1

Urban Wastewater Composition

Domestic wastewater contains about 99.9% water. The residual part is comprised of dissolved suspended organic and inorganic matter, as well as microorganisms. These 0.1% are what constitute water pollution and require that it be treated.

1.1. Main physicochemical parameters of raw wastewater

When designing a wastewater treatment plant (WWTP), it is useful to determine the different compounds present in the wastewater. Treated wastewater contains solids, dissolved substances and microorganisms. These latter are the main cause of restrictions imposed on reusing wastewater because they very often contain elements that are harmful to health, such as organic micropollutants and pathogen agents such as viruses, bacteria, protozoa and helminths. The load in pathogens in raw water is essentially a function of the health status of the population from which the wastewater originates. The risk of waterborne infection by these pathogens depends on a combination of a number of factors that include their concentrations, their dispersion in water, the capacity of these intestinal agents to survive in the environment, the quality of the water purification treatment, the infective dose, the exposure and the susceptibility of the exposed population.

The main prevalent parameters in treated wastewater that deserve special attention are:

– the indicators of organic matter (biological oxygen demand and chemical oxygen demand);

– suspended solids;

– mineral substances;

– heavy metals;

– microbiology.

Carbonaceous organic matter existing in urban wastewater is made up of a heterogeneous mixture of organic compounds of various structures and variable origin, including natural organic matter, soluble microbial products, residues of pharmaceutical products, protein compounds, carbohydrates, lipids, urea, surfactants, phenols, industrial residues, bacterial degradation by-products, pesticides and endocrine disruptors.

This great heterogeneity in the composition of urban wastewater must be taken into account when reusing it to be consumed as direct or indirect drinking water or for specific industrial applications, groundwater recharge, or irrigation of food eaten raw.

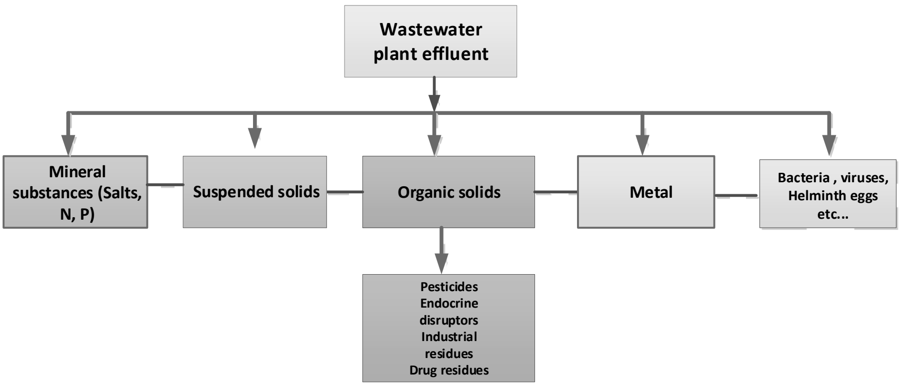

Figure 1.1. *Physicochemical and microbiological characteristics of wastewater*

The microbiological composition of wastewater is extremely variable according to the season, the origin of the collected wastewater, the population health status, etc. As a result, it contains a wide variety of microorganisms, with varying concentrations, potentially pathogenic to humans (bacteria, molds and their toxins, viruses and parasites) and likely to induce health hazards via respiratory, mucocutaneous and/or ingestion routes depending on how sensitive the person exposed is and the dose of microorganisms a person is exposed to (Michael-Kordatou 2015).

1.1.1. *Suspended solids*

Suspended solids (SS, or suspended particles) refer to insoluble solids that can be seen with the naked eye present in suspension in a liquid. Total suspended solids (TSS) is a measure of suspended solids.

Suspended solids are also studied as a "matrix" capable of adsorbing various pollutants, which can be transported along with the water in treatment plants. A distinction is made between:

– colloidal suspended solids (between 10 nm and 1 µm in size);

– dissolved solids (less than 10 nm in size).

The fine suspended particles in treated wastewater either have a natural origin in connection with precipitation or an anthropogenic origin and are then brought by atmospheric deposition and/or by urban, agricultural, domestic, rural and industrial discharges. SS can be classified according to their size and condition, chemical characteristics and settleability.

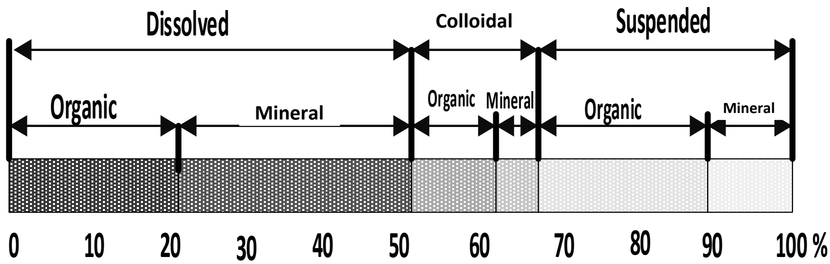

Figure 1.2. *Typical distribution of suspended solids in urban wastewater*

Raw municipal wastewater, which is a mixture of domestic wastewater and industrial wastewater, varies in composition by the hour, by the day and from one place to another. The total solid content (TSC) of raw wastewater (i.e. the sum of species of dissolved colloidal and suspended solids) depends on water consumption per capita and the nature of industrial discharges into the sewer drainage basin. The TSC value usually ranges between 500 and 1,000 $mg.L^{-1}$. A typical fractional characterization of solid matter in raw municipal wastewater into organic, inorganic, dissolved and suspended species is presented in Figure 1.2.

The inorganic particulate fraction of municipal wastewater presents a much higher density and sedimentation rate than the organic particulate fraction. It is partly eliminated during the first stage of treatment. The remaining particle size ranges from 10 mm to colloidal size. Approximately 55–65% of total suspended solids (TSS) in municipal wastewater are settleable and therefore would be removed by a gravity sedimentation process.

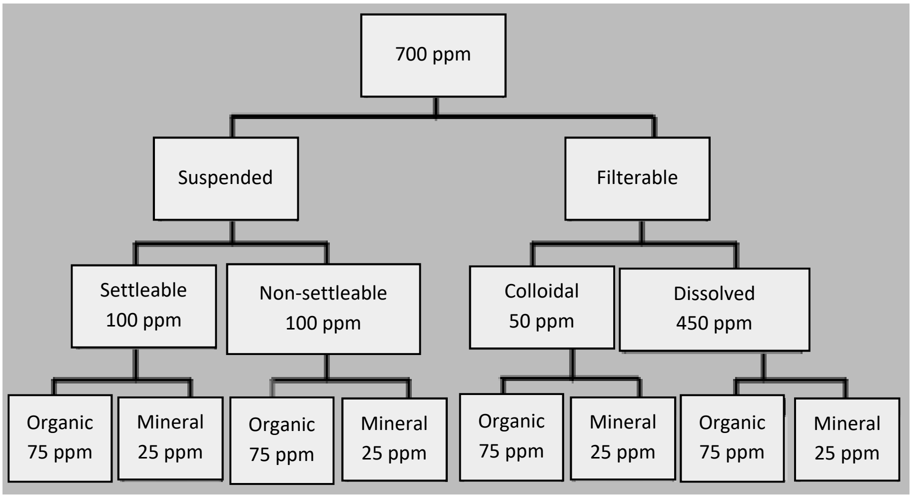

Figure 1.3. *Example of suspended solid distribution*

In general, dissolved solids are those with a diameter smaller than 10^{-3} µm, colloidal solids are those with diameters comprised between 10^{-3} and 100 µm and suspended solids are those with a diameter greater than 100 µm.

The term "particulate" is used to indicate that matter existing in wastewater is present in the form of particles. These are called suspended solids. In this context, expressions such as particulate BOD, particulate COD and particulate phosphorus serve to indicate that the parameters are related to suspended solids. In contrast, soluble BOD, COD and phosphorus are associated with dissolved substances.

Weight materials present in the effluent are subdivided into various forms which can be represented by:

– suspended solids (SS);

– volatile suspended solids (VSS);

– suspended mineral solids;

– settleable solids;

– non-settleable solids.

Suspended solids are neither solubilized nor colloidal. It may be considered that they represent an intermediate between mineral sand or coal dust particles and mineral particles of the mucilaginous type. They contain organic matter and mineral matter. Two techniques are currently used for dosing suspended solids:

– filtration separation (paper filters, membranes);

– centrifugation.

Suspended solid contents are obtained after drying at 105°C a known sample volume. SS concentrations in wastewater are highly variable, ranging from about 250 to 400 $mg.L^{-1}$. Volatile suspended solids represent the organic fraction of suspended solids. They are measured by calcination at 550°C of a sample whose SS content is already known. They make up about 70–80% of SS. When solids are submitted to a high temperature (550°C), the organic fraction is oxidized (volatilized), leaving only the inert (non-oxidized) fraction after combustion. Volatile solids represent an estimate of organic matter in solids, while non-volatile (fixed) solids represent inorganic or mineral matter.

Mineral solids represent the difference between suspended solids and volatile solids. They indicate the weight of calcination residues and correspond to the presence of salts, silica or dust, for example. Settleable solids are considered to be capable of settling during a one-hour period. The volume of solids accumulated at the bottom of a container called an Imhoff Cone is measured and expressed in $mL.L^{-1}$. The fraction that does not settle represents non-settleable solids. Suspended solids (SS) are mostly biodegradable in nature. The largest proportion of pathogen microorganisms contained in wastewater are transported by SS. In treated water, the limit concentration of suspended solids of 35 $mg.L^{-1}$ requires much more advanced treatment to deal with health risks and applied technologies. For example, excessive suspended solids can lead to complications in the transport and distribution of effluents as well as clogging of irrigation systems.

1.1.2. *Organic matter indicators*

The organic matter present in wastewater is an important parameter that is the source of the main problems of water pollution. The organic substances present (Pessoa and Jordao 1982) in wastewater are mainly composed of:

– protein compounds (≈ 40%);

– carbohydrates (≈ 25 to ≈ 50%);

– oils and fats (≈ 10%);

– urea, surfactants, phenols, pesticides and others (< 10%).

Carbonaceous organic matter (organic carbon-based) present in wastewater can be divided into several fractions:

– suspended (particles);

– dissolved (soluble);

– inert;

– biodegradable.

Direct or indirect methods are used for the quantification of organic matter:

– indirect method: measurement of oxygen consumption:

- biochemical oxygen demand (BOD),

- ultimate biochemical oxygen demand (uBOD),

- chemical oxygen demand (COD);

– direct method: measurement of total organic carbon (TOC).

1.1.2.1. *Biological oxygen demand (BOD_5)*

The main ecological effect of organic pollution in a body of water is the decrease in the concentration of dissolved oxygen. The wastewater pollution potential is evaluated by measuring oxygen consumption. This quantification can be obtained by stoichiometric calculations of the oxidation reactions of organic matter. If the substrate is, for example, glucose ($C_6H_{12}O_6$), the amount of oxygen required to oxidize this substance can be easily calculated. This is the principle of theoretical oxygen demand (TOD).

However, wastewater exhibits great heterogeneity in its compositions, and it is not possible to establish a list of all its constituents and to calculate the oxygen demand according to the chemical oxidation reactions of each of them. The solution is to measure in the laboratory the oxygen consumption by a standard volume of wastewater in a given time interval. The concept of biological oxygen demand (BOD) was thus introduced. BOD represents the amount of oxygen needed to oxidize organic (biodegradable) matter by biological means (oxidation of biodegradable organic matter by bacteria). Biological oxygen demand (BOD) is a water pollution index used for evaluating the biodegradable fraction of the carbon pollutant load in wastewater, and is generally calculated after five days at 20°C and protected from light: this is referred to as BOD_5.

BOD is an evolutionary phenomenon in the sense that it enables the study of the behavior of an organic load in the presence of microorganisms. BOD can only be determined if the existing microorganisms are able to simulate the organic substances present in the water.

The evolution of a sample of urban wastewater reveals a representation of organic matter that is always identical, in two successive stages (see Figure 1.2):

– The first stage relates to carbon compounds. It initiates quickly and is completed in 20 days, working at a temperature of 20°C.

– The second stage corresponds to the oxidation of nitrogenous compounds, which only initiates after the tenth day, due to the slow kinetics of the bacteria that convert ammoniacal nitrogen into nitrites and nitrates.

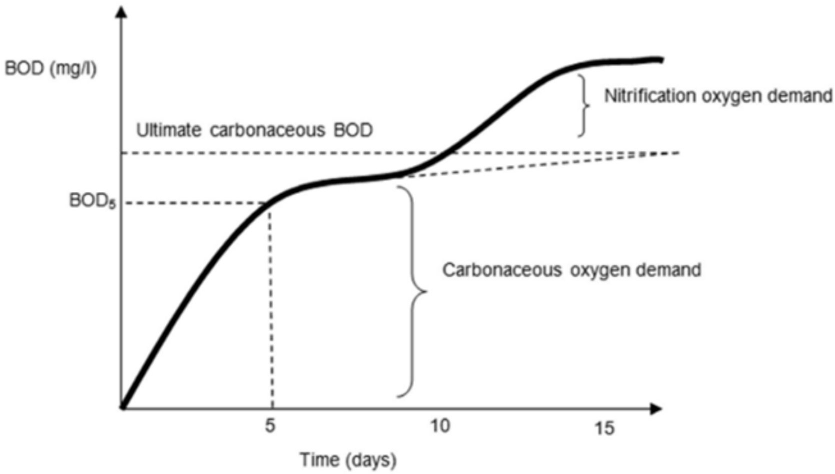

Figure 1.4. *BOD evolution at 20°C*

In fact, studies have resulted in a mathematical law of BOD evolution:

$$BODu = BOD\infty\ (1\text{-}e^{-kt})$$

where the BOD∞ is the BOD corresponding to the total mineralization of carbonaceous organic matter and k is a variable factor of about 0.2 for urban wastewater.

Therefore, at 20°C, on the first day, 20.6% of the BOD of the first stage disappears, on the second day 20.6% of the remaining BOD disappears, that is, 20.6% of the remaining 79.4%. On the third day, 20.6% of this remaining fraction disappears, etc., as shown in Table 1.1.

Oxidation time (days)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
% oxidation (1st stage) at 20°C	20.6	37	50	60	68.4	75	80	84
Oxidation time (days)	**9**	**10**	**12**	**14**	**16**	**18**	**20**	**25**
% oxidation (1st–2nd stage) at 20°C	87	90	94	96	97.5	98.4	99	99.7

Table 1.1. *O_2 consumption over time in a wastewater sample*

In practice, BOD measurements are limited to oxygen demand during the first five days: this is the BOD_5 measured at 20°C. It should be noted that the daily fraction of consumed BOD is different depending on the temperature, as shown in Table 1.2.

Temperature °C	5	10	15	20	25	30
% consumed	10.9	13.5	16.7	20.6	25.2	30.5

Table 1.2. *Consumed BOD according to incubation temperature*

In practice, complete stabilization takes several days (about 20 days or more for domestic wastewater). This corresponds to the ultimate biological oxygen demand (uBOD). However, to shorten the duration of the laboratory test and obtain results in a reasonable time, standardization was established.

BOD is determined after five days, hence the term BOD_5. The test is carried out at a temperature of 20°C. Ultimate BOD is expressed in BOD_{20}.

The BOD test follows a fairly simple protocol. BOD is commonly determined using the dilution method. It consists of diluting the water to be analyzed in a certain ratio, with clean water saturated with dissolved oxygen. The first step consists of determining the quantity of dissolved oxygen remaining after this mixture, because there is an immediate demand from the first minutes, caused by mineral or organic reducing substances.

A second fraction of this mixture is stored at 20°C for five days, after which the amount of residual oxygen is determined.

The product of the difference ($O_{22}(0)$ – $O_2(t)$) multiplied by the dilution ratio gives BOD_5.

Dilution water is the subject of much controversy. Some authors recommend the use of rural river water, as long as this water is not itself polluted. When no clean river water is available, an attempt is made to reproduce it from distilled water by addition of nutrient salts such as phosphates (sodium and potassium) and chlorides (calcium and magnesium; ammonium and ferric). These results allow the obtaining of synthetic dilution water. This water is next aerated to saturation and then used for analysis.

When the water to be analyzed is low in organic substances, the dilution water is inoculated with pure bacterial strains in a defined quantity, or by adding a fraction of river water. Some even suggest not washing the incubation vials so that there is self-seeding. This may be the case when it is always the same type of water that is analyzed (continuous controls of influents and effluents at the treatment plant).

It is recommended that different dilutions be done with several samples to obtain the true BOD value. The analysis is actually subject to variations due to pH and the presence of toxic substances (metal ions, organic products) that inhibit bacterial activity in the measurement vial and reductants (such as mercaptans, for example).

For wastewater, some practical aspects require a few adaptations. The high concentration of organic matter quickly consumes all the dissolved oxygen and prevents any analysis. Dilutions are then applied in order to reduce the concentration of the organic matter, such that the oxygen consumption on the 5th day is lower than the oxygen initially present in the sample. Domestic wastewater has a BOD_5 of approximately 300 $mg.L^{-1}$, meaning that 1 liter of wastewater is associated with a consumption of about 300 mg of oxygen, in five days.

The main benefits of BOD testing are:

– an approximate indication of the biodegradable fraction of the wastewater;

– an indication of the pollution level in the wastewater;

– an indication of the rate of oxygen consumption with respect to time;

– an approximate determination of the amount of oxygen required to biochemically stabilize the organic matter present.

The following limitations have, however, been mentioned (Marais and Ekama 1976):

– Low BOD_5 levels can be observed when microorganisms responsible for decomposition are not adapted to the existing organic substances.

– Heavy metals and other toxic substances can inhibit microorganisms.

– The $uBOD/BOD_5$ ratio varies according to wastewater.

Despite these limitations, the BOD test is still widely used, especially if we bear in mind that the design criteria for many wastewater treatment processes are expressed in terms of BOD and that the legislation on effluent discharges and the assessment of the compliance with discharge standards are normally based on BOD. This parameter represents a good indicator of the biodegradable organic matter content of water. BOD is used to measure the quality of water in a jar: surface water (rivers, lakes, etc.) and wastewater (wastewater treatment plants, industrial discharges, etc.). It can be considered as an indirect indication of biodegradable organic carbon.

The COD test is increasingly used for design, mathematical modeling and performance evaluation purposes.

1.1.2.2. *Chemical oxygen demand (COD)*

Chemical oxygen demand (COD) is the measure of the amount of oxygen required to chemically oxidize the oxidizable organic and inorganic matter contained in a sample. COD is measured using an energetic oxidant such as potassium dichromate in an acidic medium, maintained hot for two hours. It is estimated that this oxidation destroys 90–95% of the compounds. This parameter gives an estimate of the amount of pollutants present in the wastewater. The value obtained is therefore an indirect indication of the level of organic matter present. It is one of the most widely used methods for assessing the overall load of organic substances.

COD measures all oxidizable substances, whether they are biodegradable or not (persistent organic pollutants or organometallics). The main difference between these two tests is that BOD is related to the biochemical oxidation of organic matter achieved entirely by microorganisms, whereas COD corresponds to the chemical oxidation of organic matter, obtained by a powerful oxidant (potassium dichromate) in an acidic medium.

The main benefits of the COD test are:

– The test only lasts two hours.

– Due to its quick fast response, the test can be used for operational control.

– The test results provide an indication of the oxygen required for the stabilization of organic matter.

– The test makes it possible to establish stoichiometric relationships with oxygen.

– The test is not affected by nitrification, giving an indication of the oxidation of carbonaceous organic matter only.

The main limitations of the COD test are:

– The biodegradable and inert fractions of organic matter are oxidized. Consequently, the test may overestimate the oxygen needed during biological wastewater treatment.

– Some reduced inorganic constituents could be oxidized and interfere with the result.

However, COD also applies to compounds that play no role in the oxygen deficit in a waterway. These are, for example, mineral halides (except fluorine). It is often recommended that measuring the COD be performed before the BOD, in order to estimate the proportion of dilutions to be performed.

In domestic wastewater, the COD/BOD_5 ratio varies between 1.7 and 2.4. Depending on the value of this report, conclusions can be drawn about the biodegradability of wastewater and the treatment process to be implemented (Braile and Cavalcanti 1979):

– a COD/BOD_5 ratio smaller than 3: the biodegradable fraction is high; good indication of biological treatment;

– a high COD/BOD_5 ratio (greater than 3.5 or 4): the inert fraction (non-biodegradable) is high, justifying the interest of a physicochemical process.

For non-agricultural uses, a biological treatment may be used for nitrogen removal if it could not be completely treated at the treatment plant.

Table 1.3 gives some values of the BODs and CODs of some organic products found in water, expressed in mg $O_2.g^{-1}$ of product.

Organic substance	**O_2 theoretical consumption**	**BOD_5**	**BOD_{20}**	**COD**
Acetamide	1,080	745	–	–
Acetone	2,210	544	–	2,210
Acetophenone	2,532	518	–	2,532
Adipic acid	1,423	598	1,115	1,337
Acetic acid	1,066	700	900	1,066
Propionic acid	1,513	1,300	1,400	1,497
Valeric acid	2,039	1,400	1,900	1,957
Citric acid	686	420	610	–
Amylic acid	2,727	1,265	1,732	–
Benzoic acid	1,967	1,250	1,400	1,967
Succinic acid	1,305	419	607	1,305

Organic substance	O_2 theoretical consumption	BOD_5	BOD_{20}	COD
Butyric acid	1,818	1,150	1,450	1,808
Fumaric acid	827	175	–	818
Gallic acid	1,320	80	–	–
Glutaric acid	1,212	433	1,241	–
Uric acid	667	300	–	–
Lactic acid	1,067	540	960	1,067
Oxalic acid	178	100	115	178
Phthalic acid	1,444	1,000	–	–
Salicylic acid	1,623	950	1,250	1,623
Undecanoic acid	2,879	537	–	1,986
Tartaric acid	533	350	460	522
Stearic acid	2,929	117	–	878
Palmitic acid	2,875	58	–	SO5
Myristic acid	2,807	56	–	842
Capric acid	2,604	234	–	2,213
Methyl alcohol	1,500	960	1,260	1,485
Ethyl alcohol	2,090	1,350	1,800	2,027
Benzyl alcohol	2,519	1,550	1,950	2,418
Butyl alcohol	2,594	1,505	1,885	2,403
Octyl alcohol	2,950	1,088	–	2,891
Lauric alcohol	3,096	619	–	1,269
Cetyl alcohol	3,173	–	–	1,075
Hexanol	2,823	790	–	2,653
Phenol	2,383	1,700	2,000	2,383
Naphthol	2,542	932	1,598	2,313
Benzene	3,076	377	–	1,156
Glycerol	1,217	307	–	584
Resorcinol	1,890	1,150	–	–
Lactose	1,066	580	860	–
Glucose	970	580	860	850
Starch	1,185	680	900	–
Peptone	1,407	149	–	–

Organic substance	O_2 theoretical consumption	BOD_5	BOD_{20}	COD
O-cresol	2,519	1,600	1,800	–
Hydroquinone	1,890	478	–	1,871
Glycol	1,290	400	1,080	1,150
Glycerine	1,217	720	940	–
Dextrin	1,185	520	840	–
Aniline	2,660	1,760	–	–

Table 1.3. *BOD and COD of a few organic substances in mg.g^{-1} of product*

If all organic matter is biodegradable, we must have:

$$COD/BOD_{20} = 1$$

If the material is not all biodegradable, we have:

$$COD/BOD > 1$$

The theoretical oxygen demand of a Cx Hy Oz product is as follows:

$$C_x H_y O_z + nO_2 \rightarrow x\, CO_2 + y/2\, H_2O$$

The theoretical oxygen demand in g of oxygen per mole of product is:

$$32\,((4x + y - 2z)/4)\ g\ O_2/mole$$

1.1.2.3. *Total organic carbon (TOC)*

To be more accurate with treated wastewater, the recommendation is to use total and dissolved organic carbons (TOC and DOC) which are much more reliable. Organic carbon can even be measured in-line and in situ. It indicates the total amount of organic matter expressed as carbon (mg.L^{-1}) contained in a solution. It makes it possible to directly know the organic load of a water. Dissolved organic carbon (DOC) represents the organic matter remaining after filtration on membranes of 0.45 micrometers.

In wastewater reuse and depending on how the water produced will be used, the total organic carbon is an important parameter used for monitoring the overall levels of the existing organic compounds. It reflects the importance of having a general indicator of the approximate level of organic contamination that is easy to measure.

The most common measurement principle consists of burning the sample in a pure oxygen atmosphere and then directing the combustion gases towards an analyzer connected to a recorder, the response of which is proportional to the amount of organic carbon present. In general, the device is calibrated with solutions of pure products, at concentrations close to the sample to be analyzed. The main combustion gas is CO_2. It is formed by the combustion of organic products, after being conveyed through a tube furnace at 800–900°C.

When analyzing TOC, the following elements are measured:

– TC = total carbon;

– TIC = total inorganic carbon (bound to carbonates);

– POC = purgeable organic carbon;

– NPOC = non-purgeable organic carbon;

– DOC = dissolved organic carbon;

– POC = particulate organic carbon.

Volatile organic compounds have a low boiling point and can be purged from a solution by injecting gas into a sample.

In summary, we have:

– TOC = TC – TIC;

– TOC = POC + NPOC.

Generally speaking, the following relation is used:

$$TOC = DOC + POC$$

After membrane filtration during which all POC is removed, it follows that:

$$TOC = DOC$$

This principle is also used after on-material filtration when turbidity is < 2 NTU. DOC is found in colloids, mainly humic acids, fulvic acids, and small molecules resulting from the decomposition of organic matter (proteins, lipids, carbohydrates). The DOC is the most widely used indicator for designing, mathematical modeling and performance evaluation.

Generally speaking, the term "particulate" is used to indicate that solids are present in the form of suspended solids. Within this context, terms such as particulate BOD, COD, phosphorus, etc., are used to indicate that they are related to suspended solids. On the other hand, soluble BOD, COD and phosphorus are associated with dissolved solids.

This test is mainly used in research or in detailed evaluations of wastewater characteristics, due to the high cost of equipment.

1.1.3. *Nitrogen*

In its cycle in the biosphere, nitrogen alternates between various forms and states of oxidation, resulting from various biochemical processes (Table 1.4).

Form	Formula	Oxidation state
Molecular nitrogen	N_2	0
Organic nitrogen	Variable	Variable
Free ammonia	NH_3	−3
Ammonium ion	NH_4^+	−3
Nitrite ion	NO_2^-	+3
Nitrate ion	NO_3^-	+5

Table 1.4. *Different forms and states of nitrogen oxidation*

Nitrogen is a component of great importance in terms of water pollution control, for the following reasons:

– Water pollution:

- Nitrogen is an essential nutrient for algae leading, under certain conditions, to the phenomenon of eutrophication of lakes and reservoirs.

- Nitrogen may result in the consumption of dissolved oxygen in the receiving medium due to processes of conversion of ammonia into nitrites and nitrites into nitrates.

- Nitrogen in nitrate form is associated with diseases such as methemoglobinemia.

– Wastewater treatment:

- Nitrogen is an essential nutrient for microorganisms responsible for wastewater treatment.

- Nitrogen, in processes of conversion of ammonia into nitrites and nitrate into nitrites (nitrification), which can occur in a WWTP, leads to oxygen and alkalinity consumption.

- Nitrogen is involved in the process of converting nitrate into nitrogen gas (denitrification).

– In raw domestic wastewater, the predominant forms are organic nitrogen and ammonia. Organic nitrogen corresponds to the amino groups. Ammonia mainly originates from urea, which is rapidly hydrolyzed and rarely found in raw wastewater. These elements are determined in the laboratory by the Kjeldahl method, leading to total Kjeldahl nitrogen (KTN).

In brief:

KTN = NH_3 + organic nitrogen

NT (total nitrogen) = KTN + NO_2^- + NO_3^- = NH_3 + organic nitrogen + NO_2^- + NO_3^-

The distribution of ammonia in wastewater can be schematically represented in Figure 1.5. It can be seen that the fraction of oxidized nitrogen NOx (nitrite + nitrate) is very low, or even non-existent in raw wastewater. KTN can be subdivided into a soluble fraction (dominated by ammonia) and a particulate fraction (associated with suspended organic matter).

Ammonia exists in solution as an ion (NH4+) and in free non-ionized form (NH3), according to the following dynamic equilibrium:

$$NH_3 + H^+ \leftrightarrow NH_4^+$$

Free ammonia *Ionized ammonia*

At the output of the treatment plants, several forms of nitrogen can therefore be found:

– oxidized nitrogen: nitrites and nitrates;

– non-oxidized forms: Kjeldahl nitrogen including organic nitrogen and ammoniacal nitrogen (NH_4^+);

– ammonifiable organic nitrogen;

– refractory organic nitrogen.

Depending on the country’s regulations, the residual form of nitrogen may be ammonical if the discharge standards do not enforce a limit value for this parameter. As a result, its presence in organic or ammoniacal form results in oxygen consumption in the natural environment and modifies the living conditions of the environment.

In France, where nitrogen regulations are rather strict, nitrification and denitrification must be optimally controlled.

Total nitrogen includes Kjeldahl nitrogen (KTN KN), nitrites and nitrates (see Figure 1.5).

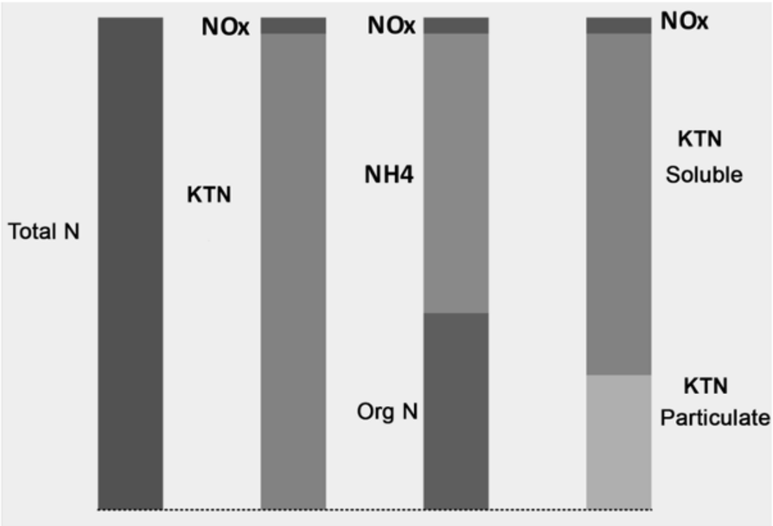

Figure 1.5. *Distribution of nitrogen forms in untreated domestic wastewater (IAWQ 1995)*

The relative distribution introduces the following values, depending on pH values:

– pH < 8: nearly all ammonia is in NH_4^+ form;

– pH = 9.5: about 50% NH_3 and 50% NH_4^+;

– pH > 11: nearly all ammonia is in NH_3 form.

It can be seen that, in the usual pH range, close to neutrality, the ammoniac existing in wastewater is practically in ionized form. Water temperature also influences this distribution. At a temperature of 25°C, the proportion of free ammonia to total ammonia is about twice as high compared with a temperature of 15°C.

The following formula can be used to calculate the proportion of free ammonia relative to total ammonia present in water, as a function of temperature and pH (Emerson et al. 1975):

$$\frac{\text{free NH3}}{\text{total NH3}}\ \% = \left(1 + 10^{0.09018+\left(\frac{2{,}729.92}{T+273}\right)-\text{pH}}\right)^{-1} \text{ x } 100$$

where T = wastewater temperature (°C).

Table 1.5 and Figure 1.6 show the variation in the proportions of free NH_3 and NH_4^+.

pH	T° = 15°C		T° = 20°C		T° = 25°C	
	% NH_3	% NH_4^+	% NH_3	% NH_4^+	% NH_3	% NH_4^+
6.50	0.09	99.91	0.13	99.87	0.18	99.82
7.00	0.27	99.73	0.40	99.60	0.57	99.43
7.50	0.86	99.14	1.24	98.76	1.77	98.23
8.00	2.67	97.33	3.82	96.18	5.38	94.62
8.50	7.97	92.03	11.16	88.84	15.25	84.72
9.00	21.50	78.50	28.43	71.57	36.27	63.73
9.50	46.41	53.59	55.68	44.32	64.28	35.72

Table 1.5. *Proportion of free and ionized NH_3 as a function of pH and temperature*

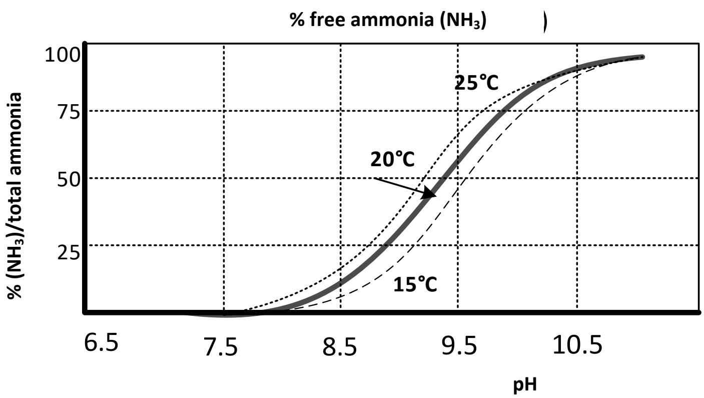

Figure 1.6. *Percentage of free NH_3 as a function of pH and temperature*

1.1.4. *Phosphorus*

Total phosphorus in domestic wastewater is present in phosphate form, classified as follows:

– inorganic (polyphosphates and orthophosphates): main source of detergents and other household chemicals;

– organic (bound to organic compounds): physiological origin.

Phosphorus in detergents is present, in raw wastewater, in the form of polyphosphates or, after hydrolysis, in orthophosphate form.

Orthophosphates are directly available to the biological metabolism. The forms in which orthophosphates are present in water depend on the pH and include PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^{-}$, H_3PO_4.

In wastewater, the predominant form is HPO_4^{2-}.

Polyphosphates are more complex molecules, with two phosphorus atoms or more. Polyphosphates are transformed into orthophosphates by hydrolysis, which despite its slow process, takes place in transport pipelines. Phosphorus in detergents can account for up to 50% of the total phosphorus in wastewater.

Phosphorus in wastewater can be in solid form:

– soluble phosphorus (mainly inorganic): mainly polyphosphates and orthophosphates (inorganic phosphorus), as well as a small fraction corresponding to phosphorus bound to soluble organic matter in wastewater;

– particulate phosphorus (fully organic): bound to particles of organic matter in wastewater.

The importance of phosphorus is associated with the following aspects:

– phosphorus is an essential nutrient for the growth of microorganisms. Domestic wastewater generally contains adequate levels of phosphorus;

– phosphorus is an essential nutrient for algae growth, eventually leading, under certain conditions, to the eutrophication of lakes and reservoirs. It is estimated that from 20 $\mu g.L^{-1}$, the effect of P on algae growth is noticeable.

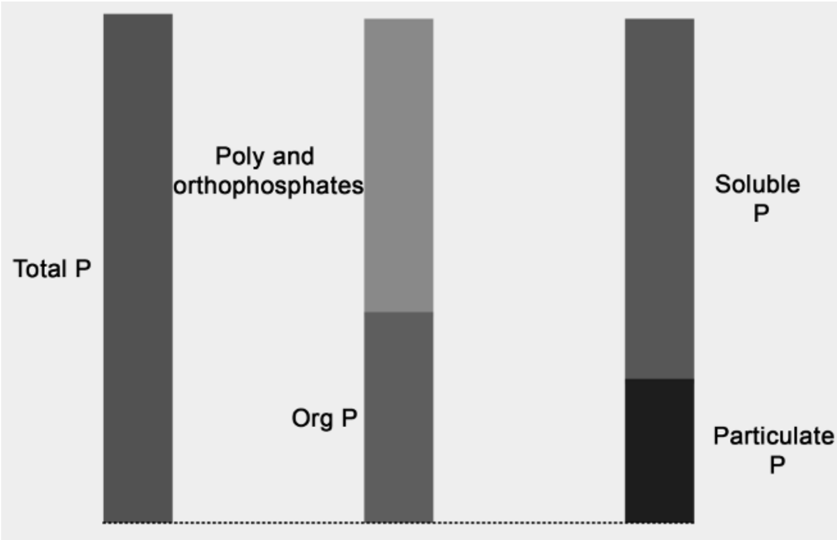

Figure 1.7. *Distribution of phosphorus forms in urban wastewater*

1.1.5. *Micropollutants*

1.1.5.1. *Pesticides and endocrine disruptors*

Over the past decade, a large number of scientific articles have quantified and described the performance of wastewater treatment plants in relation to these substances. It thus appears that treated water still contains a concentration that most often cannot be overlooked. This is expected because the equipment available to the treatment plants is not effective enough to remove these substances.

The most represented families of pesticides include:

– organophosphates (chlorpyrifos, malathion) which have an insecticidal effect by way of blocking acetylcholinesterase (disruption of the transmission of nerve impulses);

– organochlorines (DDT, chlordecone, lindane), insecticides affecting the nervous system by disrupting nerve impulses at the synapses. These compounds are now all banned in France but are widely encountered in the environment (as, for instance, in the West Indies, where chlordecone is causing problems);

– carbamates (aldicarb, carbofuran), which act on the nervous system in the same way as organophosphates;

– triazines (atrazine, simazine), which have herbicidal properties by blocking the transmission of electrons and light energy;

– phenylureas (diuron, isoproturon), which have a mode of operation comparable to that of triazines;

– chloroacetanilides (metolachlor, acetochlor), which act on plants by inhibiting elongases, enzymes involved in lipid synthesis (long-chain fatty acids);

– aryloxyacids (2.4 D, mecoprop, dichlorprop), which disrupt auxin AIA (indole 3-acetic acid) regulation, a phytohormone essential for plant growth;

– sulfonylureas (chlorsulfuron, nicosulfuron), which affect the synthesis of branched-chain amino acids (valine, leucine, isoleucine) through the inhibition of acetolactate synthetase.

Main families	**Compounds**
Drug molecules	Anxiolytics, analgesics, anti-inflammatories, antibiotics, lipid-lowering agents, antihypertensives, anti-epileptics, anticancer, iodinated contrast agents, etc.
Cosmetics and personal care products	Musks (galaxolide, tonalid), triclosan, etc.
Endocrine disruptors	Hormones, alkylphenols, BPA, phthalates, flame retardants, etc.
Illegal drugs	Cocaine and metabolites, THC, amphetamines, etc.
Pesticides	Insecticides, herbicides
Priority substances (Appendix X of the WFD) and hazardous substances (RSDE notification 050109)	PAH, dichloromethane
Disinfection by-products	Trihalomethanes, haloacetic acids

Table 1.6. *Main families and organic compounds*

Agricultural activity is not the only source of pesticide emissions. Among other things, secondary sources include the maintenance of public spaces, golf courses, private use, the annual control of mosquitoes in wetlands, the veterinary sector, road maintenance, etc.

1.1.5.2. *Hormones*

Natural (estradiol and its derivatives) and synthetic (ethynylestradiol in contraceptive pills) animal hormones are excreted by humans and end up in wastewater. Natural estrogen hormones comprise estrone (E1), 17α-estradiol (E2) and estriol (E3). The most important synthetic hormone, as it is used in birth control pills, is 17α-ethynylestradiol (EE2). Women of reproductive age excrete at least 50 μg.J^{-1} of estrogen hormones in their urine. This level can exceed 5 mg.J^{-1} for a pregnant woman. Men also excrete it, but in much smaller proportions, of approximately a few micrograms. Testosterone is also excreted but efficiently removed by mineralization in wastewater treatment plants (Barbier 2011).

	Plant influents		**Plant effluents**	
	Min	**Max**	**Min**	**Max**
A				
Estradiol	0.61	51.7	0.04	19.1
Estrone	0.90	17.6	0.04	3.62
Estriol	1.11	14.9	0.22	3.14
Ethinyl E2	1.03	48.49	0.3	14.89
B				
Estradiol	0.4	14.41	0.17	2.2
Estrone	0.13	19.8	0.06	7.75
Estriol	0.45	18	0.09	3.58
Ethinyl E2	0.77	4.09	0.3	1.47
C				
Estradiol	0.25	36.2	0.003	2.69
Estrone	2.19	13.2	0.2	6.09
Estriol	0.8	9.75	0.17	3.65
Ethinyl E2	1.07	8.89	0.46	2.93

Table 1.7. *Estrogen concentrations in influents and effluents of a few wastewater treatment plants*

The concentrations are therefore highly variable. However, the removal rates are high, of about 70–85%, except for ethinylestradiol, where it varies from half to two-thirds.

Other products that are likely to have such effects include alkylphenol ethoxylates (APEOs), which are found in industrial cleaning products, paints and some coatings. Of these products, octylphenol and nonylphenol have an endocrine disrupting effect that has been analyzed up to concentrations as high as 10 μg.L^{-1}. Phthalates such as DEHP (dietylhexylphthalate) and BBP (butylbenzylphthalate) range from 0.2 to 1 μg.L^{-1}.

Substance	Family	Efficiency (%)	Wastewater – influents (µg.L^{-1})	Treated water – effluents (µg.L^{-1})
4-nonylphenol	Alkylphenol	84 (60–100)	15.7	1.3
Pentabromodiphenyl ether	PBDE	98 (90–100)	0.39	Nc
Atrazine	Pesticide	2 (1–30)	0.02	0.02
4-t-octylphenol	Alkylphenol	88 (70–100)	5.6	0.21
Bisphenol A	Phenolated polymer	60 (30–90)	0.16	0.06
4-NP1EO	Alkylphenol	88 (70–100)	9.0	0.47
DEHP	Phthalate	92 (80–100)	52.8	4

Table 1.8. *Efficiency of wastewater treatment plants for some endocrine disruptors (Lopez and Laurent 2013)*

Drug residues are among the main xenobiotics present plant effluents and in the environment. Not all of them are endocrinic disruptors but some are. Among the most common products, we can find carbamazepine, an anticonvulsant, and lopromides, which are contrast agents for radiology. Finally, antibiotics should also be of particular concern because they are found in all aquatic compartments and accumulate in sediments.

1.1.5.3. *Polybrominated flame-retardant compounds*

The most common are polybrominated diphenyl ethers (PBDEs), the different types of hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA). These are lipophilic, bioaccumulative persistent organic substances.

1.1.5.4. *Perfluorinated compounds*

The two compounds that receive the most attention are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). They are hydrophobic and lipophobic.

1.1.5.5. *Parabens*

Because of their ubiquity, parabens are found in almost all wastewater.

1.1.6. *Parametric influence in the treatment system*

The characteristics of the effluents to be treated must necessarily be validated by 24-hour measurement campaigns (in dry and rainy weather). These campaigns make it possible to verify the ratios of the different parameters to each other: DCO/DBO_5; MES/DBO_5; DBO_5/KTN; $N\text{-}NH_4/KTN$; DCO/Pt; MVS/MES and DCO/KTN.

Unit operations will be based on these ratios (Table 1.9), and the influence of each of them will be taken into account during the treatment process.

Ratio	Urban effluent	Observation
COD/BOD_5	2.2–2.4	Indicates the influent biodegradability
TSS/BOD_5	0.8–1.2	Will affect the % MVS of the effluent and sludge production
BOD_5/KTN	4–5	Will affect the treatment of nitrogen and the conditions of the biological mechanisms
$N\text{-}NH_4/KTN$	0.6–0.8	Indicates the degree of ammonification of the influent
COD/Pt	45–50	Indicates the potential biological treatment of phosphorus
VSS/SS	0.65–0.75	Will influence sludge production, the design of the aerated lagoon, clarifier design and sludge treatment
COD/NTK	8–12	Influences the denitrification treatment and the interest of an anoxia zone separated from the aeration tank

Table 1.9. *Parametric influence on the treatment system*

Wastewater flow rates are 150–250 L/inhabitant, depending on the agglomeration, the quality and specificities of the distribution networks and how water is used.

The average concentrations in wastewater are 453 $mg.L^{-1}$ of total solids, 217 mg.L of total volatile solids, 145 $mg.L^{-1}$ of suspended solids, 120 mg.L of suspended volatile solids, 288 $mg.L^{-1}$ of chemical O_2 demand and 147 $mg.L^{-1}$ of biochemical O_2 demand for a pH varying from 6.8 to 7.5.

Volatile and non-volatile fatty acids account for 8.5–20 $mg.L^{-1}$ and 0.1–1 $mg.L^{-1}$, respectively; higher fatty acids represent 2/3 of the fatty acid content and proteins and amino acids 45–50% of total nitrogen.

The $BOD_5/N/P$ ratio is in average 100/5/5 with about 10% fat, 40% nitrogenous solids and 50% carbohydrates.

The minimal composition considered in price calculations is, for a PE, 60–70 g of dissolved BOD5, 120 g of COD and 70–90 g of SS (1/3 mineral solids (MS), 2/3 organic solids (MVS)). The fraction of suspended solids in raw municipal wastewater is generally about 72 g per population equivalent.

Parameters	**Quantity (g)**	**Concentration ($mg.L^{-1}$)**
Suspended solids (SS)	90	100–400
Oxidizable solids (OS)*	57	–
DBO_5	60	150–500
COD	120	300–1,000
$N\text{-}NH_4$	15	30–100
Total phosphorus	4	–
Inhibitory materials	0.2	–
METOX**	0.23	–
AOX***	0.05	–
Fat	22	–

*MO = (COD+ 2BOD)/3; **METOX: total toxic metals; ***AOX: adsorbable organohalogenated compounds on activated carbon.

Table 1.10. *Overall characteristics of domestic wastewater*

Equivalence	**METOX number**
1 g Zn	1
1 g Cu or Ni	5
1 g Pb or As	10
1 g Hg	50
1 g Cd	50

Table 1.11. *METOX equivalence of some heavy metals*

The legislation defines the concept of population equivalent as "the biodegradable organic load with a five-day biochemical oxygen demand (BOD_5) of 60 grams of oxygen per day" (European Directive 91-271).

1.2. Microbiological parameters: pathogenic organisms and indicators of fecal contamination

The role of bacteria as testimony of pollution has been known for a long time as various links have been established between water contamination, the presence of bacteria and diseases registered in individuals in the immediate vicinity of the discharge area. It has been estimated that every human being eliminates several billion germs per gram of faces every day, including 100–500 million coliforms. Therefore, in patients with enteritic disease, pathogenic bacteria reach high levels in the stool (250,000 to several billion salmonellae per gram). In paratyphics, the figures recorded are much higher and can reach several hundred billion per gram of fecal matter.

Most microorganisms play a variety of roles, including the transformation of constituents in biogeochemical cycles. Another important aspect in terms of biological wastewater quality is that related to the transmission of diseases by pathogenic organisms.

The groups of pathogenic organisms are:

– bacteria;

– viruses;

– protozoa;

– helminths.

A water-related disease is defined as any adverse, significant or widesperad effect on humans, such as death, disability, disease or disorder, directly or indirectly caused.

It is in the digestive system that the first reactions are born, in particular the development of digestive parasitosis, of which there are two main families: protozoa and metazoans. Protozoa include amoebas, flagellates and ciliates. Metazoans include cestodes (or tænia), nematodes and trematodes.

Another category of bacteria comprise mycobacteria, responsible for tuberculosis. Koch's bacillus is eliminated through the feces and is found in particular in wastewater discharged by sanatoriums and specialized hospitals. The population of Koch's bacilli can be very large in some cases: 10,000 germs per ml of water, or 100,000 bacilli per ml of sludge.

There are also many other bacteria such as those responsible for abscesses (flavobacterium, chromobacterium, semitia) and also for cholera germs or clostridium-type bacteria (brought by rainwater runoff on contaminated crop fields).

Wastewater also contains viruses present in stools of healthy, sick or recovering carriers. The main viruses found belong to the enterovirus group, which are located in the human intestine (poliovirus, echo, reovirus), or to virus A of viral hepatitis. Polioviruses can reach a concentration of 100,000 units per gram in feces, and the figures are of the same order of magnitude for other viruses, although not all authors agree on the significance of these values.

Wastewater also carries plant and animal parasites and parasite eggs and cysts. Various analyses conducted have highlighted the presence, for example, of saccomyces, torula, candida, trichosporon, ascomycetes, etc.

The quantities of these parasites greatly vary and fall within a very broad range (10,000–100,000 colonies per liter of water). Microbiological pollution becomes very dangerous when wastewater is discharged into a receiving environment that can be used as a leisure spot (seawater, lake, river, etc.).

There are a large number of diseases that can be contracted, and the most typical are ocular diseases, nasopharyngeal and optic disorders, cutaneous manifestations, impetigos, furunculosis and mycosis.

Intestinal diseases and diseases due to viruses should also be included. Consequently, we can be certain that microbiological pollution is serious, insidious and a constant nuisance for the individual, in particular when wastewater is discharged upstream or into environments likely to be recreational places.

Table 1.12 provides a limited inventory of the origins of and diseases caused by organisms in wastewater.

<table>
<tr><th>Organism</th><th colspan="2">Description</th></tr>
<tr><td rowspan="10">Bacteria</td><td colspan="2">Unicellular organisms.
Existing in different shapes and sizes.
Responsible for the degradation of organic solids and nitrogen products in wastewater treatment plants.
Responsible for waterborne or food-borne diseases.</td></tr>
<tr><td>E. coli (enterotoxin)</td><td>Diarrhea</td></tr>
<tr><td>Leptospira</td><td>Fever, epidermal hepatitis, meningitis</td></tr>
<tr><td>Salmonella</td><td>Gastroenteritis, vomiting</td></tr>
<tr><td>Shigella</td><td>Colitis, colic, diarrhea</td></tr>
<tr><td>Yersinia enterocolitica</td><td>Fever, enteritis, erythema</td></tr>
<tr><td>Pseudomonas aeruginosa (pyocyanic bacillus)</td><td>Urinary tract inflammation</td></tr>
<tr><td>Staphylococcus</td><td>Abscess, airways inflammation</td></tr>
<tr><td>Streptococcus</td><td>Local infection, endocarditis, otitis</td></tr>
<tr><td>Klebsiella pneumoniae</td><td>Pneumonia</td></tr>
</table>

Virus	Pathogenic and difficult to eliminate during water treatment. Includes enteroviruses, viruses hepatitis A, Norwalk-type gastroenteric virus, reovirus and rotavirus, adenovirus.
Protozoans Enteric	Some enteric protozoa are pathogenic and have been associated with outbreaks of drinking water-related diseases. The protozoa of greatest concern are *Giardia* and *Cryptosporidium*. These organisms can be found in water as a result of direct or indirect contamination with human or animal feces. The transmission of *Giardia* and *Cryptosporidium* often occurs on a person-to-person basis.
Helminths	Parasitic worms in the digestive tract of humans or animals; they release a large number of eggs with a shell consisting of several membranes of varying thickness into the external environment. This resistant structure allows them to achieve the biological cycle and to perpetuate their infesting power. These microscopic eggs vary in size from 30 to 160 μm. Intestinal helminthiases are animal and human parasitic diseases caused by helminth infestation. These pathogens can be transmitted to humans through direct contact with wastewater, indirectly through the consumption of crops irrigated with this wastewater, or through food products of animal origin.

Table 1.12. *Most frequently analyzed microorganisms in wastewater*

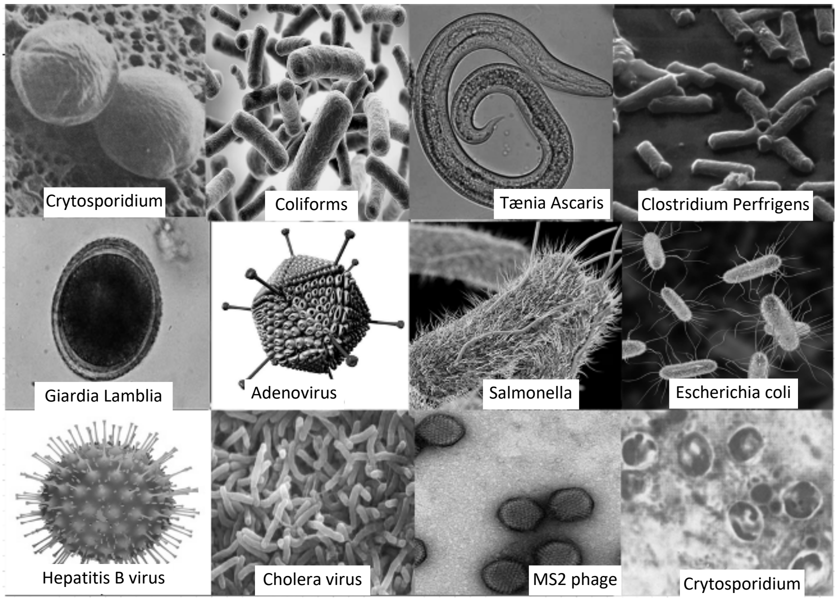

Figure 1.8. *Some microorganisms found in wastewater*

1.2.1. *Bacteria*

1.2.1.1. *Coliforms*

The most commonly mentioned microorganisms are bacteria of the coliform group. These are present in large quantities in human stool. Each individual excretes an average of 10^{10}–10^{11} cells per day (Branco 1979).

About 1/3–1/5 of the weight of human stool is made up of coliform bacteria. In wastewater, the concentration of coliforms is much higher than that of other pathogenic bacteria. In wastewater, coliforms have a higher resistance than the majority of pathogenic enteric bacteria. This is an important characteristic and one of the reasons why they are a good indicator of fecal contamination.

The coliform group of bacteria consists mainly of species of the genera *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella* and includes fecal coliforms where *Escherichia coli* is the predominant species. Many of the coliforms are capable of growing outside the gut, especially in warm climates; as such, their number is not a suitable measure to be used as a monitoring parameter for wastewater reuse systems. The fecal coliform test can also include some non-fecal organisms that can grow at 44°C; therefore, the number of *E. coli* is the most satisfactory indicator parameter for wastewater use in agriculture.

Coliform removal mechanisms in treatment plants are the same as those used for pathogen bacteria. As such, the removal of pathogenic bacteria is usually associated with the removal of coliforms. However, other pathogenic organisms (such as protozoan cysts and helminth eggs) can be removed by different mechanisms.

Bacteriological techniques for the detection of coliforms are fast and affordable compared to those of pathogens.

1.2.1.2. *Fecal streptococci*

The group of fecal streptococci includes two main genera: *Enterococcus* and *Streptococcus*. The genus *Enterococcus* comprises many species, most of them of fecal human origin; however, some species are of animal origin. All *Enterococcus* have a high tolerance to adverse environmental conditions. Fecal streptococci rarely multiply in polluted waters and are more resistant than *E. coli* and coliform bacteria. For these reasons, they are sometimes used as indicators for bathing waters (WHO 2006).

The relation between fecal coliform values and fecal streptococci (FC/FS ratio) is used in some countries to give an indication of the origin of contamination, whether mainly human or animal. High FC/FS values would suggest mainly human contamination, while low FC/FS values would suggest mainly animal contamination. These are special cases despite the fact that these relationships are not applicable in every case, and may give wrong indications of contamination in various reservoirs. This group of organisms comprises species primarily associated with animals (*Streptococcus bovis* and *S. equinus*), other species with a wider distribution (such as *S. faecalis* and *S. faecium*, present in humans and other animals) as well as two biotypes (*S. faecalis var liquefaciens* and a typical *S. faecalis* that hydrolyzes starch) that appear to be ubiquitous, occurring in both polluted and unpolluted environments. Counting the number of fecal streptococci in effluents is a simple routine procedure but it has the following limitations: 1) the possible presence of non-fecal biotypes in the natural microflora of crops may affect their usefulness in assessing the bacterial quality of crops irrigated with wastewater; 2) the lower survival of fecal streptococci at high temperatures than at low temperatures. Further studies are still needed on using fecal streptococci as an indicator in tropical conditions and especially for comparing survival with that of salmonella.

1.2.1.3. *Sulfito-reducing clostridia*

Clostridium perfringens are the most representative species of this group, normally occurring in feces. The concentration of *Clostridium perfringens* is lower than that of *E. coli*. This bacterium is a sporulated anaerobe, exclusively fecal, normally used for detecting intermittent or past water pollution, due to the prolonged survival of its spores. Although this lengthy survival is generally considered unsuitable for normal purposes, it can be very useful in wastewater reuse studies, because *Clostridium perfringens* may prove to have survival characteristics similar to viruses or even helminth eggs.

Clostridium spores can survive in water much longer than organisms in the coliform group and are more resilient to disinfection. Their presence in disinfected water may indicate treatment failure.

Pathogen bacterial concentration in treated wastewater falls between 10^6 and 10^7 bacteria per 100 mL^{-1}. That of enterobacteriaceae is about 10^5–10^6 u.100 mL^{-1}, with 10^3–10^4 for streptococci and 10^3–10^4 100 mL^{-1} for clostridia.

1.2.1.4. Salmonella *spp.*

Several species of *Salmonellae* may be found in the raw wastewater of an urban community in a tropical developing country, including *S. typhi* (typhoid fever causation agent) and many others. It is estimated (Doran et al. 1977) that a count of 7,000 *Salmonellae*/liter is typical in a tropical urban sewer with a similar number of Shigella, and possibly 1,000 *Vibrio cholera*.L^{-1}. *Shigella* spp. and *V. cholera* are both more quickly killed in the environment, therefore if salmonella can be removed, then the majority of other bacterial pathogens will have been removed as well.

1.2.2. *Enteric viruses*

1.2.2.1. *Enterovirus*

About 140 types of enteric virus diseases have been described and are potentially present in wastewater. All viruses are pathogenic to humans and cause a wide spectrum of diseases and symptoms. Let us mention enteroviruses such as polioviruses, reoviruses, rotaviruses, adenoviruses, astroviruses, caliciviruses such as Norwalk's agents, and hepatitis A and E viruses. Enteric viruses are present in smaller numbers than bacteria in wastewater and do not multiply in this environment. Nonetheless, they usually have a low infective dose and can survive for several months in water under certain conditions.

The concentration of enteroviruses in treated wastewater is between 10^3 and 10^4 u.L^{-1}.

Enteric virus diseases are human pathogens most likely to contaminate groundwater (irrigation, groundwater recharge), especially since their extremely small size (20–100 nm) enables them to infiltrate soils, eventually reaching aquifers. Depending on the physicochemistry and virus-specific factors (such as size and isoelectric point, for example), viruses can easily travel in soils and persist for several months in soils and subsoils.

Enteroviruses have also proved to be more resistant to disinfection than bacterial indicators. They give rise to serious diseases, such as poliomyelitis and meningitis, or to a range of minor diseases such as respiratory infections. Although there is no strong epidemiological evidence that these diseases spread through wastewater irrigation systems, there is some risk and we should know to what extent viruses are removed by existing and new treatment processes, especially under tropical conditions. Virus counts can only be performed in a dedicated laboratory, since the required cell culture techniques are highly sensitive to bacterial and fungal contamination.

Pathogen protozoa and enteric viruses are considered priority microbial contaminants because of their important role in waterborne disease outbreaks and associated public health risks, their lengthy survival in the environment, their resilience to conventional water disinfection processes and the often weak or non-existing correlation with the traditional bacterial water quality indicator.

1.2.2.2. *Rotaviruses*

These viruses are known to cause gastrointestinal problems and, although generally present in fewer numbers than enteroviruses in wastewater, they are known to be more persistent; it is therefore necessary to establish their survival characteristics in relation to the enterovirus and relatively to indicating organisms in wastewater. It has been claimed that the removal of viruses in wastewater treatment occurs in parallel with the removal of suspended solids, because most viral particles are associated with solids. Consequently, suspended solids must be measured in treated effluents as a routine.

1.2.2.3. *Bacteriophages*

Bacteriophages can be representative of the presence of viruses, due to their similarities to human enteric viruses. Bacteriophages are specific viruses that infect bacteria, such as, for example, coliphages, which infect *E. coli*. The number of coliphages can be greater in wastewater, due to their fast reproduction rate resulting from the aggression of bacterial cells. They can be representative as indicators of wastewater contamination and, because of their greater persistence compared to bacterial indicators, and also as additional indicators of the effectiveness of the treatment or protection of groundwater.

1.2.3. *Enteric protozoa*

Enteric protozoa are found in wastewater in forms that are highly resistant to environmental conditions and disinfection methods. Similar to viruses, they do not reproduce outside of their host and present a low infective dose compared to bacteria. *Cryptosporidium* and *Giardia* protozoa are among the main agents responsible for diarrheal diseases in humans. The protozoa most commonly infecting humans are *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium*, *Cyclospora*, *Microsporidia* and others. Their resistant forms (8–13-µm *Giardia* cysts and 4–6-µm *Cryptosporidium* oocysts) are excreted in large numbers by infected animals or humans and are ubiquitous in surface waters. Enteric protozoa are resistant to harsh environmental conditions and chemical disinfectants commonly used in water treatment plants to reduce bacterial contamination. Several studies have found little or no correlation between bacterial fecal inflammation and protozoan concentration. These observations highlight the need for regular monitoring of reused water and the implementation of technologies adapted to the removal of these microorganisms for effective user protection.

1.2.4. *Helminth eggs*

Helminths do not replace bacterial indicators. However, nematode eggs such as *Ascaris*, *Trichuris* or *Necator americanus* can be used as indicators of other helminths (cestodes, trematodes and other nematodes), which are removed in water and wastewater treatment by the same mechanism (e.g. sedimentation), thus being indicators of treatment effectiveness. Helminth nematodes such as *Roundworms* *lumbricoides* and *Trichuris trichiura*, flatworms, cestodes such as *Tænia saginata* and trematodes such as *Schistosoma mansoni* are also microorganisms potentially pathogenic to humans that can be found in wastewater. The result of being exposed to the pathogenic microorganisms previously mentioned depends on several factors related to the host (susceptibility and degree of immunity), the pathogenic microorganism and the environment.

Helminth eggs are an important parameter when evaluating the use of treated wastewater for irrigation, in which workers may have direct contact with contaminated water and consumers may consume raw or unpeeled vegetables.

There are no surrogate indicators for helminth eggs, and these are directly determined during laboratory tests.

Helminth eggs can be eliminated with physical operations, such as sedimentation, which take place, for example, in stabilization ponds. Eggs may or may not be viable, and viability may be altered by specific disinfection processes.

Table 1.13 shows the averages observed with the bacteriological concentrations in the effluents of treatment plants (conventional activated sludge system).

Microorganism	**Concentration (u.100 mL^{-1})**
Total coliforms	10^6–10^7
Fecal coliforms	10^5–10^6
Streptococci	10^3–10^4
Enterococci	10^3–10^4
Clostridiums perfringens	10^3–10^4
Enteroviruses	10^2–10^3
Cryptosporidium parvum oocysts	10^2–10^4
Giardia lamblia cysts	10^2–10^4
Helminth eggs	0–10^2

Table 1.13. *Average bacteriological concentrations in wastewater treatment plant effluents (conventional activated sludge system)*

These values are average concentrations observed at different wastewater treatment plants around the world. However, they can be different depending on seasons when the pollutant flow is significant such as when animals are slaughtered, or a reduction in the flow rate for a higher pollutant load, etc. It should be noted that to achieve a decrease of these parameters, physical (membranes) or chemical barriers will be necessary.

1.2.5. *Fecal contamination indicators: assessment in treated wastewater*

Given the great diversity of pathogen microorganisms present in water and the limitations of detection methods, acceptable levels in treated wastewater are instead established for microorganisms indicating fecal or treatment contamination.

The important criteria that should be considered when selecting an indicator microorganism (Government of Canada 2021) are:

– The fecal pollution indicator organism must be present only if there is fecal contamination and it must not reproduce outside its host.

– The survival of the indicator microorganism to treatment and in the environment must be equal to or greater than that of the target microorganism.

– Detection and counting should be fast and inexpensive.

– The indicator microorganism must be non-pathogenic to humans.

No indicator available at the moment meets all these criteria, which is why different microorganisms and parameters are used as indicators for specific purposes.

Indicator organisms are used as indicators of the efficiency of the removal of pathogen agents in the wastewater treatment process. The pathogenic organisms that can be represented are bacteria and viruses, since they are eliminated by the same mechanisms as coliform bacteria. Protozoan cysts and helminth eggs, which are mainly removed by physical mechanisms, such as sedimentation and filtration, are not adequately represented by coliform bacteria as indicators of the efficiency of the treatment. There are various other indicator organisms, each with its advantages and disadvantages.

The most commonly used indicators of fecal contamination are: total coliforms (TC), fecal coliforms (FC) or thermotolerant coliforms, namely *Escherichia coli* (EC).

Fecal coliforms (FC) are a group of bacteria originating primarily from the intestinal tract of humans and other animals. This group includes the genus *Escherichia* and, to a lesser extent, *Klebsiella*, *Enterobacter* and *Citrobacter* species.

For decades, bacteria (e.g. fecal coliforms and *E. coli*) were used to identify fecal contamination and indicate the likely presence of microbial pathogens in the water. However, their concentrations are seldom well correlated with those of pathogens.

Therefore, bacterial indicators can signal the likely presence of pathogens in the water, but they cannot accurately predict their level of occurrence. Compared to viruses and protozoa, bacterial indicators are less persistent in the aquatic environment and less resilient to disinfection mechanisms and other treatment processes.

The total coliform group (TC) is a large group of bacteria that have been isolated in water samples and in polluted and unpolluted soils and plants, as well as in the feces of humans and other warm-blooded animals. This group has been widely used in the past as an indicator and continues as such in some areas, although issues related to the emergence of non-fecal bacteria are a problem (Thomann and Mueller 1987). Fecal coliforms (FC) are a group of bacteria primarily originating from the intestinal tract of humans and other animals. This group includes the following genera: *Escherichia* and, to a lesser extent, *Klebsiella*, *Enterobacter* and *Citrobacter* species. The FC test is performed at high temperatures, allowing the removal of bacteria of non-fecal origin. However, even under these conditions, the presence of non-fecal (freely living) bacteria is possible, although in lower numbers than total coliforms.

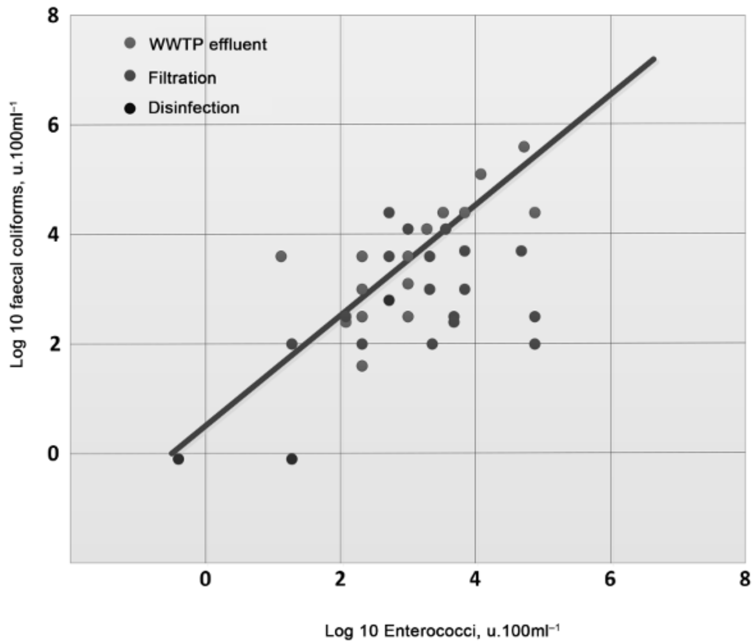

Figure 1.9. *Fecal coliforms/Enterococci correlation. For a color version of this figure, see www.iste.co.uk/gaid/wastewaterengineering1.zip*

In wastewater, *E. coli* is the predominant organism within the fecal coliform group (thermotolerant) and fecal coliforms (thermotolerant) are the predominating coliforms within total coliforms.

Therefore, even a positive fecal coliform test does not guarantee that the contamination is actually fecal. For this reason, fecal coliforms have recently been referred to as thermotolerant coliforms because they are resistant to the high testing temperatures, but are not necessarily fecal. *E. coli* is the only bacterium that guarantees contamination that is exclusively fecal. As such, there is a current trend to mainly use *E. coli* as an indicator of fecal contamination. Nonetheless, its detection does not guarantee that the contamination is of human origin, since *E. coli* can also be found in the feces of other animals. Its detection in the laboratory is very simple, established mainly by fluorogenic methods.

There is no quantifiable link between TC and pathogen microorganisms (Rose et al. 2004). However, their presence can mean the presence of other pathogen bacteria.

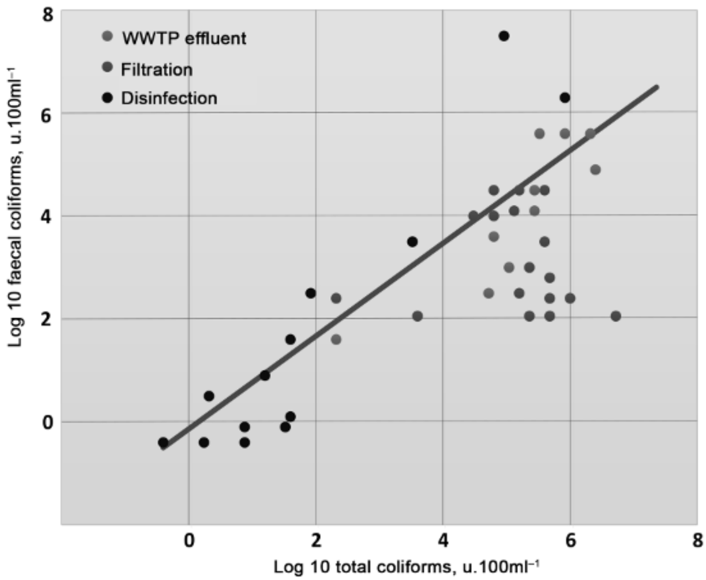

Figure 1.10. *Fecal coliforms/total coliform correlation. For a color version of this figure, see www.iste.co.uk/gaid/wastewaterengineering1.zip*

Coliforms are not good indicators of the presence of viruses or parasites (protozoa and helminths), which would be more persistent in the environment (50% reduction time in surface water concentration: coliforms = 0.9 days, cryptosporidium = up to 150 days) and more resistant to some treatments such as disinfection and have lower infective doses. To this end, other indicators such as coliphages (for viruses), *Clostridium perfringens* (for viruses and parasites), enterococci (for viruses and parasites) and a physicochemical parameter such as turbidity (for parasites) are suggested. Some countries or international organizations have added, in addition to the criteria for coliforms, criteria to be respected for parasites and viruses in reused wastewater. Despite their limitations, it should be observed that fecal coliforms and *Escherichia coli* have several advantages and are the microorganisms most widely utilized as indicators at the present time.

Microorganisms	Pollution indicators
Escherichia coli	Fecal pollution indicator Potential presence of enteric pathogens
Enterococci	Fecal pollution indicators Indirect probability of the presence of enteric viruses
F-RNA phages	Indicators of enteric virus contamination Indicators as substitutes for viruses to test the effectiveness of a process
B. fragilis phages	Fecal contamination indicators Used as microbial source monitoring parameters
Clostridium perfringens sp.	Fecal pollution indicator Indicator as a substitute for viruses and protozoa (cysts and oocysts) to test the effectiveness of a disinfection process

Table 1.14. *Examples of contamination indicators and effectiveness monitoring*

As process indicators, phage groups are often successfully used as substitutes for enteroviruses for the evaluation of the efficiency of water treatment processes. This is the case for filtration and disinfection (Rose et al. 2004).

As tracers, bacteriophages are often used both in the field and in the laboratory as biocolloids for estimating the fate and transport of surface and groundwater pathogen viruses. This use of phages as substitutes for the transport of pathogen agents applies to the evaluation of the potential health risk of pathogens in groundwater and to the evaluation of the effectiveness of treatment processes for the removal of pathogens in water.

Table 1.15 shows the values obtained in influents and effluents of wastewater treatment plants in France, where discharges are made into receiving environments located in sensitive areas.

Quality parameter ($mg.L^{-1}$)	WWTP influent	WWTP effluent	
		Sensitive area 1	Sensitive area 2
Biochemical O_2 demand, BOD_5	200–300	15	9
Chemical O_2 demand, COD	400–600	55	39
Suspended solids	250–400	19	12
$N\text{-}NH_4^+$	35–50	3	2
$N\text{-}NO_3^-$	–	8	4
$P\text{-}PO_4^{3-}$	8–20	2	1.5

Table 1.15. *Data from a few factories on treatment performance in France*

Microorganisms	Load per capita ($Org.hab^{-1}.j^{-1}$)	Concentration (Org/100 mL)
Total coliforms	10^{10}–10^{13}	10^{7}–10^{10}
Fecal coliforms (thermotolerant)	10^{9}–10^{12}	10^{6}–10^{9}
E. coli	10^{9}–10^{12}	10^{6}–10^{9}
Fecal streptococci	10^{7}–10^{10}	10^{4}–10^{7}
Protozoan cysts	$< 10^{7}$	$< 10^{4}$
Helminth eggs	10^{3}–10^{6}	10^{0}–10^{3}
Virus	10^{5}–10^{7}	10^{2}–10^{4}

Table 1.16. *Estimation of microorganism concentration in urban wastewater*

1.3. References

Barbier, G. (2011). Perturbateurs endocriniens, le temps de la précaution. Senate Parliamentary Office Report, no. 765, filed 12 July, Paris.

Birks, R., Colbourne, J., Hills, S., Hobson, R. (2004). Microbiological water quality in a large in-building water recycling facility. *Water Sci. Technol.*, 50(2), 165–172.

Bitton, G. (2005). *Wastewater Microbiology*. John Wiley & Sons, New York.

Blumenthal, U.J., Mara, D.D., Peasey, A., Ruiz-Palacios, G., Scott, R. (2000). Guidelines for the microbiological quality of treated wastewater used in agriculture: Recommendations for revising WHO guidelines. *Bull. World Health Organ.*, 78(9), 1104–1116.

Braile, P.M. and Cavalcanti, J.E.W.A. (1979). *Manual de tratamento de águas residuárias industriais*. CETESB, São Paulo.

Branco, S.M. and Rocha, A.A. (1979), *Poluiçao, proteçao e usos multiplos de represas*, Ed. Edgard Blücher, Sao Paolo.

Chauret, C., Springthorpe, S., Sattar, S. (1999). Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. *Can. J. Microbiol.*, 45, 257–262.

Chu, W.H., Wang, J.Y., Kao, C.M. (2003). A simplified risk-based approach for process screening in municipal wastewater reclamation and reuse. *Water Sci. Technol.*, 47(1), 257–262.

Cloete, T.E., Rose, J., Nel, L.H., Ford, T. (eds) (2004). *Microbial Waterborne Pathogens*. IWA Publishing, London.

Cooper, R.C. and Olivieri, A.W. (1998). Infectious disease concerns in wastewater reuse. In *Wastewater Reclamation and Reuse*, Asano, T. (ed.). CRC Press, New York, 489–520.

Crook, J. (1998). Water reclamation and reuse criteria. In *Wastewater Reclamation and Reuse*, Asano, T. (ed.). CRC Press, New York, 627–703.

Devaux, I., Gerbaud, L., Planchon, C., Bontoux, J., Glanddier, P.Y. (2001). Infectious risk associated with wastewater reuse: An epidemiological approach applied to the case of Clermont-Ferrand, France. *Water Sci. Technol.*, 43(12), 53–60.

Doran, J.W., Alexander, M. (1977). Microbial formation of volatile selenium compounds in soil. *Soil Sci. Soc. Am. J.*, 41, 69–73.

Emerson, K., Russo, R.C., Lund, R.E., Thurston, R.V. (1975). Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *J. Fish. Res. Board Can.*, 32(12), 2379–2383.

Garcia, A., Yanko, G., Widmer, G. (2002). *Giardia* cysts in tertiary-treated wastewater effluents: Are they infective? *Water Environ. Res.*, 74, 541–544.

Gardner, E.A. (2003). Some examples of water recycling in Australian urban environments: A step towards environmental sustainability. *Water Sci. Technol. Water Supply*, 3(4), 21–31.

Gennaccaro, A., McLaughlin, M.R., Quintero-Betancourt, W., Huffman, D., Rose, J.B. (2003). Infectious *Cryptosporidium parvum* oocysts in final reclaimed effluent. *Appl. Environ. Microbiol.*, 69(8), 4983–4984.

Gerba, C.P. and Rose, J.B. (2003). International guidelines for water recycling: Microbiological considerations. *Water Sci. Technol. Water Supply*, 3(4), 311–316.

Government of Canada (2021). Recommandations au sujet de la qualité des eaux utilisées à des fins récréatives au Canada : document de synthèse. Santé Canada, Ottawa.

Haas, C.N. (2000). Epidemiology, microbiology, and risk assessment of waterborne pathogens including *Cryptosporidium*. *J. Food Prot.*, 63(6), 827–831.

Howard, G., Pedley, S., Tibatemwa, S. (2000). Quantitative microbial risk assessment to estimate health risks attributable to water supply: Can the technique be applied in developing countries with limited data? *J. Water Health*, 4(1), 49–65.

IAWQ (1995). Activated sludge model. Scientific and Technical Report no. 2.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.B. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ. Sci. Technol.*, 36(6), 1202–1211.

Krikelis, V., Spyrou, N., Makoulatos, P., Serie, C. (1984). Seasonal distribution of enteroviruses and adenoviruses in domestic sewage. *Can. J. Microbiol.*, 31, 24–25.

Law, I.B. (1996) Rouse Hill – Australia's first full scale domestic non-potable reuse application. *Water Sci. Technol.*, 33(10/11), 71–78.

Lazarova, V., Hills, S., Birks, R. (2003). Using recycled water for non-potable, urban uses: A review with particular reference to toilet flushing. *Water Sci. Technol. Water Supply*, 3(4), 69–77.

Lopez, B. and Laurent, B. (2013). Campagne exceptionnelle d'analyses des substances présentes dans les eaux souterraines. Report BRGM/RP 61853FR, ONEMA – BRGM, Paris.

Lopman, B.A., Reacher, M.H., van Duijnhoven, Y., Hanon, F.-X., Brown, D., Koopmans, M. (2003). Viral gastroenteritis outbreaks in Europe, 1995–2000. *Emerg. Infect. Dis.*, 9(1), 90–96.

Marais, G.R. and Ekama, G.A. (1976). The activated sludge process. Part 1 – Steadystate behaviour, *Water S.A.*, 2(4), 164–200.

Marsalek, J., Schaefer, K., Exall, K., Brannen, L., Aidun, B. (2002). *Water Reuse and Recycling*. Canadian Council of Ministers of the Environment, Winnipeg, Manitoba.

Maunula, L., Miettinen, I., von Bonsdorff, C.-H. (2005). Norovirus outbreaks from drinking water. *Emerg. Infect. Dis.*, 11(11), 1715–1721.

Michael-Kordatou, I., Michael, C., Duan, X., He, X., Dionysiou, D.D. (2015). Dissolved effluent organic matter: Characteristics and potential implications in wastewater treatment and reuse applications. *Water Res.*, 77, 213–248.

Olivieri, A.W., Eisenberg, D.M., Soller, J.A., Suffet, I.H., Froines, J., Cooper, R.C., Gagliardo, P.F. (1998). Comparative chemical risk assessment of repurified water from an advanced water treatment facility and a raw drinking water supply. In *Water Reuse 98, Conference Proceedings*, 135–147.

Ottosson, J. (2002). Faecal contamination of greywater – Assessing the treatment required for hygienically safe reuse or discharge. In *2nd International Symposium on Ecological Sanitation*, Lübeck, 7–11 April.

Ottosson, J. and Stenstrom, T.A. (2003). Faecal contamination of greywater and associated microbial risks. *Water Res.*, 37, 645–655.

Payment, P., Plante, R., Cejka, P. (2001). Removal of indicator bacteria, human enteric viruses, *Giardia* cysts, and *Cryptosporidium* oocysts at a large wastewater primary treatment facility. *Can. J. Microbiol.*, 47, 188–193.

Pessöa, C.A. and Jordao, E.P. (1982). *Tratamento de esgotos domesticos*. ABES, São Paulo.

Rose, J.B., Haas, C.N., Regli, S. (1991). Risk assessment and control of waterborne giardiasis. *Am. J. Public Health*, 81(6), 709–713.

Rose, J.B., Dickson, L.J., Farrah, S.R., Carnahan, R.P. (1996). Removal of pathogenic and indica-tor microorganisms by a full-scale water reclamation facility. *Water Res.*, 30(11), 2785–2797.

Rose, J.B., Farrah, S., Harwood, V.J., Levine, A.D., Lukasik, J., Menendez, J., Scott, T.M. (2004). *Reduction of Pathogens, Indicator Bacteria and Alternative Indicators by Wastewater Treatment and Reclamation Processes.* Water Environment Research Foundation/International Water Association, Alexandria.

Schaub, S.A. and Oshiro, R.K. (2000). Public health concerns about caliciviruses as waterborne contaminants. *J. Infect. Dis.*, 181(Suppl. 2), S374–S380.

Scott, T.T., McLaughlin, M.R., Harwood, V.J., Chicukula, V., Levine, A., Gennaccaro, A., Lucasik, J., Farrah, S.R., Rose, J.B. (2003). Reduction of pathogens, indicator bacteria and alternative indicators by wastewater treatment and reclamation processes. *Water Sci. Technol. Water Supply*, 3(4), 247–252.

Storey, M.V. and Ashbolt, N.J. (2003). A risk model for enteric virus accumulation and release from recycled water distribution pipe biofilms. *Water Supply*, 3(3), 93–100.

Thomann, R. and Mueller, J.A. (1987). *Principles of Surface Water Quality Modeling and Control*. Harper and Row, London.

Toze, S. (2004). Literature review on the fate of viruses and other pathogens and health risks in non-potable reuse of stormwater and reclaimed water. Australian Water Conservation and Reuse Research Program, Commonwealth Scientific and Industrial Research Organisation.

Trevett, A.F., Carter, R.C., Westrell, T., Bergstedt, O., Stenstrom, T.A, Ashbolt, N.J. (2003). A theoretical approach to assess microbial risks due to failures in drinking water systems. *Int. J. Environ. Health Res.*, 13(2), 181–197.

Westrell, T., Schonning, C., Stenstrom, T.A., Ashbolt, N.J. (2004). QMRA (quantitative microbial risk assessment) and HACCP (hazard analysis and critical control points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Sci. Technol.*, 50(2), 23–30.

WHO (2006). A compendium of standards for wastewater reuse in the Eastern Mediterranean Region. Recommendations, World Health Organization, Geneva.

Yates, M. and Gerba, C. (1998). Microbial considerations in wastewater reclamation and reuse. In *Wastewater Reclamation and Reuse*, Asano, T. (ed.). CRC Press, New York, 437–487.

York, D.W. and Walker-Coleman, L. (2000). Pathogen standards for reclaimed water. *Water Environ. Technol.*, 12(1), 58–61.