxygen (DO) refers to the level of free, noncompound oxygen (O₂) dissolved in water or other liquids. The bonded oxygen in water (H₂O) is in a compound and does not count toward dissolved oxygen levels. DO is an important parameter in assessing water quality because of its influence on the organisms living within a body of water. Oxygen gets into water by diffusion from the surrounding air, by aeration, or as a waste product of photosynthesis. DO is essential to the survival of organisms in a stream. The presence of oxygen is a positive sign and the absence of oxygen is a sign of...
severe pollution. Waters with consistently high dissolved oxygen are considered to be stable aquatic systems capable of supporting many different kinds of aquatic life (Davis and Cornwell, 2012; Weiner and Matthews, 2003).

### 1.7.3 Oxygen-Demanding Materials

Anything that can be oxidized in the receiving water with consumption of molecular oxygen is termed oxygen-demanding material. These materials are usually biodegradable organic compounds but also include some inorganic compounds. The consumption of DO poses a threat to higher forms of aquatic life. The critical level of DO varies greatly among species. For example, brook trout may require 7.5 mg/L of DO, while crab may survive at 3 mg/L. Oxygen-demanding materials in domestic sewage come primarily from human waste and food residue. Almost all naturally organic matters such as animal droppings, crop residue, or leaves, which get into water from nonpoint sources, contribute to the depletion of DO (Davis, 2010; Davis and Cornwell, 2012; Weiner and Matthews, 2003).

### 1.7.4 Biochemical Oxygen Demand

Biochemical oxygen demand (BOD) is the most commonly used method for measuring the quantity of organic oxygen-demanding materials. BOD is a measure of the quantity of oxygen used by aerobic microorganisms (need molecular oxygen for living) in the oxidation of organic matter present in a given water sample at certain temperatures over a specific time period. The BOD value is most commonly expressed in milligrams of oxygen consumed per liter of sample during a prescribed period of incubation at 20°C and is often used as an indicator of the degree of organic pollution of water.

Natural sources of organic matter include plant decay and leaf fall. However, plant growth and decay may be unnaturally accelerated when nutrients and sunlight are overly abundant due to human influence. Urban runoff carries pet wastes from streets and sidewalks; nutrients from lawn fertilizers; and leaves, grass clippings, and paper from residential areas, which increase oxygen demand. Oxygen consumed in the decomposition process robs other aquatic organisms of the oxygen they need to live. Organisms that are more tolerant of lower dissolved oxygen levels may thrive in a diversity of natural water systems including aerobic bacteria. Most of them feed on dead algae and other dead organisms and are part of the decomposition cycle. Algae and other organism can produce oxygen through photosynthesis using the energy of sun. At night they use up oxygen in respiration. Therefore, there is a continuous competition between the consumption of oxygen and its production.
through photosynthesis. The rate of oxygen consumption and therefore BOD is affected by a number of variables: temperature, pH, the presence of certain kinds of microorganisms, and the type of organic and inorganic material in the water (Davis and Cornwell, 2012; Weiner and Matthews, 2003).

### 1.7.5 Measurement of BOD in the Lab (BOD Test)

The most commonly used test for BOD is called BOD$_5$. This test provides a measure of the oxygen consumed in the biological oxidation of organic material in a sample at 20°C over a period of 5 days. In the 5-day BOD test (BOD$_5$), the sample of water is suitably diluted (in case of wastewater samples) with well-oxygenated water and an inoculum of microorganisms is introduced. The initial oxygen concentration is measured and the sample stored in darkness (to avoid photosynthetic oxygen generation) at 20°C for 5 days. Over the 5-day period, the oxygen concentration is measured at regular intervals and at the end of the 5-day period. The difference in oxygen concentration between the beginning and the end of the 5-day test period, taking due account of dilution, gives the BOD$_5$ (American Public Health Association (APHA), 2012). The concentration of oxygen could be measured using the traditional Winkler titration method (APHA, 2012) or is done automatically with modern BOD apparatus equipped with manometer and DO sensor. BOD$_5$ method is the most widely used method for assessment of water quality (Weiner and Matthews, 2003).

### 1.7.6 Carbonaceous and Nitrogenous BOD

The amount of dissolved oxygen used by microorganisms in order to oxidize organic material in the absence of nitrification is called carbonaceous BOD or CBOD. However, additional amounts of dissolved oxygen could be depleted from the sample due to nitrification process, that is, the conversion of ammonia (NH$_3$) to nitrate (NO$_3^-$) by nitrogenous bacteria which is called nitrogenous BOD or NBOD.

The forms of nitrogen in urine and proteins (urea, uric acids, ammonia, amino acids, and nitrates) are nutrients for algae and aquatic plant growth. The nitrogenous waste in municipal and industrial sewage is used by autotrophic bacteria, and they use a significant amount of oxygen as an energy source and convert ammonia to nitrates. The described BOD$_5$ test in Section 1.7.5 gives value for both carbonaceous and nitrogenous BOD. Thus, if we wish to determine the value of CBOD only, nitrification suppressor or inhibitor such as ATU (Allylthiourea) should be added (Davis and Cornwell, 2012).
1.7.7 Chemical Oxygen Demand

Another closely related test to BOD is the chemical oxygen demand (COD) test which is also considered as an important indicator of water quality. COD is a measurement of the oxygen required to oxidize soluble and particulate organic matter in water. This test gives the electron donating capacity of practically all the organic compounds in the sample, biodegradable or non-biodegradable and soluble or particulate.

The COD of a wastewater, in general, is greater than the BOD because more compounds can be oxidized chemically than can be oxidized biologically.

The method involves using a strong oxidizing chemical, potassium dichromate Cr₂O₇²⁻, to oxidize the organic matter in solution to carbon dioxide and water under acidic conditions.

The sample is then digested for approximately 2 h at 150°C. The amount of oxygen required is calculated from the quantity of chemical oxidant consumed (APHA, 2012; Davis, 2010).

1.7.8 Nitrogen and Phosphorous in Water

Nitrogen and phosphorus are important elements that could be present in water and affect water quality fundamentally since they are essential nutrients for biological growth.

1.7.8.1 Chemistry of Nitrogen

Nitrogen occurs in five major forms in aquatic environments: organic nitrogen, ammonia, nitrite, nitrate, and dissolved nitrogen gas.

Ammonia is one of the intermediate compounds formed during biological metabolism and, together with organic nitrogen, is considered an indicator of recent pollution.

Aerobic decomposition of organic nitrogen and ammonia eventually produces nitrite (NO₂⁻) and finally nitrate (NO₃⁻). Therefore, high nitrate concentration may indicate that organic nitrogen pollution occurred far enough upstream that the organics have had time to oxidize completely. Similarly, nitrate may be high in groundwater after land application of organic fertilizers if there is sufficient residence time (and available oxygen) in the soils to allow oxidation of the organic nitrogen in the fertilizer (Andrews et al., 2004; Manahan, 2000).

Because ammonia and organic nitrogen are pollution indicators, these two forms of nitrogen are often combined in one measure, called Kjeldahl
Nitrogen (APHA, 2012). Nitrate and nitrite could be determined separately by spectrophotometric methods (APHA, 2012).

1.7.8.2 Nitrogen Cycle
The nitrogen cycle (Fig. 3) is the biogeochemical cycle that describes the transformations of nitrogen and nitrogen-containing compounds in nature. Although there is an abundance of nitrogen in the atmosphere, most plants cannot use this form of nitrogen. Instead, nitrogen must be in its “fixed” form, or in a compound, in order to be usable by plants. To get to this fixed form, nitrogen must first go through the cycle. The steps of the nitrogen cycle are the following: Nitrogen Fixation, Nitrification, Denitrification, Ammonification, and Assimilation.

**Nitrogen Fixation** occurs when atmospheric nitrogen is converted to ammonia by a pair of bacterial enzymes called nitrogenase present in a few species of bacteria, including cyanobacteria. Although ammonia (NH₃) is the direct product of this reaction, it is quickly ionized to ammonium (NH₄⁺).

**Nitrification** is the two-step process in which ammonia is converted to nitrites (NO₂⁻) and then to nitrates (NO₃⁻). Two different genera of bacteria that are present in the soil oxidize the ammonia into inorganic forms of nitrogen: these are *Nitrosomonas* and *Nitrobacter*.

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**Fig. 3** Nitrogen cycle.
**Denitrification** is the process of reducing nitrate and nitrite, highly oxidized forms of nitrogen available for consumption by many groups of organisms, into gaseous nitrogen. It can be thought of as the opposite of nitrogen fixation. Certain types of bacteria are responsible for this transformation.

**Assimilation** is the process by which living organisms incorporate $\text{NO}_3^-$ and $\text{NH}_4^+$ (ammonium) formed through nitrogen fixation and nitrification. Plants take in this form of nitrogen through their roots and incorporate them into nucleic acids and plant protein. Animals are then able to receive and utilize the nitrogen from plant tissues through consumption.

**Ammonification** occurs when a plant or animal dies or excretes waste. Decomposers, such as bacteria and fungi, first break down the proteins in the organic matter. This releases ammonia, which dissolves with water in the soil. Ammonia then combines with a hydrogen ion to create ammonium.

**Denitrification** is the process in which microorganisms, such as bacteria, break down nitrates to metabolize oxygen. This releases nitrogen gas back into the atmosphere, completing the cycle (Andrews et al., 2004; Manahan, 2000).

### 1.7.8.3 Chemistry of Phosphorous

Phosphorus is found in the earth and rocks, and it is taken up by plants and animals. Phosphorus is a very important chemical because it is essential in the formation of DNA. In water bodies, phosphorus occurs almost entirely as organic phosphate and inorganic orthophosphate or polyphosphates (APHA, 2012; Davis and Cornwell, 2012).

Phosphorus is usually measured as total phosphorus (all forms combined) or dissolved phosphorus. Dissolved orthophosphate ($\text{PO}_3^{\text{2-}}$) is an important indicator of water pollution because it is easily and rapidly taken up by bacteria, and therefore is almost never found in high concentrations in unpolluted waters. The various phosphorus forms can be measured analytically by spectrophotometric techniques (APHA, 2012).

### 1.7.8.4 Phosphorous Cycle

Phosphorous cycle could be defined as the biogeochemical cycle that describes the movement of phosphorus through the lithosphere (solid earth), hydrosphere (water), and biosphere (living organisms) (Manahan, 2000; Ruttenberg, 2003). The global phosphorus cycle (Fig. 4) could be summarized in the following steps:

1. Phosphorus starts out in rocks and then phosphates are washed off the rocks over a period of time by weathering and rain. After that, phosphate is distributed into soil and water.
Fig. 4 Phosphorous cycle.
2. Plants then take up phosphate from the soil, and animals may consume these plants. When this happens, the phosphate becomes imbedded into organic molecules (DNA). When plants and animals die and decompose, phosphate will return to the soil.

3. Organic forms of phosphate can be made accessible to plants by bacteria which break down the organic matter. Once this happens, the phosphate is changed to an inorganic form of phosphorus (this is known as mineralization).

4. Once the phosphorus is in the soil, it can be transferred to waterways and then oceans. After this phosphorus can be integrated into sediments and rock and the cycle starts again.

The cycle is largely affected by the production and use of fertilizers made from phosphorus and animal manure that contains phosphorus. Soil cannot absorb all the fertilizers, so it eventually gets washed away with other surface runoff. This will cause an increased amount of phosphorus in the water bodies. Consequently, eutrophication will occur.

1.7.9 Eutrophication

Eutrophication occurs when extra nitrate and phosphate leach into a body of water and cause excessive growth of microorganisms.

When phosphorus and nitrogen are introduced into the lake, either naturally from storm runoff, or from a pollution source, the nutrients promote rapid growth of algae on the surface layer of the lake. When the algae die, they drop to the lake bottom and become a source of carbon for decomposing bacteria. Aerobic bacteria (that use oxygen for living) will use all available dissolved oxygen in the process of decomposing this material, and the dissolved oxygen may be depleted enough to cause the bottom of the lake to become anaerobic. As more and more algae die, and more and more dissolved oxygen is used in their decomposition, the middle layer of the lake may also become anaerobic (do not need molecular oxygen for living). When this happens, aerobic biological activity will be restricted on the surface layer only. The increasing frequency of this condition over the years is called eutrophication (Davis and Cornwell, 2012; Manahan, 2000; Weiner and Matthews, 2003).

Natural eutrophication may take thousands of years. However, eutrophication process may be shortened to as little as a decade as a result of excessive use of chemicals that provide nutrients such as fertilizers, detergents, and pesticides (Davis and Cornwell, 2012).
1.8 Water Treatment

Water treatment is any process that makes water more suitable for a specific end use. The end use could be drinking or cooking, industrial supply, irrigation, fire fighting, river flow maintenance, water recreation, or just safe discharge of water to the environment.

Generally speaking, the characteristics of water and the final intended use of water determine the degree of treatment and the treatment method (Davis, 2010; Davis and Cornwell, 2012; Weiner and Matthews, 2003).

Mostly, the focus is on water treatment plants that produce safe drinking water and wastewater treatment plants that treat water for safe discharge or future reuse.

1.8.1 Drinking Water Treatment

Drinking water treatment plant could be classified into:
- **Disinfection plant** which is used for high-quality water source to ensure that water does not contain pathogens
- **Filtration plant**: this is usually used to treat surface water
- **Softening plant** which is used to treat groundwater

Typical filtration plant is shown in Fig. 5 which is designed to remove odors, color, and turbidity as well as bacteria and other contaminants. Filtration plant employs the following steps:

a. **Rapid mixing**: where chemicals are added and rapidly dispersed through the water

b. **Flocculation**: Chemicals like alum (aluminum sulfate) are added to the water both to neutralize the particles electrically and to make them come close to each other and form large particles called **flocs** that could more readily be settled out

![Fig. 5 Filtration treatment plant.](image-url)
c. **Sedimentation**: During sedimentation, floc settles to the bottom of the water supply, due to its weight

d. **Filtration**: Once the floc has settled to the bottom of the water supply, the clear water on top will pass through filters of varying compositions (sand, gravel, and charcoal) and pore sizes in order to remove fine particles that were not settled, such as dust, parasites, bacteria, viruses, and chemicals

e. **Disinfection**: involves the addition of chemicals in order to kill or reduce the number of pathogenic organisms

Softening plants utilize the same operational unit as filtration plants but use different chemicals in order to remove water hardness (Davis, 2010; Davis and Cornwell, 2012; Weiner and Matthews, 2003).

### 1.8.2 Wastewater Treatment

Municipal wastewater treatment may involve three major categories: primary treatment, secondary treatment, and advance treatment. Fig. 6 shows degrees of wastewater treatment.

- **Primary treatment**: the goal is to remove pollutants which will either settle or float in a clarifier tank. Primary treatment will typically remove 60% of the raw sewage suspended solids and 35% of the BOD$_5$. Soluble pollutants are not removed in this process.

- **Secondary treatment**: the major goal is to remove BOD$_5$ that escapes the primary process and to provide additional removal of suspended solids. Secondary treatment is typically achieved using biological processes. These processes could occur naturally in wastewater. Nevertheless, secondary treatments are designed to speed up these natural processes. Although secondary treatment may remove 85% of BOD$_5$ and suspended solids, it does not remove significant amount of nitrogen, phosphorous, or heavy metals, nor does it completely remove pathogenic bacteria and viruses.

- **Advance treatments or tertiary wastewater treatment**: these processes are normally applied when secondary treatment is not adequate. Some of these processes may remove up to 99% of BOD$_5$ and phosphorous, all suspended solids and bacteria, and 95% of nitrogen. They can produce sparkling clean, colorless, and odorless effluent indistinguishable in appearance from high-quality drinking water. Some of these processes involve: Filtration or **application of adsorbent** such as activated carbon in order to remove persistent organic pollutants. Tertiary treatment may involve **addition of chemicals** such as ferric chloride or alum to
Fig. 6 Degrees of wastewater treatment.
enhance the removal of phosphorous. **Nitrogen control** step could be added to the tertiary treatment plant in order to facilitate the removal of nitrogen. This step could be accomplished biologically or chemically. The biological step is called nitrification/denitrification. Specific types of bacteria must be present to cause these two reactions to occur. The chemical process is called ammonia stripping; in this process nitrogen is removed in the form of ammonia by raising the pH of the solution by addition of base such as lime to covert ammonium ion to ammonia (Davis, 2010; Davis and Cornwell, 2012).

2. **WATER MICROBIOLOGY**

2.1 **Introduction**

Microbiology is the science devoted to the study of organisms that are too small to be seen by the naked eye. These microorganisms constitute a large and diverse group of free-living forms that exist as single cells or cell clusters. Being free living, microbial cells are distinct from the cells of animals and plants as the latter are not able to live alone in nature but only in characteristic groups. A single microbial cell, generally, is able to carry out its life processes of growth, respiration, and reproduction independently of other cells, whether of the same kind or of different kinds. Microorganisms could be bacteria, viruses, algae, fungi, or protozoa (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016). In the following sections we will present an overview about microorganisms, their role in water, their classification, the main processes they perform, disease-causing microorganisms, and the role of microorganisms in wastewater treatment. Most of the examples and discussion in the following sections will be assigned to bacteria as they outline the group of the most important microorganisms when encountering water and wastewater treatment; they can be pathogens or can help in wastewater treatment.

2.2 **Taxonomy and Classification**

Despite their complexity and variety, all living cells can be arranged into two major groups, prokaryotes and eukaryotes, based on certain structural and functional characteristics. In general, prokaryotes are structurally simpler and smaller than eukaryotes. The DNA (genetic material) of prokaryotes is usually in a single, circularly arranged chromosome and is not surrounded by a membrane; therefore, they do not have true nucleolus. They also lack
membrane-enclosed organelles (specialized structures that carry on various activities). Their cell walls almost always contain the complex polysaccharide peptidoglycan. They usually divide by binary fission. During this process, the DNA is copied, and the cell splits into two. On the other hand, the DNA of eukaryotes is found in multiple chromosomes in a membrane-enclosed nucleus. Plants and animals are entirely composed of eukaryotic cells (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

Taxonomy is the science of classifying living organisms. Taxonomy is a basic and necessary tool for scientists, providing a universal language of communication. In a broader sense taxonomy consists of three separate but interrelated parts: classification, nomenclature, and identification.

Classification is the arrangement of organisms into groups (taxa, singular taxon), nomenclature involves assignment of names to taxa, and identification is the experimental determination of taxon to which an isolate belongs.

In the currently accepted classification of life, there are three main domains which are eukarya, bacteria, and archaea. Animals, plants, fungi, and protists are kingdoms in the domain eukarya. The domain bacteria include all of the pathogenic prokaryotes as well as many of the nonpathogenic prokaryotes found in soil and water.

The organisms are classified as species. Species is a group of closely related organisms that breed among themselves. A group of species that differ from each other in certain ways but are related genetically consist a genus. Related genera make up a family. A group of similar families constitutes an order, and a group of similar orders makes up a class. Related classes, in turn, make up a phylum or division. All phyla or divisions that are related to each other make up a kingdom, and related kingdoms are grouped into a domain. The hierarchal classification of organisms showing the eight major taxonomical ranks in addition to the phylogenetic tree of life are presented in Fig. 7 (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016). According to this classification, the full taxonomical position of a bacterium (Escherichia coli) can be described as follows (Willey et al., 2016):

- **Domain:** Bacteria
- **Kingdom:** Bacteria
- **Division:** Proteobacteria
- **Class:** Schizomycetes
- **Order:** Eubacteriales
- **Family:** Enterobacteriaceae
- **Genus:** Escherichia
- **Species:** coli
2.2.1 Nomenclature of Microorganisms

Nomenclature refers to the naming of microorganisms. Two kinds of names are usually given to bacteria, common name and scientific name.

The common or casual name for a microorganism varies from country to country and is usually known in the local language. For example, tubercle bacillus, typhoid bacillus, gonococcus are common names for communication at the local level.

The scientific name is the international name that is accepted throughout the world. Scientific nomenclature of microorganisms is governed by the International Committee on Systematic Bacteriology and published as Approved List of Bacterial Names in the *International Journal of Systematic Bacteriology* (Tortora et al., 2010). By accepted taxonomic conventions, the order names end in *ales* (e.g., the order Eubacteriales), family names end in *aceae* (e.g., the family Enterobacteriaceae), and the tribe names end in *eae* (e.g., the tribe Proteae). The order, family, and tribe names begin with capital letters. The genus name also begins with capital letter, but species name (e.g., *coli*) begins with running letter and not capital letter. Both the genus

![Fig. 7 The phylogenetic tree of life and taxonomy.](image-url)
(e.g., *Escherichia*) and species names are either italicized or underlined when written in the text. The scientific name of the bacterium when written for the first time is written in full (e.g., *Escherichia coli*), but later mentioned in an abbreviated form (e.g., *E. coli*). Because this system gives two names to each organism, the system is called **binomial nomenclature**. When bacteria are referred to as a group, their names are neither capitalized nor italicized nor underlined (e.g., streptococci) (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

### 2.2.2 Major Groups of Microorganisms

In the microbial world, **bacteria** and **archaea** are prokaryotes which constitute a vast group of very small unicellular organisms. Other microorganisms such as **fungi**, **protoza**, and **algae** are eukaryotes. **Viruses**, as noncellular elements, do not fit into any organizational scheme of living cells. They are genetic particles that replicate but are unable to perform the usual chemical activities of living cells (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

#### 2.2.2.1 Bacteria

The majority of prokaryotes are bacteria. The thousands of species of bacteria are differentiated by many factors, including morphology (shape), chemical composition (often detected by staining reactions), nutritional requirements, biochemical activities, and sources of energy (sunlight or chemicals). Bacteria comprise the highest population of microorganisms in wastewater treatment plants (Gerardi, 2006; Mara and Horan, 2003). Therefore, special attention will be given to this group of microorganisms in the following sections. Before that, a brief synopsis about the other types of microorganisms will be presented.

#### 2.2.2.2 Archaea

Bacteria and archaea may look similar since they are both prokaryotes. However, their chemical composition is different. Archaea do not have peptido-glycan in their cell walls. They often live in extreme environments and carry out unusual metabolic processes (Tortora et al., 2010; Willey et al., 2016).

#### 2.2.2.3 Fungi

Fungi are eukaryotes. Their cells have a distinct nucleus containing the cell’s genetic material and surrounded by a special envelope called the nuclear membrane. True fungi have cell walls composed primarily of a substance
called chitin. Fungi are nonphotosynthetic heterotrophic microorganisms. They are obligate aerobe. In general fungi are multicellular; however, yeast is a unicellular form of fungi. Fungi can be reproduced by a variety of methods including fission, budding, and spore formation. The unicellular forms of fungi, yeasts, are oval microorganisms that are larger than bacteria. The most typical fungi are molds which form visible masses called mycelia. The cottony growths sometimes found on bread and fruit are mold mycelia. Organisms called slime molds have characteristics of both fungi and amoebas. Fungi form normal cell material using one half the nitrogen amount required by bacteria. In nitrogen-deficient wastewater they are found to be predominant over bacteria (Davis and Cornwell, 2012; Gerardi, 2006; Mara and Horan, 2003; Tortora et al., 2010).

2.2.2.4 Algae
Algae are a group of eukaryotic microorganism. They might be unicellular or multicellular. The cell walls of many algae are composed of a carbohydrate called cellulose. Algae have chlorophyll in their cells; therefore, they can perform photosynthesis. They reproduce sexually or asexually. Algae are abundant in fresh and salt water, in soil, and in association with plants (Davis and Cornwell, 2012; Gerardi, 2006; Hogg, 2005; Tortora et al., 2010).

2.2.2.5 Protozoa
Protozoa are unicellular eukaryotic organisms. Most protozoa are free living and solitary, but some do form colonies. Most protozoa are aerobic chemotrophs (see Section 2.2.3), but some including amoebae and flagellates can survive anaerobic conditions. They reproduce by binary fission. They often consume bacteria. They are desirable in wastewater effluent because they act as polisher in consuming bacteria (Gerardi, 2006; Hogg, 2005; Tortora et al., 2010).

2.2.3 Classification of Microorganisms According to Their Relationship to Environmental Factors
Microorganisms could be classified according to the energy and carbon source. Carbon is the basic building block for cell synthesis. Energy must be obtained from outside the cell to enable synthesis to proceed. If the microorganism uses organic material as a supply for carbon, it is called heterotrophic. Autotrophs require only CO₂ to supply their carbon needs. Organisms that rely only on the sun as source of energy are called phototrophs. Chemotrophs extract energy from organic or inorganic oxidation/reduction reactions.
Organotrophs use organic material, while lithotrophs oxidize inorganic compounds (Davis, 2010; Davis and Cornwell, 2012).

According to the ability to utilize oxygen as a terminal electron acceptor in oxidation–reduction reactions, microorganisms could be classified as obligate aerobes which are the microorganisms that must have oxygen as the terminal electron acceptor. Obligate anaerobes are microorganisms that cannot survive in the presence of oxygen; they cannot use oxygen as a terminal electron acceptor. Facultative aerobes are able to grow with or without oxygen being present. Under anoxic conditions (depleted dissolved oxygen), facultative aerobes utilize nitrates (NO\textsubscript{3}–) and nitrites (NO\textsubscript{2}–) as the terminal electron acceptor. Nitrate nitrogen is converted to nitrogen gas in the absence of oxygen. This process is called anoxic denitrification (Davis, 2010; Davis and Cornwell, 2012; Mara and Horan, 2003).

According to the preferred temperature regime, four temperature ranges are used to classify microorganisms. Those that best grow at temperatures below 20°C are called psychrophiles. Mesophiles grow best at temperatures between 25°C and 40°C. Between 45°C and 60°C, the thermophiles grow best. Above 60°C stenothermophiles grow best. The growth range of facultative thermophiles extends from the thermophilic range into the mesophilic range (Davis, 2010; Davis and Cornwell, 2012).

2.3 Bacterial Cell: Size, Shape, Arrangement, and Structure

Bacteria come in a great many sizes and several shapes. Most bacteria range from 0.2 to 2.0 μm in diameter and from 2 to 8 μm in length (Tortora et al., 2010). Most bacteria of medical importance range from 0.2 to 1.5 μm in diameter and from 2 to 5 μm in length (Parija, 2012). The basic shapes of bacteria are spherical coccus (plural: cocci, meaning berries), rod-shaped bacillus (plural: bacilli, meaning little staffs), and spiral (see Fig. 8 for the different shapes of bacteria).

Cocci are usually round but can be oval, elongated, or flattened on one side. When cocci divide to reproduce, the cells can remain attached to one another. Cocci that remain in pairs after dividing are called diplococci; those that divide and remain attached in chainlike patterns are called streptococci. Those that divide in two planes and remain in groups of four are known as tetrads. Those that divide in three planes and remain attached in cubelike groups of eight are called sarcinae. Those that divide in multiple planes and form grapelike clusters or broad sheets are called staphylococci.
**Bacilli** divide only across their short axis. Most bacilli appear as single rods. **Diplobacilli** appear in pairs after division, and **streptobacilli** occur in chains.

**Spiral** bacteria have one or more twists; they are never straight. Bacteria that look like curved rods are called **vibrios**. Others, called **spirilla**, have a helical shape, like a corkscrew, and fairly rigid bodies. Another group of spirals are helical and flexible; they are called **spirochetes**. Spirochetes move by means of axial filaments, which resemble flagella but are contained within a flexible external sheath. In addition to the three basic shapes, there are star-shaped cells, rectangular cells, flat cells, and triangular cells (Hogg, 2005; Madigan et al., 2014; Parija, 2012; Tortora et al., 2010; Willey et al., 2016).

Fig. 9 shows the features of a typical bacterial cell. Bacterial cell consists of the following main components (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016):

a. **The cytoplasm and its contents**: are inside the cell membrane and are mostly water by composition. However, they have a semifluid nature due to a suspension of carbohydrates, enzymes, inorganic ions, lipids, and proteins. Within this suspension we can find the following:

   - **The nuclear region** which consists mostly of genetic material in one large and circular chromosome. In addition to the chromosome, some bacteria have small molecules of genetic material called **plasmids** that may include genes encoding toxins or resistance to antibiotics.
Ribosomes are small spherical bodies that serve as sites for protein synthesis.

Inclusions which accumulate certain nutrients when they are plentiful and use them when the environment is deficient.

Endospores which form in certain bacteria such as Bacillus and Clostridium. They are dormant forms of the cell that are highly resistant to extremes of temperature, pH, and other environmental factors, and germinate into new bacterial cells when conditions become more favorable.

b. The cell membrane or plasma membrane is a flexible semipermeable membrane that surrounds the cytoplasm. The cell membrane contains two different layers that regulate the movement of substances in and out of the cell. The outer layer is hydrophilic (water loving), while the inner layer is hydrophobic (water fearing). Together, these layers form a protective and regulating barrier between the cytoplasm and the environment.

c. Cell wall is an extremely porous and rigid structure that lies outside the cell membrane. The cell wall performs three functions. Firstly, it provides protection. Secondly, it maintains the characteristic shape of the cell. Thirdly, it prevents the cell from bursting when fluids flow into the cytoplasm.
d. A variety of external structures: bacterial cells might have several external structures such as the axial filament, capsule, flagella, glycocalyx, and pili. All external structures perform specific functions, but these structures are not necessarily found in all bacteria.

Based upon chemical composition of the cell walls, there are two types of bacteria, Gram negative and Gram positive. Gram-positive bacteria retain the crystal violet stain after the Gram-staining technique due to their thick cell wall layer of peptidoglycan. Gram-negative bacteria on the other hand, cannot retain the crystal violet stain due to their thin cell wall that has a very thin layer of peptidoglycan, and this layer is reinforced with a second membrane on top. Gram-negative bacteria are stained red with Safranin at the end of the Gram-staining technique (Parija, 2012; Tortora et al., 2010; Willey et al., 2016).

In the following section we will take a look at the main microbial activities that are necessary for their life, namely; metabolism, respiration, and bacterial growth.

2.4 Microbial Metabolism

Metabolism is the term used to describe all the biochemical reactions that take place inside a cell; it includes those reactions that release energy into the cell, and those that make use of that energy.

Most microorganisms obtain their energy from the nutrients they take into the cell. These microorganisms break the nutrients into smaller molecules, and then use these molecules for synthesis of new cellular components. They release the chemical energy stored in the nutrients and use it later to perform other processes.

On the other hand, some microorganisms obtain the energy needed for metabolism from the sun by means of photosynthesis (Davis and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

Catabolism is the term used to describe reactions that break down large molecules forming smaller molecules, usually associated with a release of energy. Anabolism is the term used to describe reactions involved in biosynthesis of macromolecules, usually requiring an input of energy (Davis and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

The energy released in catabolism is used by the microorganisms in different aspects (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016):
a. to synthesize new cellular components such as nucleic acids, polysaccharides, and enzymes
b. to transport certain substances into the cell from its surroundings
c. for the cell to grow and multiply
d. for cellular movement
e. to maintain the structural integrity of the cell by repairing any damage to its constituents

The process of metabolism involves the use of some important molecules such as enzymes, and electron and proton carriers.

Biosynthesis and metabolic processes in the cell are generally catalyzed by molecules that are called enzymes which are large molecules usually protein, specific to a particular reaction or group of reactions. Enzymes increase the rate of the reaction by lowering its activation energy through providing alternative pathway for the reaction. Enzymes bring substrates together at spatial places on their surface called active sites. Therefore, chances of effective interaction between substrates are enhanced and the rate increases (Tortora et al., 2010; Willey et al., 2016).

Coenzymes may assist the enzyme by accepting atoms removed from the substrate or by donating atoms required by the substrate. Some coenzymes act as electron carriers, removing electrons from the substrate and donating them to other molecules in subsequent reactions. Many coenzymes are derived from vitamins. Two important coenzymes are encountered while discussing metabolism are nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+); both are derivatives of the B vitamin niacin, and each can exist in an oxidized and a reduced form.

\[
\text{NAD}^+ + H^+ + 2e^- \rightleftharpoons -\text{NADH}
\]
\[
\text{NADP}^+ + H^+ + 2e^- \rightleftharpoons \text{NADPH}
\]

NAD+ is primarily involved in catabolic reactions, while NADP+ is primarily involved in anabolic reactions. The flavin coenzymes, such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), contain derivatives of the B vitamin riboflavin and are also electron carriers. Cytochromes are another type of electron carriers. Cytochromes are proteins with an iron-containing group that could exist as Fe^{2+} or Fe^{3+} (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

Energy taken up by the cell, either in the form of nutrients or sunlight, must be converted into a usable form. The most usable form of energy is a compound called adenosine triphosphate (ATP). ATP is by far the most
important of a class of compounds known as high-energy transfer. ATP stores energy derived from catabolic reactions (such as breaking down of glucose) and releases it later to drive anabolic processes (such as synthetic reactions) and perform other cellular works. ATP molecule (Fig. 10) consists of an adenine, a ribose, and three phosphate groups. The terminal phosphate group requires a lot of energy for its formation and is often referred to as a high-energy phosphate bond. When this bond is broken, the same large amount of energy is released, so when ATP is broken down, adenosine diphosphate (ADP) is formed, and energy is released to drive anabolic reactions.

The process of adding or removing a phosphate group is called **phosphorylation** or **dephosphorylation**, respectively (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

### 2.5 Cellular Respiration

The term cellular respiration refers to the biochemical pathway by which cells release energy from the chemical bonds of food molecules and provide that energy for the essential processes of life. All living cells must carry out cellular respiration. Respiration could be aerobic or anaerobic according to the availability of oxygen. Prokaryotic cells carry out cellular respiration within the cytoplasm or on the inner surfaces of the cells. In eukaryotic cells the mitochondria are the site of most of the reactions. The energy currency of these cells is ATP, and one way to view the outcome of cellular respiration is as a production process for ATP.
Prokaryotes take the carbohydrates into their cytoplasm, and through a complex series of metabolic processes, they break down the carbohydrate and release the energy. The energy is generally not needed immediately, so it is used to combine ADP with phosphate ions to form ATP molecules. During the process of cellular respiration, carbon dioxide is given off as a waste product. This carbon dioxide can be used by photosynthesizing cells to form new carbohydrates. Also in the process of cellular respiration, oxygen gas is required to serve as an acceptor of electrons. This oxygen gas is identical to the oxygen gas given off in photosynthesis (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

The overall mechanism of cellular respiration involves four subdivisions:

a. **Glycolysis**, in which glucose is oxidized to two molecules of pyruvate with the synthesis of two molecules of ATP. Glycolysis is summarized in Fig. 11. After glycolysis, two options are present depending on the amount of available oxygen. In the presence of oxygen, the cell will undergo aerobic respiration. Without a supply of oxygen, as in an anaerobic environment, the cell will undergo fermentation (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

b. **Krebs cycle, citric acid cycle (CAC), or the tricarboxylic acid (TCA) cycle**: which results in formation of (per two molecules of pyruvic acid) two ATP molecules, six NADH molecules, and two FADH$_2$ molecules and six CO$_2$ molecules. The NADH and the FADH$_2$ will be used in the electron transport system (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016). The main steps of Krebs cycle could be summarized as in Fig. 12.

c. **Electron transport**: In this step NADH and FADH produced in both glycolysis and the Krebs cycle are now being used. Electron transfer takes place in mitochondria in eukaryotic cells. The process is the same in prokaryotes, but it just happens at the main cell membrane. Cytochromes and coenzymes act as carrier molecules and transfer molecules. They accept high-energy electrons and pass the electrons to the next molecule in the system. At key proton-pumping sites, the energy of the electrons transport protons across the cell membrane to the outside (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

d. **Chemiosmosis**, in which the energy given off by electrons is used to pump protons across a membrane and provide the energy for ATP synthesis.

During cellular respiration (Krebs cycle reactions, the electron transport system, and chemiosmosis), 36 molecules of ATP can be produced for each
glucose molecule. Additional two ATP molecules are produced through glycolysis, so the net yield is 38 molecules of ATP. These ATP molecules may then be used in the cell for its needs. However, the ATP molecules cannot be stored for long periods of time, so cellular respiration must constantly continue in order to regenerate the ATP molecules as they are used. Each ATP molecule is capable of releasing 7.3 kcal of energy per mole (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

When oxygen is not available as final electron acceptor, the process of respiration after glycolysis cannot continue as described above. Therefore, an alternative pathway should be present such as fermentation or anaerobic respiration.
Fermentation is an anaerobic pathway for breaking down glucose in which organic carbon is the terminal electron acceptor, one that is performed by many types of organisms and cells. In fermentation, the only energy extraction pathway is glycolysis, with one or two extra reactions tacked on at the end. 

Fermentative organisms use NADH and other cofactors to produce many different reduced metabolic by-products, often including hydrogen gas (H₂). These reduced organic compounds are generally small organic acids and alcohols derived from pyruvate, the end product of glycolysis. Examples include ethanol, acetate, lactate, and butyrate. Fermentative organisms are very important industrially and are used to make many different types of food products. The different metabolic end products produced by each specific bacterial species are responsible for the different tastes and properties of each food.

Fig. 12 Krebs cycle.
Many microorganisms are **facultative anaerobes**, meaning they can switch between aerobic respiration and anaerobic pathways (fermentation or anaerobic respiration) depending on the availability of oxygen (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

In the process of **anaerobic respiration**, carbohydrate can be metabolized by a process that utilizes oxidative phosphorylation via an electron transport chain, but instead of oxygen serving as the terminal electron acceptor an **inorganic molecule** such as nitrate or sulfate is used. In addition, other organisms may turn to this form of respiration if oxygen is unavailable (**facultative anaerobes**).

Anaerobic respiration is not as productive as its aerobic counterpart in terms of ATP production, because electron acceptors such as nitrate or sulfate have less positive redox potentials than oxygen. Anaerobic respiration tends to occur in oxygen-depleted environments such as waterlogged soils. It must be underlined here that anaerobic respiration is **not** the same as fermentation. The latter process does not involve the components of the electron transport chain and generate much smaller amounts of energy (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

### 2.6 Anabolism and Use of Energy in Biosynthesis

Most organisms obtain energy by oxidizing complex organic compounds produced by other organisms, while other microorganisms are capable of producing the complex organic compounds from simple inorganic substances in a process called **photosynthesis**.

Photosynthesis involves two major processes:

- **The light reaction** or **energy fixing reaction** in which light energy from the sun is converted to chemical energy stored in ATP molecule. The organisms use the light energy, water molecules, NADP+, ADP, and phosphate to produce ATP, NADH, and oxygen molecule.

- **The Dark reaction** or **carbon fixing reaction**. In this reaction, organisms use ATP and NADH produced in the light reaction plus carbon dioxide absorbed from the atmosphere to produce carbohydrates, mainly glucose (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

Carbon fixation reaction is usually referred to as CO₂ fixation or **Calvin cycle**. Calvin cycle (Fig. 13) is the cycle in which carbon is fixed, reduced, and utilized. It involves enzymatically regulated chemical
The reaction used by autotrophic microorganisms for CO₂ fixation. The cycle produces phosphoglycerate and the glyceraldehyde-3-phosphate molecules which are subsequently used to synthesize all the organic compounds needed by the cell. Each turn of the cycle results in the fixation of one molecule of CO₂. Therefore, to synthesize one glucose molecule, the cycle must operate six times (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016). Fig. 13 shows the net outcomes of six cycles.

The energy generated in the catabolic pathways will be used by cells to manufacture the cellular components (biosynthesis) such as: nitrogenous substances, including proteins and nucleic acids, carbohydrates, including complex polysaccharides (such as capsules and the peptidoglycan of the cell wall), and Phospholipids which are components of the cytoplasmic membrane.

An autotrophic bacterium that can synthesize all its cellular constituents from CO₂ and simple inorganic compounds obviously is said to have great biosynthetic ability. In the same way, a heterotrophic bacterium that uses glucose as the only carbon and energy source, ammonium sulfate as the nitrogen and sulfur source, and a few additional inorganic compounds also has great biosynthetic capability. From these nutritional substances, the microorganisms
can manufacture anything they need. Bacteria are particularly adept at converting anything they can get hold of into any other item they need.

Glycolysis and the Krebs cycle produce a number of intermediates that can be used in amino acids synthesis. Amino acids can be converted to proteins or they can be incorporated in the production of nucleotides to form nucleic acids. These intermediates can be used also to form more complex carbohydrates. Acetyl CoA from Glycolysis can be oxidized to form fatty acids. Glucose can be converted into just about anything. Fatty acids and amino acids can be catabolized to feed into glycolysis or the Krebs cycle. These pathways are largely reversible and interconnected as shown in Fig. 14. Bacteria are extremely resilient, they just need something to work with, and they can manufacture the rest. For example, if they start with protein, they will break it down to amino acids, convert that into pyruvic acid, and be able to form carbohydrates (Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

Fig. 14 The interconnected anabolic and catabolic pathways.
2.7 Bacterial Growth

Bacterial growth refers to an increase in cell number, not in cell size. Bacterial growth is a complex process involving numerous anabolic and catabolic reactions. Ultimately, these biosynthetic reactions result in cell division which is done mostly by binary fission as shown in Fig. 15.

2.7.1 Bacterial Growth Requirements

The requirements for microbial growth can be divided into two main categories: physical and chemical. Physical requirements include temperature, pH, and osmotic pressure.

Chemical requirements include macronutrient and micronutrients (Davies, 2005; Davis and Cornwell, 2012; Gerardi, 2006; Hogg, 2005; Madigan et al., 2014; Mara and Horan, 2003; Parija, 2012; Tortora et al., 2010; Weiner and Matthews, 2003; Willey et al., 2016). Macronutrients include the elements that are involved largely in the composition of bacterial cells, such as carbon, hydrogen, nitrogen, oxygen, phosphorous, and sulfur. These elements are required in large quantities.
Micronutrients, on the other hand, contain minor elements such as Ca, Fe, K, Mg, and Na; organic compounds such as vitamins; and trace elements such as Co, Mn, Mo, Ni, and Zn. These micronutrients are required in relatively small quantities for most bacteria. Some bacteria such as methane-forming bacteria require sulfur and some minor and trace elements such as Co, Fe, and Ni in quantities, two to five times greater than other bacteria. Calcium is required in large amounts by Gram-positive bacteria for the synthesis of cell walls (Davis and Cornwell, 2012; Tortora et al., 2010).

2.7.2 Growth in Pure Culture and Growth Curve

Microbial culture is a method for increasing the number of organisms by letting them reproduce in controlled media. In a pure culture medium, cells are placed in a liquid medium in which the nutrients and environmental conditions are controlled to allow the reproduction of single species of microorganisms. If the medium supplies all nutrients required for growth and environmental parameters are optimal, the increase in numbers or bacterial mass can be measured as a function of time. The representation of the increase in numbers or bacterial mass as a function of time is called a growth curve. Several distinct growth phases can be observed within a growth curve as can be seen in Fig. 16. These includes the lag phase, the exponential or log phase, the stationary phase, and the death phase. Each of these phases represents a distinct period of growth that is associated with typical physiological changes in the cell culture (Davies, 2005; Davis

Fig. 16 Growth curve.
and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

The first phase observed under batch conditions is the lag phase in which the growth rate is essentially zero. When an inoculum is placed into a fresh medium, growth begins after a period of time called the lag phase. The lag phase is thought to be due to the physiological adaptation of the cell to the culture conditions (Davies, 2005; Davis and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

The second phase of growth observed in a batch system is the exponential phase. In this phase, the most rapid growth occurs. Exponential increase in the number of bacterial cells is observed in the growth curve. During exponential growth, the rate of increase of cells in the culture is proportional to the number of cells present at any particular time (Davies, 2005; Davis and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

The third phase of growth is the stationary phase. The stationary phase in a batch culture can be defined as a state of no net growth. Carbon and energy source or an essential nutrient becomes completely used up; therefore, there is no net growth in stationary phase. However, cells still grow and divide. This is because dying cells can lyse and provide a source of nutrients (Davies, 2005; Davis and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

The final phase of the growth curve is the death phase, which is characterized by a net loss of culturable cells. Even in the death phase there may be individual cells that are metabolizing and dividing, but more viable cells are lost than are gained so there is a net loss of viable cells. The death phase is often exponential, although the rate of cell death is usually slower than the rate of growth during the exponential phase (Davies, 2005; Davis and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

2.7.3 Growth in Mixed Culture
In nature, pure cultures do not exist, rather mixtures of species compete and survive within the limits set by the environment. Population dynamic is the term used to describe the time-varying success of the various species in competition. It is expressed quantitatively in terms of relative mass of microorganisms.

The prime factor determining the dynamic of the various microbial populations is the competition for food. The second most important factor is the predator–prey relationship.
The relative success of a pair of species competing for the same substrate is a function of the ability of species to metabolize the substrate. The more successful species will be the one which metabolizes the substrate more completely. In so doing, it will obtain more energy for synthesis and consequently will achieve greater mass.

Because of their relatively smaller size and thus larger surface area per unit mass which allow a more rapid uptake of substrate, bacteria will predominate over fungi. For the same reason, fungi predominate over protozoa.

When the supply of soluble organic substrate becomes exhausted, the bacterial population is less successful in reproduction and the predator population predominates. In a closed system with an initial inoculum of mixed microorganisms and substrate, the predominant populations will cycle as the bacteria give way to higher-level organisms that in turn die for lack of food and are then decomposed by different sets of bacteria. In an open system such as wastewater treatment plant or river, with a continuous inflow of new substrate, the predominant populations will change through the length of the plant (Davis and Cornwell, 2012; Hogg, 2005; Madigan et al., 2014; Tortora et al., 2010; Willey et al., 2016).

2.7.4 Microbial Biofilms

In nature, microorganisms seldom live in the isolated single-species colonies as we see on laboratory plates. They more typically live in communities called biofilms. Biofilms are an assemblage of microbial cells; in this assemblage cells stick to each other and often these cells adhere irreversibly. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilms contain also DNA and proteins that are often informally called slime.

Biofilms could be attached to a wide variety of surfaces, such as a rock in a pond, a human tooth, mucous membrane, indwelling medical devices, or water system piping.

Bacteria start to divide by binary fission. After their growth, those bacteria belonging to the same species will produce and secrete saccharide. This saccharide will form the extra polymer substance (EPS). EPS is very adhesive and is the protective membrane of a biofilm.

Living in biofilm community provides bacteria with resistance to antibiotic penetration. In addition, it allows bacteria to survive in low nutrient medium. If viewing an intact biofilm under microscope, one may immediately find that bacteria in biofilms do not randomly stick together, but rather form a well-organized community with numerous specialized configurations.
Cell-to-cell signaling or **quorum sensing (QS)** has been demonstrated to play a role in biofilm formation and cell attachment and detachment from biofilms. QS allows bacterial cells to communicate. It can occur within a single bacterial species as well as between diverse species.

QS is highly associated with cell number. Induction of QS gene system requires the accumulation of signaling molecules called **autoinducers such as** acyl homoserine lactones (**AHLs**) or **peptide pheromone**. For example, Gram-negative bacteria adapt luxI/luxR gene signaling system using AHLs autoinducers. Lux gene is initially expressed in low levels, leading to the production of small number of lux proteins including luxI and luxR. luxI code for synthesis of autoinducer AHL. Autoinducer molecules rapidly diffuse out of the cell. The more the number of cells is in a given space, the faster the inducers build up and the more likely they reenter the cells, bind to receptor, and stimulate the expression of luxR. LuxR protein will stimulate the expression of many genes including those for inducer synthesis. The produced gene could trigger many cellular activities such as the formation of biofilms. It has been suggested that biofilm formation results from a developmental program of gene expression. In addition, biofilm development and maintenance have been shown to require a wide range of genetic determinants and to involve bacterial subpopulations carrying out different functions. Quorum sensing was found to play a significant role in coordinating biofilm formation for many species (Donlan, 2002; Irie and Parsek, 2008; Li and Tian, 2012).

Biofilms also might be useful in wastewater treatment plants. The floc that forms in certain types of sewage treatment is an example. Many wastewater treatment plants include a secondary treatment stage in which wastewater passes over biofilms grown on filters, which extract and digest organic compounds. In such biofilms, bacteria are mainly responsible for removal of BOD from water, while protozoa and rotifers are mainly responsible for removal of suspended solids (SS), including pathogens and other microorganisms. On the other hand, biofilms could have adverse effect on wastewater treatment process. For instance biofilm is a major drawback in the performance of membrane bioreactor (MBR) (Davis, 2010; Tortora et al., 2010; Weiner and Matthews, 2003).

### 2.8 Health Risks Related to Microorganisms in Water

The pathogenic microbiological agents that may be present in water intended for human consumption are essentially bacteria, viruses, or
protozoa, most of them derived from human or animal waste. The consequences of exposure to pathogenic bacteria, viruses, and pathogens in the water may vary. The most common symptoms are nausea, vomiting, and diarrhea. Infants, children, older people, as well as people with compromised immune systems may have more serious symptoms. In extreme cases, some pathogens can infect the lungs, skin, eyes, nervous system, kidneys, or the liver, and the effects may be more severe, chronic, or even fatal.

The presence of pathogenic microorganisms in tap water is linked to a lack of protection of the resource, to a lack of water treatment, or to backflow into the drinking water system.

Worldwide, the presence of microorganisms in water kills millions of people every year. It is estimated that half of the world’s population contract diseases that are the direct consequence of polluted water (typhoid fever, cholera, dysentery, toxoplasmosis, and cryptosporidiosis). Thousands of children die every day from contaminated water.

Microbial waterborne diseases also affect developed countries. In the United States, it has been estimated that 560,000 people suffer from severe waterborne diseases yearly, and 7.1 million suffer from a mild to moderate infections, resulting in estimated 12,000 deaths a year (Cabral, 2010; WHO, 1997, 2017). The most important bacterial diseases transmitted through water are listed in Table 5 (Cabral, 2010).

According to the WHO in 2015, 71% of the global population (5.2 billion people) use safe drinking water that is free from contamination.

| Disease                        | Causal Bacterial Agent                                           |
|-------------------------------|-----------------------------------------------------------------|
| Cholera                       | *Vibrio cholerae*, serovarieties O1 and O139                     |
| Gastroenteritis caused by vibrios | Mainly *Vibrio parahaemolyticus*                               |
| Typhoid fever and other serious salmonellosis | *Salmonella enterica* subspecies *enterica* serovar Paratyphi |
|                               | *Salmonella enterica* subspecies *enterica* serovar Typhi       |
|                               | *Salmonella enterica* subspecies *enterica* serovar Typhimurium |
| Bacillary dysentery or shigellosis | *Shigella dysenteriae*                                           |
|                               | *Shigella flexneri*                                              |
|                               | *Shigella boydii*                                                 |
|                               | *Shigella sonnei*                                                 |
| Acute diarrheas and gastroenteritis | *Escherichia coli*, particularly serotypes such as O148, |
|                               | O157, and O124                                                   |
However, 844 million people lack even a basic drinking water service, including 159 million people who are dependent on surface water.

Safe and readily available water is important for public health, whether it is used for drinking, domestic use, food production, or recreational purposes. Improved water supply and sanitation, and better management of water resources can boost countries’ economic growth and can contribute greatly to poverty reduction.

2.9 Control of Microorganisms

In some situations, it is necessary for us to destroy, or at least limit, microbial growth because of the undesirable consequences of the presence of microorganisms or their products.

Control of microorganisms can be achieved by a broad variety of chemical and physical methods. Some common terms are associated with microbial control such as sterilization, disinfection, antisepsis, sanitation, decontamination, and degerming.

**Sterilization** is the removal or destruction of all forms of microbial life. Sterilization is generally achieved by using physical means such as heat, radiation, and filtration. **Disinfection** usually involves the destruction of vegetative pathogens which is not the same thing as complete sterility.

**Antisepsis** is the destruction of vegetative pathogens on living tissue and the chemical used for this purpose is called an **antiseptic**. **Sanitation** is the treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels. **Decontamination** is the treatment of an object or inanimate surface to make it safe to handle. On the other hand, **degerming** involves removal of microbes from a limited area, such as the skin around an injection site. Degerming is mostly a mechanical removal by an alcohol-soaked swab.

Some other terms are associated with treatments that cause the outright death of microbes. **Biocides** or **germicides** are the agents that kill microorganisms; a **fungicide** kills fungi; a **virucide** inactivates viruses. **Antibiotic** which is a metabolic product produced by one microorganism that inhibits or kills other microorganisms. **Static**, on the other hand, is the agent that is static in action and will inhibit the growth of microorganisms (McDonnell and Russell, 1999; Russell, 2002; Tortora et al., 2010).

Biocidal activity depends upon several factors, notably, concentration, period of contact, pH, temperature, the type, nature, and numbers of microorganisms to be inactivated, and the presence of interfering material.
Microorganisms vary considerably in their response to antiseptics and disinfectants. Viruses with lipid envelopes are the least susceptible, followed by gram-positive bacteria, viruses without envelopes, fungi, including most fungal spores, then gram-negative bacteria, vegetative protozoa, cysts of protozoa, mycobacteria, endospores of bacteria, and finally prions which have the highest resistance (McDonnell and Russell, 1999; Russell, 2002; Tortora et al., 2010).

Disinfection is a very crucial step in water treatment processes as well as many industries since it aims at destroying all harmful microorganisms. Disinfection could be accomplished through the use of chemicals, ultraviolet radiation, boiling water, or steam. In practice, the term disinfection is most commonly applied to the use of a chemical (a disinfectant) to treat an inert surface or substance. Several categories of disinfectants can be used for commercial and industrial purposes such as chlorine compounds, alcohols, aldehydes, phenols, and hydrogen peroxide.

Disinfection is the final component in most water treatment processes. Disinfection serves two purposes: as a final stage of treatment, in order to remove any remaining microorganisms that have survived the preceding processes; it may also provide partial protection against recontamination within distribution. Disinfection step in water treatment plants is commonly done with chlorination, ultraviolet (UV) radiation, or ozonation (Davis, 2010; Davis and Cornwell, 2012; Tortora et al., 2010; Weiner and Matthews, 2003). Chlorination which is the most common disinfectant in water and wastewater treatment plants is very powerful in eliminating coliforms or *E. coli*. However, it was found that some pathogens, especially viruses and protozoan (*Giardia lamblia* and *Cryptosporidium*), are more resistant than coliforms to chlorination. This emerges the need for using alternative disinfectant (i.e., chlorine dioxide) or coupling of more than one disinfectant in the disinfection steps.

### 2.10 Indicators of Water Microbiological Quality

The occurrence of microbial pathogens is often difficult to determine due to many factors. Pathogens are usually present in low numbers so it is necessary to monitor waters using “indicator” organisms, e.g., *E. coli*, *fecal*, *thermotolerant*, or *total coliforms*. The assumption of this approach is that the presence of bacterial indicator organisms is associated with the presence of microbial pathogens.

The traditional indicator group for all microbial pathogens has been the coliform bacteria. Their ease of enumeration, crude relationship to pathogen
and disease occurrence, and the lack of better indicators led to the adoption of coliform counts in most international standards documents. During the last 20 years, numerous shortcomings of the use of coliforms as indicators of water-borne disease have been identified, and possible alternative indicators have been suggested; however, one all-purpose indicator has not emerged.

For example, *Pseudomonas aeruginosa* are alternative indicator for the traditional total coliforms, *Candida albicans* are alternative indicator for *E. coli*, and *Bifidobacteria* are alternative indicator for *Fecal streptococci* (Russell, 2002; Tortora et al., 2010).

These indicators are distinguished using various methods based on enumeration procedures such as membrane filtration (MF) or multiple tube dilution or most probable number (MPN). These methods will be discussed briefly in the following section.

### 2.11 Microbiological Water Analysis

The detection and enumeration of water quality indicators currently relies on the use of a few basic methods: membrane filtration (MF), multiple tube or most probable number (MPN) method, the standard plate count (SPC) or total bacterial count, and presence–absence (PA) test.

It should be noted that defined enzyme substrate technologies (DST) have been incorporated into each of these methods (PA, MPN/MTF, and MF) and are increasingly used in microbiological testing generally, both clinically as well as for food and water analysis and monitoring.

Each method has its advantages and disadvantages and has its applications according to the type of information needed and the type of water sample to be tested (Mara and Horan, 2003; Russell, 2002; Smith et al., 2013; Tortora et al., 2010).

#### 2.11.1 Membrane Filtration Method

In the membrane filtration (MF) method, a measured volume of sample is passed through a membrane filter of known pore size that retains bacteria on or near the filter surface. The colonies that develop after incubation on a selective medium are presumed to have originated from individual bacteria, thus representing a direct count of the number of bacteria in the original sample. The MF method is often the method of choice in the analysis of most water types (Russell, 2002; Smith et al., 2013; Tortora et al., 2010).

#### 2.11.2 Multiple Tube or Most Probable Number Technique

The multiple tube technique (MTF) or most probable number (MPN) technique involves three stages: the initial stage that is presumptive because
the acid and gas, or growth/no growth, reactions may be caused by organisms other than those being selected; the **confirmed test** (the second stage which is usually the extent of testing), and the **completed test** (the third stage which is used for total coliform analyses only). Although the completed test provides the greatest reliability, the workload of the testing laboratory and the time for all tests to be completed may restrict its use (Russell, 2002; Smith et al., 2013; Tortora et al., 2010).

The MTF or MPN technique involves the inoculation of measured volumes of decimal dilutions of a sample into replicate tubes containing an inverted Durham tube of a selective liquid medium. The tubes are incubated at 35°C or 37°C for 24 h. Positive tubes can be identified by the presence of gas in the Durham tube. Negative tubes are reincubated for a further 24 h. At the end of this period, the tubes are checked again for gas production. Gas production at the end of either 24 or 48 h incubation is presumed to be due to the presence of coliforms in the sample. Confirmation test should be carried out after each incubation in order to confirm the presence of thermotolerant (fecal) coliform. The positive results of the confirmed test with the aid of probability tables are then used to obtain the most probable number (MPN) (Russell, 2002; Smith et al., 2013; Tortora et al., 2010).

### 2.11.3 Standard Plate Count

The viable aerobic and facultative anaerobic, heterotrophic bacterial count of water is generally enumerated by either **pour plate** or **surface plate** technique. The procedures provide data on the bacterial population capable of growth at the incubation temperature–time combinations used and do not estimate the total bacterial population present in the water.

It is the most frequently used method of measuring bacterial populations. An important advantage of this method is that it measures the number of viable cells. A plate count is done by either the pour plate method or the spread plate method.

In the **pour plate** method either 1.0 mL or 0.1 mL of dilutions of the bacterial suspension is introduced into a Petri dish. The nutrient medium, in which the agar is kept liquid by holding it in a water bath at about 50°C, is poured over the sample, which is then mixed into the medium by gentle agitation of the plate. When the agar solidifies, the plate is incubated. With the pour plate technique, colonies will grow within the nutrient agar as well as on the surface of the agar plate.

**Spread plate** method is frequently used instead. A 0.1 mL inoculum is added to the surface of a prepoured, solidified agar medium. The inoculum is then spread uniformly over the surface of the medium with a specially
shaped, sterilized glass or metal rod. This method positions all the colonies on the surface and avoids contact between the cells and melted agar.

Some recent methods include a fluorescent agent so that counting of the colonies can be automated. At the end of the incubation period, the colonies are counted by eye, a procedure that takes a few moments and does not require a microscope as the colonies are typically a few millimeters across (APHA, 2012; Russell, 2002; Smith et al., 2013; Tortora et al., 2010).

### 2.11.4 Presence–Absence Procedure

The presence or absence of pathogens, or other traditional indicator organisms, is frequently determined economically by presence–absence test (PA), which is a simplification of the multiple-tube procedure that permits qualitative information, as opposed to quantitative methods.

The PA test involves analyses of one large test volume of water in a single culture bottle to obtain qualitative information. The utility of the procedure is its logistical simplicity relative to MF or MPN techniques. As regulatory limits for drinking waters are commonly <1 mL coliform or E. coli/100 mL, PA format analysis allows such determinations by simply analyzing for the presence of the respective organism in that volume of sample with minimal processing (APHA, 2012; Russell, 2002; Smith et al., 2013).

### 2.12 The Role of Microorganisms in Wastewater Treatment Plants

The basic objectives of wastewater treatment are to remove oxygen-demanding organic wastes and to remove nutrients to levels where photosynthetic organisms in receiving waters are limited in their growth.

Biological, wastewater treatment plants are simply biological amplifiers. The plants permit organisms (biomass or sludge), primarily bacteria, to increase in number by using the pollutants (substrates and nutrients) in the wastewater and converting them to new organisms (biomass or sludge) and nonpolluting wastes and less polluting wastes (Davis, 2010; Davis and Cornwell, 2012; Gerardi, 2006; Weiner and Matthews, 2003).

There are three basic categories of biological treatment: aerobic, anaerobic, and anoxic. **Aerobic biological treatment** involves contacting wastewater with microbes and oxygen in a reactor to optimize the growth and efficiency of the biomass. The microorganisms catalyze the oxidation of biodegradable organics and other contaminants such as ammonia, generating innocuous by-products such as carbon dioxide, water, and excess biomass (sludge).
Anaerobic (without oxygen) and anoxic (oxygen-deficient) treatments are similar to aerobic treatment, but use microorganisms that do not require the addition of oxygen. These microorganisms use compounds other than oxygen to catalyze the oxidation of biodegradable organics and other contaminants, resulting in safe by-products.

The three individual types of biological treatment technologies (aerobic, anaerobic, or anoxic) can be run in combination or in sequence to offer greater levels of treatment. Regardless of the type of system selected, accomplishing effective biological treatment is guaranteed by developing and maintaining an acclimated, healthy biomass, sufficient in quantity to handle maximum flows and the organic loads to be treated (Davis, 2010; Davis and Cornwell, 2012; Gerardi, 2006; Weiner and Matthews, 2003).

2.12.1 Secondary Treatment Processes

A typical wastewater treatment plant comprises three phases of treatment (Fig. 6): primary, secondary, and tertiary. Secondary treatment is mainly biological and may contain several processes.

The major goal of secondary treatment is to remove BOD that escapes primary treatment and to provide further removal of suspended solids. However, due to the increasing recognition of the adverse effects of nutrients, secondary treatment often includes treatment of nitrogen and phosphorus.

The main unit processes that are employed in secondary treatment are aerobic oxidation, nitrification, denitrification, phosphorous removal, and activated sludge selector. The objectives of these processes may be summarized as (Davis, 2010; Davis and Cornwell, 2012; Gerardi, 2006; Weiner and Matthews, 2003):

- **Aerobic oxidation** which is concerned with the removal of BOD. This process is accomplished by aerobic heterotrophic bacteria.

- **Nitrification** which involves the oxidation of ammonia to nitrate. Aerobic autotrophic bacteria must be predominant to carry out nitrification. Two genera are commonly recognized. Ammonia is oxidized to nitrite by *Nitrosomonas*. Nitrite is oxidized to nitrate by *Nitrobacter*.

- **Denitrification** involves the reduction of nitrate to nitric oxide, nitrous oxide, and nitrogen gas. A large number of genera are capable of denitrification, either heterotrophic or autotrophic. *Pseudomonas* is the most common species.

- **Phosphorous removal**: in this process phosphorus is incorporated into cell biomass that is subsequently removed from the process. Genera such as *Acinetobacter*, *Arthrobacter*, *Aeromonas*, *Nocardia*, and *Pseudomonas* are responsible for this transformation.
Selector: is a bioreactor design that favors the growth of floc-forming bacteria instead of filamentous bacteria so that the biomass has better settling and thickening properties.

The unit processes for biological treatment may be either suspended growth, where microorganisms are suspended in the wastewater or attached growth, where the microorganisms grow on a solid surface.

Suspended growth methods include activated sludge processes, aerated lagoons, and aerobic digestion. Attached growth methods include trickling filters, Rotating Biological Contactors, and Fixed-film Reactors (Davis, 2010).

2.12.2 Activated Sludge Method vs Membrane Bioreactor

One of the most commonly used methods of secondary treatment are activated sludge systems. The term activated sludge refers to particles produced in wastewater by the growth of organisms in aeration tanks. Aerobic bacteria are the most common organisms in activated sludge. However, facultative bacteria along with higher organisms such as fungi and protozoa can be present. The exact composition of bacteria depends on the reactor design, environment, and wastewater characteristics.

The sludge microorganisms oxidize the organic carbon in the wastewater to produce new cells, carbon dioxide, and water. As microorganisms grow, they form particles that clump together. These particles (floc) are allowed to settle to the bottom of the tank, leaving a relatively clear liquid free of organic material and suspended solids. The effluent can be discharged into a river or treated in a tertiary treatment facility if necessary for further use.

There is a large variety of design; however, in principle all activated sludges consist of three main components: an aeration tank, which serves as a bioreactor; a settling tank or clarifier for separation of activated sludge solids and treated wastewater; and a return activated sludge equipment to transfer settled activated sludge from the clarifier to the influent of the aeration tank. The mixture of activated sludge and wastewater in the aeration tank is called mixed liquor. The average amount of time that microorganisms are kept in the system is called solids retention time (SRT). The time that a fluid particle remains in the reactor is called hydraulic retention time (HRT).

Activated sludge processes show many advantages such as: resistant to organic and hydraulic shock loads, high reduction of BOD and pathogens, and high effluent quality. On the other hand, these processes have some disadvantages such as: high energy consumption, high capital and operating costs, prone to complicated chemical and microbiological
problems, and sludge and possibly effluent require further treatment and/or appropriate discharge.

**Membrane bioreactor (MBR) technology** has emerged as a wastewater treatment technology of choice over the activated sludge process. It overcomes the drawbacks of the conventional activated sludge process.

MBRs have two fundamental process arrangements: (1) integrated systems that have membranes immersed in an activated sludge reactor and (2) separate systems that have a membrane module placed outside the reactor. Immersed membranes are the most popular arrangement.

The membranes (which could be flat sheet or tubular) have porosities ranging from 0.035 to 0.4 μm (which is considered between micro- and ultrafiltration). When immersed in wastewater, the membrane forms a barrier for impurities, while allowing pure water molecules to pass. Process arrangements for implementation of MBR for nitrification, nitrogen removal, and complete biological nutrient removal could be also found.

The MBR technology provides the following advantages over activated sludge process: it eliminates the requirement of large space for settling tank (or clarifier) which is replaced by the membrane, thereby reducing plant footprint which is usually 30%–50% smaller than an equivalent conventional activated sludge facility; it produces high-quality effluent with higher volumetric loading rates; it needs shorter hydraulic retention time (HRT), and longer solid retention times (SRT); it leads to less sludge production. A membrane bioreactor is able to process significantly higher sludge concentrations (10–20 g/L) and lower reactor volumes, compared to conventional activated sludge systems.

However, the use of MBR technology has disadvantages, including higher energy costs, the need to control membrane fouling problems, and potential high costs of periodic membrane replacement.

**Membrane fouling** is believed to be a major drawback of MBR, as it significantly reduces membrane performances and membrane lifespan, leading to an increase in maintenance and operating costs and low treatment efficiency. Membrane fouling in MBRs is attributable to suspended particulates in activated sludge that includes biomass of microorganisms and cell debris, colloids, solutes, and sludge flocs. These materials deposit onto the membrane surface and into the membrane pores, clogging the pores, and leading to a decline in the permeability of the membrane. When the clogging of membrane is due to accumulation of biomass of microorganisms, it is referred to as membrane biofouling.

Membrane biofouling is caused by several physicochemical and biological processes, which are highly dependent on the composition of feedwater,
membrane characteristics, operation conditions, and microorganisms present. **Biofilms** formation is thought to be essential in membrane biofouling. Therefore, controlling biofilm formation in membranes can prevent MBR failures.

In order to overcome the fouling problem, several operational strategies have been employed; however, membrane fouling remains an issue of investigation in MBR operation.

Understanding the biofouling phenomenon in MBR is crucial to be able to control this problem. Hence, it is essential to characterize the microbial community involved in the development of mature biofilms in MBR.

Biofilm formation begins with the colonization of bacteria and subsequent surface attachment. The initial colonization starts with the detection of **quorum sensing** (QS) signal molecules (such as AHLs) from the surrounding environment. The QS regulates the bacterial group behaviors in a cell density-dependent way and is closely associated with biofouling of the membrane in MBR treating wastewater. Thus, characterization of activated sludge AHLs will provide an additional insight into the bacteria involved in MBR. Also it helps in developing more effective strategies to deal with the biofouling problem in MBRs, for example, by blocking of intercellular communication to prevent biofilm formation (Lade et al., 2014; Yeon et al., 2009).

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FURTHER READING

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