Handbook of Water Analysis

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Main Parameters and Assays Involved with the Organic Pollution of Water

Publication details
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Published online on: 29 Jul 2013

How to cite :- Lorena Vidal, Claudia E. Domini, Antonio Canals, 29 Jul 2013, Main Parameters and Assays Involved with the Organic Pollution of Water from: Handbook of Water Analysis CRC Press
Accessed on: 09 Nov 2023

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17

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Lorena Vidal, Claudia E. Domini, and Antonio Canals

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17.1 Introduction

The estimation of the organic contamination in a water sample is a complex and delicate problem that involves several determination assays because the organic matter is present under diverse chemical compounds and degradation states. The global organic matter balance cannot be obtained by considering a single method, but it must be done by a comparison of the results obtained by different methods [1]. An additional difficulty lies in the fact that, in general terms, no single parameter can be used to quantify the organic matter content.

In principle, the carbonated matter is used as a nutrient by aerobic germs, and it is oxidized to carbon dioxide and water, while species such as nitrite and nitrate are used as food by, for example, nitrobacteria. In an oxygen-deficient environment, such as sewer or stale water, bacteria take oxygen not only from nitrates and nitrites but also from sulfates, with sulfur hydrogen as residual product. These oxidation
phenomena that take place in nature are very difficult to reproduce on a laboratory scale. However, some tests (e.g., biochemical oxygen demand, BOD) allow a biological appreciation of the phenomena, although there are some inherent problems, which will be discussed later.

Several chemical methods have been developed to obtain a more complete and reproducible oxidation of organic matter. Some of them are based on the use of chemical reagents and a methodology that avoids the ambiguity of biological methods. In this way, chemical oxygen demand (COD) has become one of the obtained parameters. Nevertheless, the degradation (i.e., extent and velocity) of organic substances by means of biological methods can be different from that produced by chemical methods. Therefore, the results obtained with both sorts of methods may be difficult to compare. In particular, the extent of oxidation reached when using a strong oxidizing agent (e.g., potassium dichromate) is more complete for many organic compounds than is biological oxidation, although in some cases it is not fully accomplished. As a result, the COD values obtained by this method are so high that, under biological conditions, the complete oxidation of organic matter takes a long time, and it is not always reached.

Another way to evaluate organic matter content is to measure the carbon present in a sample. Total organic carbon (TOC) is, in this case, the employed parameter. The rapid evolution of relatively complex techniques introduced in the past few years has promoted the development of these methods. These techniques show, as the most relevant advantage, applicability to almost every category of organic products, even to the most resistant oxidizing compounds. Besides, the results are obtained quickly and the determinations can be easily automated.

There are other parameters for estimating water contamination. Besides those already mentioned (i.e., BOD, COD, and TOC), total organic halide (TOX) is a very useful parameter. The combination of all these parameters would give complete information for the characterization of the organic matter present in a sample.

### 17.2 Biochemical Oxygen Demand

BOD measures the amount of oxygen (measured in mg/L) required for the oxidation of the organic matter by biological action under specific standard test conditions [2–4]. In the test for BOD determination, the oxygen required to degrade the organic compounds of a water sample by biological means is measured. The test is frequently used to evaluate the efficiency of organic matter removal after a given wastewater treatment process. Owing to its biological character, both the application of the method and the interpretation of the results are often difficult. In addition, its reproducibility is sometimes unsuitable. Other problem that can emerge is that BOD changes with time, up to 25 days. These facts led to the development of some BOD variations.

Carbonaceous BOD (CBOD) is one of these variations. In this case, the effect of the nitrifying bacteria, which can also consume some more dissolved oxygen, is avoided by means of chemical inhibition (i.e., by the addition of 2-chloro-6-(trichloromethyl)pyridine).

Nevertheless, among the BOD variations, BOD5 is the most widely used parameter. It is defined as the BOD obtained after an incubation period of 5 days. In some cases, the water is seeded with a given mass of microorganisms that will depend on their initial amount in the water sample.

Two different kinds of methodologies have been developed for BOD5 determination: the dilution method (classic method) and the instrumental methods. In both cases, the measurement of the concentration of dissolved oxygen (DO) is of crucial importance. The initial DO drops with time due to the oxidation of the organic matter by the microorganisms present either in natural or in seeded water.

#### 17.2.1 Dilution Method

In the classic method, also called the dilution method, the DO is determined via Winkler titration [3,4]. Thus, manganese sulfate is added to the sample. After the redissolution of the precipitate, the solution is titrated with thiosulfate until the color of solution changes form dark blue to clear, employing starch indicator. The volume of titrant employed corresponds to the DO value.
The classic BOD assay is rather long and requires multiple steps. First, a given sample volume is placed inside a volumetric flask and made up to a fixed total volume with distilled water. The role of dilution is to ensure that there is a mass of oxygen sufficient to avoid any decline in bacterial activity. The flask can be shaken to ensure that the water is saturated with oxygen. To keep an appropriate (optimum) medium for the development of the microorganisms, the pH of the sample should be between 6 and 8. A blank (i.e., dilution water) is also prepared, and the same treatment as for the sample is applied. When necessary, both sample and blank must be seeded with a volume of water with microorganisms. A fraction of the total solution content is stored in a covered flask, avoiding the presence of air bubbles. The sample should be kept away from all light and at 20°C. Then, DO is measured in both diluted sample and blank, at the beginning and 5 days after the preparation. Finally, the BOD₅ value can be obtained by applying the following equation:

\[
\text{BOD}_5 = \frac{(D_0 - D_5) - (B_0 - B_5)f}{P} \tag{17.1}
\]

where \(D_0\) (mg/L) is the DO of the diluted sample after preparation, \(D_5\) (mg/L) is the DO of the diluted sample after 5 days of incubation at 20°C, \(B_0\) (mg/L) is the DO of the dilution water before incubation, \(B_5\) (mg/L) is the DO of the dilution water after 5 days of incubation at 20°C, \(P\) is the decimal volumetric fraction of sample used (note that the sample is diluted), and \(f\) is the ratio of seed in the diluted sample to seed in the dilution water (i.e., \(f = (\%\text{ seed in diluted sample})/(\%\text{ seed in dilution water})\)).

If the sample and the dilution water are not seeded, Equation 17.1 can be simplified to:

\[
\text{BOD}_5 = \frac{(D_0 - D_5)}{P} \tag{17.2}
\]

By applying this method, a suitable detection limit for environmental purposes should be 1 mg/L. One factor that must be taken into account is that the presence of chlorine in the sample produces interferences in the determination of the DO value. In this case, the addition of sodium sulfite leads to a reduction in the amount of this species.

### 17.2.2 Instrumental Methods

Owing to the multiple drawbacks that the dilution method presents, an effort has been made to develop alternative methods to overcome or at least alleviate them. These processes are mainly devoted to the reduction of the total analysis time and to the improvement of the reproducibility. Moreover, the correlation between the obtained parameter and BOD₅ should be as good as possible. For this purpose, different strategies have been proposed, the most popular among them being respirometry and biosensors.

#### 17.2.2.1 Respirometric Methods

Respirometry is the measurement and interpretation of the respiration rate of activated sludge [4], and is defined as the amount of oxygen per unit of volume and time that is consumed by the microorganisms in activated sludge [5]. Respirometric methods were the first employed for the rapid determination of BOD. Unlike the dilution method, the solution is aerated and undiluted. In the respirometric method, oxygen uptake in the respirometric bottle is measured either by measuring oxygen depletion in the gas phase or by measuring the mass of oxygen that must be refilled in the headspace to maintain constant conditions. Manometric, volumetric, electrolytic, and direct-input respirometers are available [6].

Application of this method involves the measurement of the rate of decrease of the oxygen content versus time. First, a graph of oxygen concentration against time is plotted. Obviously, oxygen concentration decreases with time. After that, the rate of decreased oxygen concentration at a given time is obtained by measuring the slope of the line at that point. Then, the rate of oxygen consumption is plotted against time, and the area under the curve gives an indication of the total amount of oxygen employed in the oxidation of the organic matter. By applying this method, an analysis can be performed in less than 2 h.
Respirometric methods can be classified into a number of basic measurement principles depending on two criteria: (i) the phase where oxygen is measured (gas or liquid) and (ii) the flow regime of both gas and liquid phase, which can be either flowing or static [5]. From these criteria, the modalities are

- Static gas–static liquid
- Flowing gas–static liquid
- Static gas–flowing liquid
- Hybrid respirometer

### 17.2.2.2 Biosensors

A biosensor is defined as an analytical device that converts a biological response into an electrical signal. A biosensor is composed of a biological recognition element (microorganisms, organelles, cell receptors, enzymes, antibodies, etc.) producing a biochemical signal and a transducer (electrode, piezoelectric crystals, optode, etc.) converting the biochemical signal into an electrical signal [6]. BOD biosensors have been a research area of growing interest in the past few years [7]. As before, in most biosensors, the rate of oxygen consumption is measured, but the sample solution is not continuously aerated. Although some techniques use free cells in solution, the basic structure of biosensors generally consists of an oxygen probe and immobilized microorganisms on the electrode surface, supported by different kinds of materials. The electrode is then introduced into the water sample and DO is measured and change is correlated to the BOD value. One consequence of oxygen limitation for microbial BOD sensors is that the amount of organic material that can be biodegraded in a short-time assay is small and represents only a very small fraction of the total biodegradable organic content [8], and hence, BOD₅ cannot be easily substituted by the parameter supplied by these electrodes (i.e., BOD supplied by sensors (BOD₅)). However, in some cases, BOD₅ has good correlation with BOD₅ [7–10]. Many works on this topic have been published (Table 17.1), with encouraging results, especially promising being the experimental setups based on flow injection-type devices [45,46,48,49,52] (Figure 17.1) since they can be easily automated for online real-time monitoring/control.

### 17.2.2.3 Other Methods

In addition to the previously mentioned methodologies for determining BOD, some authors have suggested alternative systems. This section summarizes the most relevant ones found in the literature.

#### 17.2.2.3.1 Headspace BOD

Logan et al. [53,54] employed the headspace technique combined with gas chromatography (GC) to obtain a parameter that can be correlated with BOD₅ (i.e., headspace biochemical oxygen demand, HBOD). The sample is placed inside a container similar to those used in classic BOD determination, and the remaining space is filled with oxygen. Under these conditions, a high concentration of oxygen is reached, with subsequent growth in the velocity of the oxidation of the organic matter. Next, the air in the headspace is sampled with a syringe and then introduced into the gas chromatograph. Finally, the oxygen concentration is determined. The water sample is left for 3 days under the same conditions as those fixed for BOD determination, and after this period of time, the oxygen concentration in the headspace is measured again. The correlation obtained by Logan et al. is not good, but they give an estimating value of the rate of disappearance of organic matter [53,54].

To consider a BOD determination method ideal, it should meet several characteristics: (a) it must be easy to handle; (b) the sample pretreatment must be avoided; and (c) the organic matter content must be measured instantaneously.

Recently, Min et al. [55,56] have developed a new fiber optic probe to measure oxygen in the gas phase of anaerobic test tubes within a few seconds. The main advantages of the HBOD are: (i) use of nondiluted samples; (ii) faster exertion of oxygen demand (the test is completed in 2 or 3 days); and (iii) a reduced sample preparation time. In addition, HBOD test precision is typically much better than that obtained in a BOD test.
### Table 17.1

Significant Data on Various Biosensors for BOD Tests

<table>
<thead>
<tr>
<th>Reference</th>
<th>Measuring Time</th>
<th>pH</th>
<th>Electrode Life</th>
<th>Organism Yeast</th>
<th>Detection</th>
<th>Linear Range (mg O₂/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[9]</td>
<td>10–20 min</td>
<td>7.9</td>
<td>10 months</td>
<td><em>Bacillus licheniformis, Dietzia maris, and Marinobacter marinus</em></td>
<td>Fluorescent</td>
<td>0.3–40</td>
</tr>
<tr>
<td>[11]</td>
<td>18 min</td>
<td>7.0</td>
<td>17 days</td>
<td><em>Trichosporon cutaneum</em></td>
<td>Amperometric</td>
<td>0–60</td>
</tr>
<tr>
<td>[12]</td>
<td>30 s</td>
<td>6.8</td>
<td>48 days</td>
<td><em>Trichosporon cutaneum</em></td>
<td>Amperometric</td>
<td>0–100</td>
</tr>
<tr>
<td>[13]</td>
<td>15–30 s</td>
<td>7.2</td>
<td>2 days</td>
<td><em>Bacillus subtilis and B. licheniformis 7B</em></td>
<td>Amperometric</td>
<td>0–80</td>
</tr>
<tr>
<td>[14]</td>
<td>5–10 min</td>
<td>6.8</td>
<td>1–2 weeks</td>
<td><em>Trichosporon cutaneum</em></td>
<td>Fluorescent</td>
<td>0–110</td>
</tr>
<tr>
<td>[15]</td>
<td>15 min</td>
<td>7.0</td>
<td>2–8 months</td>
<td><em>Trichosporon cutaneum</em></td>
<td>Amperometric</td>
<td>0–150</td>
</tr>
<tr>
<td>[16]</td>
<td>7–20 min</td>
<td>7.0</td>
<td>3 days</td>
<td><em>Trichosporon cutaneum</em></td>
<td>Amperometric</td>
<td>0–18</td>
</tr>
<tr>
<td>[17]</td>
<td>20–30 min</td>
<td>7.2</td>
<td>Several months</td>
<td><em>Bacillus subtilis</em></td>
<td>Amperometric</td>
<td>0–70</td>
</tr>
<tr>
<td>[18,19]</td>
<td>100 s</td>
<td>6.8</td>
<td>2 months</td>
<td><em>Arxula adeninivorans</em></td>
<td>Amperometric</td>
<td>0–150</td>
</tr>
<tr>
<td>[20]</td>
<td>70 s</td>
<td>6.8</td>
<td>110 days</td>
<td><em>Arxula adeninivorans</em></td>
<td>Amperometric</td>
<td>524</td>
</tr>
<tr>
<td>[21]</td>
<td>2–3 min</td>
<td>6.9</td>
<td>40 days</td>
<td><em>Candida parapsilosis</em></td>
<td>Amperometric</td>
<td>0–30</td>
</tr>
<tr>
<td>[22]</td>
<td>5 min</td>
<td>7.2</td>
<td>7 days</td>
<td><em>Candida sp.</em></td>
<td>Voltammetric</td>
<td>2–100</td>
</tr>
<tr>
<td>[23,24]</td>
<td>5–10 min</td>
<td>6.8</td>
<td>240 days</td>
<td>Formulated uniform dehydrated microbial consortium for BOD estimation (BODSEED)</td>
<td>Electrochemical sensor</td>
<td>30–90</td>
</tr>
<tr>
<td>[25]</td>
<td>1 h</td>
<td>7.0</td>
<td></td>
<td><em>Trichosporon cutaneum, Pseudomonas putida, and Bacillus licheniformis</em></td>
<td>Amperometric</td>
<td>200</td>
</tr>
<tr>
<td>[26]</td>
<td>3–10 min</td>
<td>7.2</td>
<td>&gt;3 months</td>
<td><em>Trichosporon cutaneum and Bacillus subtilis</em></td>
<td>Amperometric</td>
<td>1.0–60.0</td>
</tr>
<tr>
<td>[27]</td>
<td>5–10 min</td>
<td>6.8</td>
<td>180 days</td>
<td><em>Enterobacter cloaca, Citrobacter amalonaticus, Pseudomonas aeruginosa, Yersinia enterocolitica, Klebsiella oxytoca, Enterobacter sakazaki, and Serratia liquefaciens.</em></td>
<td>Amperometric</td>
<td>60</td>
</tr>
<tr>
<td>[28]</td>
<td>1 h</td>
<td>7.0</td>
<td></td>
<td><em>Escherichia coli</em></td>
<td>Coulometric</td>
<td>150</td>
</tr>
<tr>
<td>[29]</td>
<td>4 min</td>
<td>7.0</td>
<td></td>
<td><em>Pseudomonas putida</em></td>
<td>Amperometric</td>
<td>0.25–10</td>
</tr>
<tr>
<td>[30]</td>
<td>2–15 min</td>
<td>7.0</td>
<td>&gt;10 days</td>
<td><em>Pseudomonas putida</em></td>
<td>Amperometric</td>
<td></td>
</tr>
</tbody>
</table>

*continued*
### TABLE 17.1 (continued)

Significant Data on Various Biosensors for BOD Tests

<table>
<thead>
<tr>
<th>Reference</th>
<th>Measuring Time</th>
<th>pH</th>
<th>Electrode Life</th>
<th>Organism Yeast</th>
<th>Detection</th>
<th>Linear Rangea (mg O2/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[31]</td>
<td>25 s</td>
<td>7.0</td>
<td></td>
<td><em>Trichosporon cutaneum</em>, <em>Klebsiella oxytoca</em> AS1, <em>Hansenula anomala</em>, <em>Pseudomonas</em> spp., <em>Bacillus subtilis</em>, <em>Torulopsis candida</em>, <em>B. subtilis</em>, and <em>Bacillus licheniformis</em></td>
<td>Amperometric</td>
<td>3.5–40</td>
</tr>
<tr>
<td>[32]</td>
<td>7.2</td>
<td>30 days</td>
<td></td>
<td>Thermally killed</td>
<td>Amperometric</td>
<td>0–40</td>
</tr>
<tr>
<td>[33]</td>
<td>15–20 min</td>
<td>7.0</td>
<td>30 days</td>
<td>Activated sludge and <em>Bacillus subtilis</em></td>
<td>Luminiscent</td>
<td>0–60</td>
</tr>
<tr>
<td>[34]</td>
<td>5–10 min</td>
<td>7.0</td>
<td></td>
<td><em>Pseudomonas putida</em></td>
<td>Amperometric</td>
<td>0–10</td>
</tr>
<tr>
<td>[35]</td>
<td>10 min</td>
<td>7.9</td>
<td>6 months</td>
<td><em>Bacillus licheniformis</em>, <em>Dietzia maris</em>, and <em>Marinobacter marinus</em></td>
<td>Fluorescent</td>
<td>0.2–40</td>
</tr>
<tr>
<td>[36]</td>
<td>20 min</td>
<td>7.5</td>
<td>8 days</td>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>Luminiscent</td>
<td>0–70</td>
</tr>
<tr>
<td>[37]</td>
<td>3–5 min</td>
<td>7.5</td>
<td>11 days</td>
<td>Consortium from anaerobic sludge</td>
<td>Luminiscent</td>
<td>1000–25,000</td>
</tr>
<tr>
<td>[38]</td>
<td>20 min</td>
<td>96 h</td>
<td></td>
<td><em>Photobacterium phosphoreum</em></td>
<td>Luminiscent</td>
<td>0–16</td>
</tr>
<tr>
<td>[39]</td>
<td>7.0</td>
<td>3 months</td>
<td></td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Luminiscent</td>
<td>0–40</td>
</tr>
<tr>
<td>[40]</td>
<td>10 min</td>
<td>7.0</td>
<td>36 days</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Visible absorption</td>
<td>1.1–22</td>
</tr>
<tr>
<td>[41]</td>
<td>15 min</td>
<td>7.0</td>
<td>14 days</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Amperometric</td>
<td>6.6–220</td>
</tr>
<tr>
<td>[42]</td>
<td>10 min</td>
<td>7.0</td>
<td>4 weeks</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Visible absorption</td>
<td>0–11</td>
</tr>
<tr>
<td>[43]</td>
<td>90 min</td>
<td>6.8</td>
<td>400 days</td>
<td>Consortium</td>
<td>Amperometric</td>
<td>0–45</td>
</tr>
<tr>
<td>[44]</td>
<td>30 min</td>
<td>7.7</td>
<td>4 h</td>
<td><em>Exiguobacterium marius</em>, <em>Bacillus horikoshii</em>, and <em>Halomonas marina</em></td>
<td>Amperometric</td>
<td>1.2–40</td>
</tr>
<tr>
<td>[45]</td>
<td>10–15 min</td>
<td>6.5</td>
<td>15 days</td>
<td>Activated sludge</td>
<td>Amperometric</td>
<td>2–60</td>
</tr>
<tr>
<td>[46]</td>
<td>3 min</td>
<td>8.0</td>
<td>30 days</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Amperometric</td>
<td>0–20</td>
</tr>
<tr>
<td>[47]</td>
<td>30–60 min</td>
<td>7.0</td>
<td>35 days</td>
<td>Activated sludge</td>
<td>Amperometric</td>
<td>15–50 (steady-state method)</td>
</tr>
<tr>
<td>[48]</td>
<td>30 min</td>
<td>7.0</td>
<td>1 week</td>
<td><em>Candida krusei</em></td>
<td>Amperometric</td>
<td>0–140</td>
</tr>
<tr>
<td>[49]</td>
<td>30–60 min</td>
<td>7.0</td>
<td>50 days</td>
<td>Consortium</td>
<td>Amperometric</td>
<td>0–20</td>
</tr>
<tr>
<td>[50]</td>
<td>10–17 min</td>
<td>6.8</td>
<td>&gt;30 days</td>
<td><em>Dabaryomyces hansenii</em></td>
<td>Amperometric</td>
<td>2.2–177</td>
</tr>
<tr>
<td>[51]</td>
<td>20 min</td>
<td>6.9</td>
<td>90 days</td>
<td><em>Aeromonas hydrophila</em></td>
<td>Amperometric</td>
<td>45</td>
</tr>
<tr>
<td>[52]</td>
<td>500–600 s</td>
<td>6.5</td>
<td>17 months</td>
<td>Naturally occurring microorganisms</td>
<td>Amperometric</td>
<td>30</td>
</tr>
</tbody>
</table>

a BOD range for which there is a linear relationship between the measured magnitude and the BOD value.
17.2.2.3.2 Direct Measurement of Absorbance

The direct measurement of any water physical property that changes as a function of the level of organic compounds should be a method close to the ideal one. Although some good results have been obtained, they are applicable only to very particular cases (i.e., a region, river, lake, etc.).

Brookman [57] measures water absorbance at 280 nm. The absorbance values are further correlated with BOD. The value of the square of the regression coefficient, $R^2$, corresponding to the correlation line are contained within the 0.74–0.76 range. By adopting this procedure, the complexity of the BOD determination is minimized. Furthermore, no extra biological media (i.e., bacteria) are required. One severe drawback of this method is the interferences produced by the solid particles present in the sample, leading to significant light diffraction and, thus, to irreproducible BOD values.

Reynolds and Ahmad [58] correlate the fluorescence signal at 340 nm emitted by a wastewater sample irradiated at 280 nm with BOD5. Regression analysis of the fluorescence and BOD data give correlation coefficients values ranging between 0.94 and 0.89. The improvement of the method is due to the fact that Reynolds and Ahmad use the water Raman emission at 309 nm as an internal standard. With this procedure, many of the errors produced by the absorbance methods are mitigated. Later, the same procedure has been extended to COD and TOC showing that fluorescence data can be used to quantify oxygen demand values (chemical and biochemical) and TOC values. In addition, the fluorescence spectra response can be apportioned to biodegradable (BOD) and nonbiodegradable (COD minus BOD) dissolved organic matter [59].

17.2.2.3.3 Spectrophotometric Determination

Recently, Sha and Ma [60] have described a high-throughput and sensitive photometric method for the determination of BOD in wastewater, depending on the measurement of the dissolved oxygen. The method is based on the reaction between $\text{I}_3^-$ and acridine red to give an ion-association complex with a characteristic absorption at 525 nm. The BOD could be calculated through the amount of dissolved oxygen before and after the seeding procedure determined by the proposed spectrophotometry. This method was found to be able to determine the BOD values of 20 waste samples within 40 min and these BOD values were in good agreement with BDO5 values.

17.3 Chemical Oxygen Demand

COD is the amount of oxygen needed to reduce the organic matter present in a water sample by chemical methods [2]. This parameter is of great importance in monitoring water quality, and it is widely employed in analytical laboratories. The advantages of this assessment are that the COD determination takes less
time and is simpler and more reproducible than that for BOD determination. Besides, BOD can be estimated from COD, although the mathematical relationship can vary greatly from one sample to another. This lack of correlation is due to the fact that some bacteria produce more complete oxidation of organic matter than any chemical oxidizing agent. Thus, for instance, *Acetobacter* is able to oxidize acetate more quickly and efficiently than it does potassium dichromate (i.e., the common oxidant used in COD determination). Meanwhile, for other organic compounds (e.g., substituted aromatic hydrocarbons), chemical oxidation is more complete than biological oxidation, since there are no bacteria capable of oxidizing them.

In the COD assay, a known excess of strong oxidant is added to the sample and, by means of an indirect determination, the mass (or concentration) of oxidant that has not been reduced by reaction with the organic matter is derived. Various methods have appeared in the literature dealing with the determination of COD in water samples. A brief description of the most commonly used methods is presented in the following sections.

### 17.3.1 Classic (Opened Reflux) Method

In the first method described for COD determination, the sample is placed in a refluxing flask [61]. Then, the oxidizing agent is set in contact with the water sample. Nowadays, potassium dichromate is the most accepted oxidant, although some others (e.g., permanganate, Ce(IV), persulfate) have also been used. Oxidation of the organic matter could be now performed. Nevertheless, there are several considerations that must be taken into account to improve the speed and accuracy of the method. On this subject, it has been observed that the use of a catalyst is advisable to increase the reaction rate. The addition of silver sulfate has been shown to reduce significantly the time required to complete organic matter oxidation. As regards the accuracy of the method, it must be borne in mind that the presence of inorganic species in the water sample that might be oxidized by dichromate (i.e., mainly chloride) could give rise to higher COD values than the real value. The addition of HgSO₄ has proven to be a good way to eliminate the chloride interference, since mercury generates very stable complexes with this anion.

Finally, to enhance the oxidizing capability of dichromate, a given volume of sulfuric acid is also added. Then follows a further heating step for a period that normally reaches 2 h. After cooling the mixture and washing down the condenser with distilled water, the dichromate that has not been reduced is titrated with ferrous ammonium sulfate, employing ferroine sulfate as indicator. The endpoint of the titration is detected by a change in the indicator color from blue-green to reddish brown. In general, the COD value is obtained by applying the following relationship:

$$\text{COD (mgO}_2/\text{L}) = \frac{(a - b)cf'8000}{V_{\text{sample}}} \quad (17.3)$$

where \(a\) (mL) is the volume of titrating solution used with the blank, \(b\) (mL) is the volume of titrating solution used with the sample, \(c\) (eq/L) is the normality of the titrating solution, \(f'\) is the titrating solution correction factor, 8000 is the equivalent weight of O₂ expressed in mg O₂/eq, and \(V_{\text{sample}}\) (mL) is the volume of the water sample analyzed.

### 17.3.2 Semimicro (Closed Reflux) Method

The principle of this method is the same as for the opened reflux (classic) method. In this case, various culture tubes sealed with PTFE caps are used. The sample and the four reagents mentioned earlier are placed inside the tubes. Blanks (i.e., reagents with distilled water) are also prepared. All the tubes are mounted on a heating block or placed inside an oven at 150°C for 2 h. After this period of time, the excess of dichromate that has not been reduced is determined against a ferrous ammonium sulfate standard solution, using ferroine as indicator.

The closed reflux method is more efficient in the oxidation of volatile organic compounds than is the opened reflux method. The reason for this is that the oxidant is in contact with these compounds for a longer time [61,62]. Besides, the closed method is cheaper, because only 2 mL of sample is required, since 5 mL is the total volume of the mixture. This means that the amounts of reagents and sample are...
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reduced by a factor of over 20 compared to the opened reflux method. In addition, this fact makes the method less contaminating and allows for the simultaneous digestion of a great number of samples (e.g., by employing an oven, up to 40–50 samples can be digested in 2 h).

The precision of this method depends on various factors, such as the chloride content. Hence, the relative standard deviation (RSD) values range from 5.6% in water without chlorides to 4.8% in water with 100 mg/L of chloride. The limits of detection (LOD) are about 3 mg O₂/L and 5 mg O₂/L for the closed and opened reflux methods, respectively.

Many variations of the semimicro method have been suggested. Most involve modifying the detection step. In this way, titration can be substituted by the spectrophotometric determination of chromium (VI) [62–64]. This is the so-called colorimetric closed reflux method. When the organic matter digestion step has been completed, the suspended solids are left to settle before the absorbance is read. Therefore, the interferences caused by sample turbidity due to the presence of inorganic particles are attenuated. Once the solid particles are removed, the absorbance signal is measured at either 445 nm [62] or 600 nm [64], and finally the concentration of nonreduced dichromate or chromium (III) that appears by reduction, respectively, is obtained. Absorbances of the sample, blank, and standards are also determined. Sample COD is obtained by interpolation from the calibration curve. Previous studies show that, with this method, the linear dynamic range (i.e., the COD range for which there is a linear correlation between the COD and the difference between the blank and sample absorbances) reaches a maximum COD value of 960 mg O₂/L [63]. Hence, when the colorimetric method is selected, samples whose COD values are higher than this value should be diluted.

The precision of the colorimetric method is slightly poorer than that for the chromium titration-based procedure. Nevertheless, in other investigations, it has been indicated that this method affords precision values up to seven times better than the opened reflux method [64].

Vyrides et al. [65] have proposed a modification of the standard closed reflux colorimetric method where chloride interferences are masked by the use of a solution of HgSO₄ prior to digestion. Comparison of the standard method with the new method, using a synthetic sewage, highlights the large errors (50–85%) of the standard method in contrast to an error of less than 10% for the proposed modified method. Another approach for COD measurement at high salinity and low organic matter samples has been recently published [66]. The purpose of this study is to determine optimum HgSO₄:Cl⁻ ratio according to the chloride concentrations of the samples and to show the importance of the strength of the digestion solution for the correct COD determination.

17.3.3 Other Discontinuous Methods

Many modifications of the two just described methods have been reported in the literature. The main goals of these are: (i) to reduce the amount of reagents employed; (ii) to increase the sample throughput; (iii) to increase the efficiency of the oxidation step; and (iv) to develop new detection methods. Table 17.2 presents the most relevant discontinuous methods for COD determination.

The conventional methods (opened reflux and closed reflux), however, show some important limitations, among which is the time-consuming digestion step (2–3 h). For this reason, many efforts have been put in to reduce the digestion time. The advantages of microwaves (MW) in the analytical laboratory are well known. Microwaves have been used for COD determination with good recoveries and for an important reduction in the digestion step. For example, microwave radiation has been employed as a good alternative to the conventional heating step in the semimicro determination of COD [96]. In this modified semimicro method, digestion time has been considerably reduced (0.5 min exposure at 1000 W MW power, and 10 min exposure at 250 W MW power). A good agreement with the usual reflux method has been obtained with selected pure organic compounds (potassium hydrogen phthalate, glutamic acid, glucose, dodecyl sulfate) and with real samples (municipal sewage, mixed tanning and chemical wastewaters, and treated wastewater from biological treatment plants). COD–MW/theroretical chemical oxygen demand (ThOD) ranging from 98.9% to 101.5% and from 88.0% to 111.9%, respectively. However, two main drawbacks are the high initial cost of the equipment and safety limitations (high temperature and pressure).

The acceleration of chemical reactions is a feature shared by MW and ultrasound (US) radiation. Power ultrasound is being extensively used in a great variety of applications such as solution degasification,
<table>
<thead>
<tr>
<th>Reference</th>
<th>Change</th>
<th>Main Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>[62]</td>
<td>Reagents</td>
<td>KCr(SO₄)₂ · 12H₂O as a chloride interference suppressor</td>
</tr>
<tr>
<td>[67]</td>
<td>Reagents</td>
<td>Cerium(IV) sulfate is used as oxidant; oxidation is less complete than with dichromate</td>
</tr>
<tr>
<td>[68]</td>
<td>Detection</td>
<td>Cr(VI) titration is carried out by potentiometric means</td>
</tr>
<tr>
<td>[69]</td>
<td>Detection</td>
<td>Cr(VI) titration is carried out by amperometric means</td>
</tr>
<tr>
<td>[70]</td>
<td>Reagents and detection</td>
<td>Chemiluminescence emitted by bacteria is decreased due to the presence of toxic products</td>
</tr>
<tr>
<td>[71,72]</td>
<td>Reagents and detection</td>
<td>Absorbance of water is correlated with COD</td>
</tr>
<tr>
<td>[73]</td>
<td>Instrumentation</td>
<td>A discontinuous microanalyzer is used</td>
</tr>
<tr>
<td>[74]</td>
<td>Reagents</td>
<td>Two advanced oxidative processes (Fe²⁺/H₂O₂/UV and H₂O₂/UV systems)</td>
</tr>
<tr>
<td>[75]</td>
<td>Detection</td>
<td>Sensor based on the in situ electrochemical generation of an aggressive strong oxidant. Fast response (few seconds)</td>
</tr>
<tr>
<td>[76]</td>
<td>Detection</td>
<td>Chemiluminescence detector measures the light emission intensity caused by luminol–H₂O₂–Cr³⁺ system</td>
</tr>
<tr>
<td>[77]</td>
<td>Detection</td>
<td>Sensor based on microfabricated Clark-type oxygen electrode and TiO₂ fine particles suspended in the sample solution. Response time of the sensor: 3–4 min</td>
</tr>
<tr>
<td>[78]</td>
<td>Detection</td>
<td>Nano-PbO₂ modified electrode as an electrocatalytic sensor. Good reproducibility and long-term stability</td>
</tr>
<tr>
<td>[79]</td>
<td>Reagents and detection</td>
<td>Newly photoelectrochemical degradation principle. COD is directly quantified by measuring the amount of electrons transferred at a TiO₂ nanoporous thin-film electrode during an exhaustive photoelectrocatalytic degradation process in a thin-layer photoelectrochemical cell</td>
</tr>
<tr>
<td>[80]</td>
<td>Reagents</td>
<td>New photocatalytic method based on a nano-TiO₂–K₂Cr₂O₇ system. COD values are calculated from the absorbance of Cr(III) produced by the photocatalytic reduction of Cr(VI)</td>
</tr>
<tr>
<td>[81]</td>
<td>Digestion</td>
<td>Closed microwave heating system. The digestion time is 15 min. Chloride ion interference can be removed up to 6000 mg Cl⁻/L</td>
</tr>
<tr>
<td>[82]</td>
<td>Digestion</td>
<td>Open microwave heating system. The digestion time is reduced from 2 h to 3–9 min</td>
</tr>
<tr>
<td>[83]</td>
<td>Digestion</td>
<td>Closed and open microwave heating are compared with ultrasound irradiation. Digestion time: 4 min, 4 min, and 1 min for closed MW, open MW, and US, respectively. Recovery values of real wastewater samples lie between 88% and 104% of the values obtained with the classical (open reflux) method</td>
</tr>
<tr>
<td>[84]</td>
<td>Digestion</td>
<td>Ultrasound irradiation. High-grade titanium alloy sonotrode indirectly irradiate water samples. Sonication time: 2 min. Recovery values of real wastewater samples lie between 50% and 60% of the values obtained with the conventional semimicro method</td>
</tr>
<tr>
<td>[85]</td>
<td>Digestion</td>
<td>Ultrasound irradiation. All-glass cylindrical sonotrode directly irradiate water samples. Sonication time: 2 min. Chloride is tolerated up to a concentration of 7000 mg Cl⁻/L. COD values obtained with real samples range between 53% and 143% of values obtained with the reference method</td>
</tr>
</tbody>
</table>
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[86] Detection and degradation
Ultrasound irradiation with boron-doped diamond electrode as electrochemical determination. It improves degradation efficiency, accelerates mass transfer process, and expands measurement range up to 23,200 mg O₂/L. It requires about 5 min to complete the oxidation.

[87] Detection
Amperometric determination using boron-doped diamond sensor. Optimal operating conditions are pH 2 and 2.5 V of applied potential. Recoveries range from 94% to 105%, with a linear range of 20–9000 mg O₂/L, a LOD of 7.5 mg/L, and 2–3 min for total analysis time per sample.

[88] Detection
Photoelectrocatalytic thin-cell reactor based on a highly effective TiO₂ nanotube array electrode. 1–5 min to complete the oxidation, wide dynamic working range (0–850 mg O₂/L), good accuracy, stability, reproducibility, and excellent correspondence with the standard method (94–104%).

[89] Digestion and detection
A novel and low-cost method based on KMnO₄–glutaraldehyde CL system. Complete analysis performed in a 96-well plate format in 40 min, a high throughput (3 × 96 samples per hour), small volume of sample (5 mL), linear range from 0.16 to 19.24 mg O₂/L and excellent LOD of 0.1 mg O₂/L. The method can also be used to determine high COD in sewage water. Recoveries range from 88% to 101%.

[90] Detection
TiO₂ nanofibers fixed in a microfluidic device for rapid determination via photoelectrocatalysis. A working electrode in a transparent microfluidic device made from poly(dimethylsiloxane). Determination limit of 0.95 mg/L and working range of 0–250 mg/L.

[91] Detection
A nano-ZnO/TiO₂ composite film as photocatalyst, which improves the charge separation efficiency and extends the spectrum range. This method is based on the direct determination of the Mn(VII) concentration change resulting from photocatalytic oxidation of organic compounds on the nano-ZnO/TiO₂ film. LOD is 0.1 mg O₂/L and linear range is from 0.3 to 10.0 mg O₂/L. Recoveries range from 93% to 101%.

[92] Detection
Near-infrared reflectance spectrometry of seston (NIRR). The analysis does not require any special reagent, catalyst, or solvent. Inherent baseline and noise features present in NIRR spectra are removed by a Savitzky–Golay derivative procedure followed by wavelet denoising and subsequent prediction of COD values in new samples. Results are deemed adequate and calculated error is 21 mg O₂/L.

[93] Digestion
Manganese(III) is used as oxidant and microwave radiation and ultrasound energy have been assessed to speed up and to improve the efficiency of digestion step. MW- and US-assisted digestion methods (1 min irradiation time) are optimized by means of experimental design. COD recoveries obtained for five real wastewater samples with MW- ranged between 86% and 97% and from 68% to 91% for US-assisted digestion.

[94] Detection
A nanostructured mixed-phase TiO₂ photoanode is used as a photoelectrochemical portable probe. A practical LOD of 0.2 mg O₂/L and a linear range of 0–120 mg O₂/L are achieved. Excellent agreement between the proposed method and the conventional method (dichromate).

[95] Digestion and detection
Combined photocatalytic system and a three-parameterized procedure for the determination of COD, with a highly oxidative reagent utilized as a photoelectron scavenger and signal indicator. A narrow and reliable analytical linear range of 0–260 mg O₂/L is achieved for 10 min determination.

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cleaning, and metal extraction. However, surprisingly, ultrasound-assisted sample preparation is still not commonly used for analytical purposes. Recently, Canals et al. [84,85] have used for the first time ultrasound energy for the determination of COD. In these works, a time-consuming one-at-a-time optimization of the different parameters affecting the sonication process allowed the decrease of the digestion time from 2 h to 2 min, resulting in COD recovery values ranging between 53% and 143% in real samples [85].

Both microwave- and ultrasound-assisted digestions have been statistically optimized (experimental design) for COD determination of wastewater samples [83]. The digestion methods evaluated were “closed microwave-assisted” (CMWD), “open microwave-assisted” (OMWD), and “ultrasound-assisted” (USD). The optimized digestion time is 4, 4, and 1 min for CMWD, OMWD, and USD, respectively. Under optimum conditions, the studied digestion methods have been successfully applied, with the exception of pyridine, to several pure organic compounds and COD recoveries for 10 real wastewater samples were ranged between 88% and 104% of the values obtained with the open reflux method used as reference. Thus, the use of ultrasound energy for COD determination seems to be an interesting and promising alternative to conventional convective–conductive and microwave-assisted digestion methods since the instrumentation is simpler, cheaper, and safer, and the digestion step faster than the ones used for the same purpose. Domini et al. [93] have presented a method that combines trivalent manganese as oxidizing reagent with microwave radiation and ultrasound energy. The developed method is an interesting alternative to conventional COD digestion methods since it is faster and more environmentally friendly than the ones used previously for the same purpose. Furthermore, both types of energies have also been combined for the simultaneous and direct irradiation of water samples to obtain a synergy between both types of energy [97]. The patented system reduces significantly the digestion time (1 min) and improves the COD recovery value of a very difficult pure organic compound (pyridine) up to 75%. Figure 17.2 shows the simultaneous and direct MW–US digestion system.

![Simultaneous and direct MW-US digestion system](image-url)

**FIGURE 17.2** Simultaneous and direct MW-US digestion system. (a) Scheme: 1, sample + reagents; 2, MW generator; 3, US generator; 4, all-glass sonotrode. (b) Photo: 5, MW unit; 6, US unit. (Adapted from A. Canals et al. Método y dispositivo que permite irradiar directamente bien de forma simultánea, consecutiva o alternativa una muestra con radiación de microondas y/o ultrasonidos. Spanish patent number: P200600502, March 06, 2006.)

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17.3.4 Methods Based on Flow Injection Analysis

Over the past 30 years, the methods based on flow injection analysis (FIA) have experienced enormous development in analytical laboratories [98]. The COD has also been one of the applications of this methodology. In these cases, the digestion and detection steps are carried out on-line. Hence, the analyte concentration is continuously determined from a liquid stream. Small volumes of sample and reagents are added at strategic points of the system. Several FIA manifolds have been developed for COD determination. The most significant characteristics of these systems are summarized below.

The first attempt to develop an FIA system for COD determination was performed in 1980 by Korenaga [99]. In this method, the heating step consists of a thermostated bath of water, oil, or polyethylene glycol in which a 20–50 m Teflon® capillary is immersed. The reagents are mixed into the flow by connections, while a given volume of the sample is inserted in the flowing carrier through injection valves. Owing to the high length of the capillary, the pressure needed for the mixture to flow through the system is high and an appropriate pump is required. When the sample leaves the bath, the absorbances are obtained spectrophotometrically [99–102]. For this purpose, a flow cell is adapted at the end of the line. The absorbance is measured at wavelengths that depend on the oxidant employed. To date, this methodology has been applied using potassium permanganate [99,100], cerium (IV) [102], and dichromate [101,103] as oxidizing agents. An application of the FIA methodology to COD determination describes the electrochemical generation of the oxidant (i.e., Co(III)) [104]. Following the FIA method, a calibration curve is obtained from the absorbances of the standard solutions. Note that, when the oxidizing agent is monitored, the absorbance obtained for the blank is always higher than that obtained for the water sample, since a fraction of the oxidant is spent in the reaction with the organic matter.

Some FIA systems based on microwave heating of the sample have been proposed for COD determination [105–108]. In these methods, the digestion step is accelerated with respect to the method of Korenaga and coworkers. Recently, a fast and fully automatic MW-assisted COD measurement device has been described [108]. The absorbance of Cr(III) generated during sample oxidation is measured at 590 nm. COD values are obtained in just 12 min and the device is absolutely controlled by a personal computer. The application range extends from 0 to 15,000 mg O₂/L, and the interference of Cl⁻ can be removed up to 8000 mg/L. Particular attention has been given to the cleaning-up of the device.

A flow injection manifold incorporating ultraviolet (UV)–photocatalytic oxidation for the spectrophotometric determination of COD in freshwaters has been reported by Dan et al. [109]. The reduction in permanganate absorbance due to oxidation of the organic compounds is determined at 524 nm and the sample throughput is 30 samples per hour. The use of UV irradiation eliminates the need for high-temperature oxidation. Besides, the methods based on spectrophotometric detection have some problems (Section 17.3.2). These have been overcome by the use of a flame atomic absorption spectrometer (FAAS) as the detector [106]. Since this is a nonspecific detector for Cr(VI), an anionic-exchange resin was placed before the FAAS instrument. The chromium(VI) that was not reduced in the digestion step is retained in the anionic resin. Then, it is eluted with the appropriate solution (e.g., 10 mol/L nitric acid) and the FAAS signal is recorded as a peak. Moreover, with this setup, the linear dynamic range lies between 50 and 10,000 mg O₂/L, and up to 50 samples per hour can be analyzed. In a more recent study, the resin has been changed by a selective Cr(VI) organic solvent extraction step [107].

An electrochemical detection system with an F-PbO₂ modified electrode for FIA to determine COD has been proposed [110]. The measuring principle is based on the current response on the modified electrode, which is proportional to the COD value. The method is characterized by short analysis time, simplicity, low environmental impact, a limited reagent consumption, and easy automation.

Recently, a photocatalytic sensor has been described for the COD determination on flow injection analysis [111]. The sensor is developed in conjunction with TiO₂ beads in a photochemical column irradiated with a UV lamp and with an oxygen electrode as the sensing part. The sensor signal is observed as a result of the detection of dissolved oxygen changes due to photocatalytic oxidation of organic compounds in the sample solution. More recently, Chen et al. [112] has suggested another TiO₂ photocatalytic sensor for COD determination by measuring the photocurrent formed at the interface of TiO₂ sensor and the flowing carrier when the sensor is illuminated with UV light (λₘₐₓ = 253.7 nm). The COD values...
obtained with the photocatalytic sensors are in excellent correspondence with values obtained using conventional methods (dichromate and permanganate). The main advantages of these TiO₂ photocatalytic sensors are their simplicity of preparation, low cost in the manufacturing process for the sensor, fast response time, acceptable lifetime, and potential for automated monitoring.

Li et al. [113] have investigated a photoelectro-synergistic catalysis oxidation of organic compounds in water on Ti/TiO₂/PbO₂ electrode coupled with FIA. The results are compared with those obtained from electrocatalysis and electro-assisted photocatalysis. The photoelectro-synergistic catalysis method obtains higher correlation, lower detection limit (15.0 mg O₂/L) and wider linear range (30–2500 mg O₂/L). Recoveries range from 81% to 103%.

Flow-injection chemiluminescence (FI–CL) has also been reported for COD determination [114,115]. FI–CL is known to be a powerful analytical technique that promises high sensitivity, wide linear range, simple instrumentation, rapid, reproducible means of detection, and a broad range of applications. In the first work [114], potassium permanganate is reduced to Mn²⁺, which is first adsorbed on a strongly acid cation-exchange resin mini-column to be concentrated during chemical oxidation of the organic compounds at room temperature. Then the concentrated Mn²⁺ is eluted and measured by the luminol–H₂O₂ CL system. The CL flow system is very simple and rapid. The FI–CL method studied by Jin et al. [115] is based on the phenomenon that luminol can be oxidized by the dissolved ozone aided by UV radiation to produce luminescence. From the difference in the CL signals between the blank and sample solutions, the COD on the sample solution can be determined. The FI–CL methods are simple and fast.

A novel on-line method based on the combination of a UV photoreactor with CL detection for indirect COD determination has been developed by Su and coworkers [116]. The UV irradiation of the sample without TiO₂ shows adequate oxidative capacity and reproducibility. Under optimum experimental conditions, a linear range of 0.2–20 mg O₂/L and LOD of 0.08 mg O₂/L is obtained. Later, Liu et al. [117] have developed a micro-flow system with CL for rapid COD determination at room temperature. A poly(methyl methacrylate) micro-flow chip with discrete microdroplet sampling is used.

An FIA system based on a boron-doped diamond (BDD) electrode as the detecting element has been developed [118]. This system promotes an environmentally friendly, in situ, and on-line COD testing system. Furthermore, the FIA system shortens the analysis period, reduces the dosage of reagents used, and improves reproducibility.

Another original FIA based on the combination of a fully automated single interface flow system, an on-line UV photocatalytic unit, and quantum dot nanotechnology is presented by Silvestre et al. [119]. The developed approach takes advantage of CdTe nanocrystal’s capacity to generate strong oxidizing species upon irradiation with UV light, which promotes a fast catalytic degradation of the organic compounds. The proposed methodology allowed COD determination between 1 and 35 mg O₂/L, with good precision and high sample frequency. The procedure was applied to COD determination in wastewater certified reference materials showing excellent agreement with the certified values.

In summary, the proposed FIA-based methods for the determination of COD have several important advantages as compared with the conventional methods discussed in Sections 17.3.1 and 17.3.2: (a) higher sample throughput, (b) enhanced response times, producing shorter startup and shutdown times, (c) simpler methodology, (d) higher precision, and (e) wider dynamic range.

## 17.4 Total Organic Carbon

TOC is defined as the amount of carbon covalently bonded in organic compounds in a water sample [2]. The TOC is a more suitable and direct expression of total organics than either BOD or COD, but it does not provide the same kind of information. If a reproducible empirical relationship is established between TOC values and either COD or BOD, the TOC can be used to estimate the respective BOD or COD values. Typical TOC values range from 0.001 to 50 mg C/L. To determine the content of organically bonded carbon, the organic molecules must be broken down to single carbon units and converted into a simple molecular form that can be quantitatively measured [1,120–138]. The instruments employed to determine TOC could be classified as on-line and off-line. The first category has several advantages over the off-line
methods, among them being (a) simplicity of the method and (b) avoidance of the errors induced by the dramatic change of TOC with time, since the on-line instruments are able to take measures very quickly.

The TOC is included within the total carbon (TC) and has many fractions that can be analyzed separately. Figure 17.3 gives an overview of the definitions of the different TC portions and their distribution.

To determine TOC, IC must be either removed from the sample (direct TOC method) or measured (indirect TOC method). With direct method, TOC value can be obtained by means of removing IC and measuring directly the TOC value, whereas on the indirect method, IC and TC are measured and TOC is obtained subtracting IC from TC. Several methods have been proposed for the direct TOC method [121,127]. IC can be eliminated by acidifying samples to pH 2 or less to convert all the fractions included in this category (see Figure 17.3) to CO₂, which is more easily removed from the water sample. For IC determination, the sample can be injected into a separate reaction chamber packed with phosphoric acid-coated quartz beads, where all the IC is converted to CO₂, which is then measured. Under these conditions, organic carbon is not oxidized and only IC is measured. Different methods have been proposed to measure the CO₂ evolved [138–145]. Nonetheless, when a gas stream is passed to purge CO₂, volatile compounds can be dragged as well. In this case, the measure in fact corresponds to the organic carbon that cannot be purged (i.e., nonpurgeable organic carbon (NPOC)). The POC is a very interesting parameter to survey whether there are some synthetic organics and must be determined to know the true TOC value (Figure 17.3). The POC is determined by sparging the sample at ambient temperature. The purgeable components can be further trapped and thermally desorbed and driven to the high-temperature zone where oxidation of CO₂ is produced, or on the other hand, trapped, thermally desorbed, and measured by gas chromatography–mass spectrometry [146]. However, the POC value is usually less than 10% of

**FIGURE 17.3** Chart of the different fractions of total carbon.
the NPOC concentration, and it is commonly neglected, assigning the NPOC value as the TOC value (Figure 17.3). In addition, if the solid fraction is not significant, then the dissolved organic carbon (i.e., DOC) value is similar to the TOC value. Finally, volatile organic carbon (VOC) and nonvolatile organic carbon (NVOC) are other parameters included with the POC and NPOC.

There are several different methods used to convert carbonaceous matter to carbon dioxide. Among others, two principal oxidation techniques, which are considered in the official methods, can be distinguished: high-temperature combustion (HTC) [147–151] and low-temperature oxidation with UV radiation [123,129–132,148,150,152–154] or heated persulfate [133,148,150,152,153]. Other alternative oxidation methods have been proposed, such as chromic acid [155–157], γ-ray radiation and electron beams [158], direct determination by ICP–AES [140], and using ozone and hydroxyl radicals [159–162].

Different detection systems can be employed for the measurement of CO₂ evolved in the oxidation process. Nondispersive infrared spectrometry (NDIR) [120–126] can be highlighted as the most used; however, thermal conductivity [137], selective membrane conductivity [163], spectrophotometry [88], acid–base titration [114], CO₂-sensitive electrode [154,165–167], gravimetry [113], flame ionization [128], ion chromatography [127], and coulometer cell [169] are also employed.

Since the past few years, only a few publications have worked on the development of new oxidation procedures [160–162] or detection systems [154,170]. Most of the efforts have been focused on improving the systems already developed, mainly by commercial brands [163,169,171–179]. As a result, there are a wider variety of many modern analytical instruments, in which the oxidation process and detection system can be selected based on the needs.

### 17.4.1 High-Temperature Combustion Methods

The most widely used method to accomplish the oxidation of carbon-containing species to CO₂ and H₂O is the catalytic (i.e., Pt, Cu, Co, Ir, their oxides or alloys) oxidation in gas phase at temperatures ranging from 680°C to 1300°C. First, the aqueous sample is homogenized and diluted as necessary and then dispensed as a stream of liquid into a combustion tube filled with a catalyst that promotes the redox reaction with oxygen, requiring very low sample volumes (i.e., from 10 to 2000 μL). Second, the carbon dioxide generated is transported in the carrier-gas streams and determined by means of an NDIR analyzer [120–126] or other detection system. Finally, milligrams of carbon per liter are obtained from a calibration curve. An ultrapure gas stream (e.g., oxygen [127], helium [128], argon [140], air, or nitrogen) is used to drive the CO₂ toward the detector.

Many commercial TOC analyzers are available [169,171–176]. These systems can be used to determine any TC fraction by selecting the appropriate automatic program. The reported significant figures are: dynamic range: 0.002–30,000 mg C/L; time for analysis: 1–10 min; precision: <1–2% (RSD); LODs: 2–4 μg C/L.

### 17.4.2 Low-Temperature Oxidation Methods

The main analytical problem encountered in determining TOC determination by HTC methods arises from the difficulty in controlling the temperature in the oxidation step, leading to deterioration in the precision of the results. Additional pitfalls of the HTC methods include [140,180] (i) appearance of long memory effects, (ii) capillary blocking when working with high-salt-content solutions or with samples containing suspended solid matter, (iii) high background levels due to carbon release from the catalyst and some other parts of the system, (iv) sometimes the too low yield of oxidation (e.g., 82% for sulfathiazole), and (v) mechanical problems caused by the sudden expansion of the carrier gas stream as it enters the high-temperature column. For these reasons, alternative low-temperature oxidation methods were proposed: UV radiation [123,129–132,148,150,152–154] or heated persulfate [133,148,150,152,153].

In the UV method, the solution is irradiated with near-ultraviolet radiation (200–400 nm) to decompose organic matter by means of a radical formation mechanism. Then the generated CO₂ is transported toward the detector with a carrier gas. To eliminate some ionic compounds that can interfere with the measurement, a membrane is placed before the detector. The detection can be carried out by
the measurement of conductivity via a sensor, by an NDIR analyzer, or by another detection method described above. Species such as TiO$_2$ [129,134,165–167] and persulfate [135,148,150,152–154] have been used as catalysts present as a diluted suspension in water.

Persulfate is also employed for the oxidation in the presence of heat [133,148,150,152,153]. The oxidation principle is that organic carbon is oxidized to carbon dioxide by persulfate and the CO$_2$ produced may be purged from the sample, dried, and transferred with a carrier gas to an NDIR analyzer, or be coulometrically titrated, or by means of another detection method.

Nowadays, these oxidation methods are well known and most of the commercially available instruments are based on them [163,171,172,174–176]. The reported significant figures are: dynamic range from 0.03 μg C/L to 50,000 mg C/L; analysis time 1–10 min; LODs: 0.03–0.5 μg C/L; precision: <1–2% (RSD).

These oxidation methods overcome most of the disadvantages of the HTC method; however, they show other drawbacks. On the one hand, the intensity of the UV light reaching the sample matrix may be reduced by highly turbid samples or with aging of the UV source, resulting in sluggish or incomplete oxidation [152]. On the other hand, large organic particles or very large or complex organic molecules such as tannins, lignins, and humic acid may be oxidized slowly because persulfate oxidation is rate-limited. Moreover, persulfate oxidation of organic molecules is slowed in samples containing significant concentrations of chloride by the preferential oxidation of chloride; at concentrations above 0.05% chloride, oxidation of organic matter may be inhibited [152].

17.4.3 Other Methods

Plasma spectrometric techniques have been hardly applied to carbon determination [181–184] and a new attempt was made to use the ICP–AES technique for the direct and simultaneous determination of TOC and IC [140]. The new system for the direct determination of TOC and IC (or total inorganic carbon (TIC)) is based on the measurement of the carbon atomic emission intensity in inductively coupled plasma atomic emission spectrometry with the aid of a semiautomatic accessory connected to the spectrometer that separates the different carbon fractions (i.e., organic and inorganic). This way, the organic matter does not undergo any preoxidation step. The system exhibits good sensitivities compared to those provided by conventional TOC and IC determination methods. The limits of detection obtained have been 0.07 and 0.0007 mg C/L in terms of TOC and IC, respectively. Furthermore, the system is able to handle high-salt-content solutions. This fact suggests that it would be possible to analyze seawater samples, avoiding some of the problems encountered with conventional methods, such as system blocking or interferences. The ICP–AES method has been successfully used in two interesting applications: (i) monitoring the efficiency of a water treatment plant; and (ii) determining the contents of dissolved carbon dioxide, on the one hand, and that of carbonate and bicarbonate, on the other, in the same sample. Other reported figures of merit are: dynamic range: 10–1000 mg C/L; precision: 1–10% (RSD).

Another procedure for the determination of TOC and its fractions in industrial effluent samples has been introduced [185]. A flow injection system using a gas–liquid transfer microreactor is developed, and adapted to a turbidimetric spectrophotometer. Samples are decomposed into glass vials in a microwave oven and a fraction of the CO$_2$ is injected into a carrier gas and pumped to a glass microreactor. With minor modifications, the system allows the determination of different carbon fractions. The advantages of the proposed procedure are simplicity, low volume of samples and reagents, high frequency of determinations, and low cost. The dynamic range is 20–800 mg C/L, and the calculated LOD is 17 mg C/L.

17.4.4 Recent Advances

One of the last advances in the oxidation processes is shown in a commercial instrument developed by BioTector [160–162,179] that uses a patented two-stage advanced oxidation technique as an alternative to the common standard oxidation methods. In this instrument, the sample is oxidized by hydroxyl radicals, which are created by exposing high pH reagents to ozone (oxidation time less than 150 s). Then, acid reagent containing 40 mg/L of manganese sulfate monohydrate catalyst is added to remove the TOC content of the oxidized sample as CO$_2$, which is sparged by the carrier gas and measured with an infrared
detector. The duration of a typical two-stage advanced oxidation reaction is less than 7 min (including both TIC and TOC measurement). The accuracy of the analyzer is ±3% of the reading and the dynamic range is from 0 to 25,000 mg C/L.

The interference coming from salt concentration up to 26–30% has been found to be negligible on the oxidation process. This process does not require any sample pretreatment such as homogenization, filtration, and/or salt removal, and it does not suffer from any clogging or contamination problems. Another advantage of this system is that no TIC carryover to the TOC analysis phase is guaranteed. In addition, the aggressive oxidation process using hydroxyl radicals cleans all contaminant and the wetted reactor parts.

Regarding the new detection systems, a tubular membrane capacitance sensor (TMCS) [154], in which wire electrodes are wrapped on a porous hydrophobic membrane tube and capacitance is measured, has been developed. The sensor has been used for the detection of CO2 evolved in the oxidation process by UV/persulfate [154]. The system used is lab-made and one sample can be measured every 8 min; the relative standard deviation for five replicates of glucose solutions containing 10 mg C/L is 2.3%. The range studied 0.73–43.8 mg C/L shows a quadratic relationship. The system is capable of detecting 800 ng C as glucose and 100 ng C as inorganic carbonate. The CO2 evolved is sensed by the TMCS as the water slowly passing through it absorbs the CO2, resulting in a change in conductance and capacitance. The sensor combines the dual function of gas collection and measurement, thus permitting a simple miniature economic device.

Another alternative to the determination of CO2 produced in the oxidation process has been developed by Fan et al. [170]. In this system, the CO2 evolved in the oxidation by potassium persulfate was purged with nitrogen, and absorbed by 50 mL scrubber solution. The carbonate is determined by means of a H2O2–luminol–uranine CL reaction. The advantage of employing this reaction is that a low concentration level of carbonate plays a significant role in the CL reaction due to the strongly synergistic enhancement effect of uranine. The developed method promises a wide linear detection range spanning four orders of magnitude and a low limit of detection of 1.2 × 10^-11 mol/L for the determination of carbonate. The linear ranges for TIC and TOC are 1.2 × 10^-6–6.0 × 10^-2 mg C/L and 0.08–30 mg C/L, respectively. Recoveries of 97.4–106.4% for TIC and 96.0–98.5% for TOC are obtained by adding 5 or 50 mg C/L to the samples. The relative standard deviations are 2.6–4.8% for TIC and 4.6–6.6% for TOC (n = 5).

In this case, the reaction of the H2O2–luminol type are greatly influenced by the presence of some heavy metal ions. However, owing to the purge and scrub process, metal ions are not present. Although the method is precise, accurate, simple, selective, and resistant to foreign interferences, it suffers from incomplete oxidation in the sample preparation procedure used when very low TOC concentration (<0.08 mg C/L) is determined.

17.4.5 Interferences

The chloride is a source of interferences in TOC determination since it scavenges the free radicals that are the principal agents of oxidation. In addition, the oxidation of chloride to chlorine can produce detector failure. Therefore, some of these methods are not advisable to determine TOC in seawater. The photodecomposition method is not suitable for refractory nonpurgeable organic compounds. Dry combustion has also been used to determine NPOC in seawater [186]. Dry combustion loses even moderately volatile material because it requires evaporating off all the water first. Fry et al. [186] relied on the natural marine sulfate salts to act as an oxidant in a fashion similar to a Kjeldahl determination. However, owing to the large sample volume required and the potential for contamination or loss in manipulations, this approach seems unlikely to catch on.

However, the system recently developed by BioTector [160–162] can determine TOC in samples that contain a salt concentration up to 30%. This improvement shows an important step to overcome the salt interference pitfall.

The relative effectiveness of the different digestion methods still remains a source of debate to this day [6,187–194] due to the inexistence of the perfect method. Although the two-stage advanced oxidation method solves most of the problems presented by the conventional oxidation methods, the HTC method is suitable for samples with higher levels of TOC that would require dilution for the various persulfate methods. Generally, it will also determine organic carbon from compounds that are chemically
refractory and not determined by persulfate methods. HTC is also desirable for samples containing solid particles, chlorides, and high levels of suspended organic carbon, which may not be efficiently oxidized by persulfate and/or UV methods. However, the HTC methods accumulate nonvolatile residues in the analyzer. Persulfate and/or UV oxidation are useful for TOC as low as 0.03–0.5 μg C/L. Since part of the range of the sensitivity of the method overlaps and other factors may dictate method choice. A method may be chosen on the basis of desired precision, ease of use, cost, interferences, and so on.

### 17.5 Total Organic Halide

Disinfection of drinking water has been practiced since the early 1900s. Chlorine has been the most widely used in many water treatment plants to inactivate microorganisms and maintain a residual concentration through the water distribution system. Despite obvious advantages in terms of controlling microbes in drinking water, chlorination also has disadvantages because of the formation of disinfection by-products (DBPs) through contact between natural organic matter (NOM) and disinfectants. Some of these products such as trihalomethanes (THMs) and haloacetic acids (HAAs) have been known to cause cancer and other toxic effects to human beings. Owing to their high toxicity, it is very important to control the concentration of these compounds. TOX is the parameter used to estimate the total amount of organic halide present in water [195]. The TOX value is a complex function of several parameters (i.e., pH, temperature, reaction time, amount of dissolved organic matter, halogen type and concentration, disinfectant type and concentration, etc.) [196,197]. However, as with some parameters just discussed, TOX does not give any information about the structure or bonds between halogen and carbon, or about the different halogenated subgroups of TOX. As for carbon, there is also a parameter called dissolved organic halide (DOX), which reflects the total amount of organic halogenated matter dissolved in a water sample.

The TOX is useful for screening a large number of samples before performing a specific, and often more complex, analysis. In addition, it is used for (a) extensive field surveying of the degree of pollution by certain classes of synthetic organic compounds in natural waters; (b) mapping the extent of organohalide contamination in groundwater; (c) monitoring the breakthrough of some synthetic organic compounds in water treatment processes; and (d) estimating the level of formation of halogenated, mainly chlorinated, organic by-products after disinfection. There is always a possibility of overestimating TOX concentration because the inorganic halide contribution should always be considered when interpreting results [198].

The method most often employed has four steps and is called the adsorption–pyrolysis–titrimetric method [197–201].

#### 17.5.1 Adsorption–Pyrolysis–Titrimetric Method

The adsorption–pyrolysis–titrimetric method for TOX measures only the total molar amount of dissolved organically bound halogen retained on the activated carbon (AC) adsorbent. Nevertheless, it yields no information about the structure or nature of the organic compounds to which the halogens are bound or about the individual halogens present. The four processes involved are: (a) all the halogenated compounds are adsorbed on granular AC; (b) adsorbed inorganic halogens are displaced by means of nitrate; (c) the AC with only the halogenated organic compounds adsorbed is pyrolyzed, and the halogens bonded to carbon are transformed to their corresponding halides (X⁻), whereas the carbon yields CO₂; and (d) X⁻ is driven to a microcoulometric titration cell, where it is quantified by measuring the current produced by silver ion precipitation of the halides. In this method, the pH of the sample must be adjusted to a value lower than 2 with sulfuric acid. If there are suspended solids, the sample must be filtered or particulates must be settled in the sample container and decant the supernatant liquid into the adsorption system. This process must be done as gently as possible to minimize the loss of volatile organo halides. Residual chlorine is reduced by adding sulfite. Then the treated solution is forced to pass through two activated-carbon columns. To displace the inorganic halide adsorbed, 2 mL of potassium nitrate is passed through the columns filled with the AC. Finally, the contents of each column are pyrolyzed separately to convert
the adsorbed organohalides to hydrogen halide (HX). Pyrolysis of the sample is accomplished in two stages. The volatile components are pyrolyzed in a CO₂-rich atmosphere at a low temperature to ensure the conversion of brominated THMs to a titratable species. The less volatile components are then pyrolyzed at a high temperature in an O₂-rich atmosphere. The effluent gases are directly analyzed in the microcoulometric titration cell. The mass of HX determined is equivalent to the milligrams of X in the injected sample. The method detects all organic halides containing chlorine, bromine, and iodine that are adsorbed by granular AC but fluorine-containing species are not determined by this method. The method is applicable to samples whose inorganic halide concentration does not exceed the organic halide concentration by more than 20,000 times and it does not measure TOX of compounds adsorbed to undissolved solids. In general, this method cannot be applied for chloride concentrations greater than 500 mg/L, although the concentrations of halogenated compounds in water are usually smaller than 100 μg/L [197,198]. The same treatment is applied to both blanks and samples.

The silver-based microcoulometric titration used in conventional TOX measurement is incapable of differentiating between halides, and therefore, the method cannot distinguish total organic chlorine (TOCl) from total organic bromine (TOBr) and total organic iodine (TOI). By convention, TOX is calculated as a molar mass of organic halides, expressed as chlorine. However, brominated and iodinated DBPs are produced from the disinfection of water in the presence of bromide and iodide. Hence, the nonspecific TOX analysis might be a biased estimator of toxicity since brominated and iodinated DBPs present higher adverse health effects than their chlorinated analogs [202,203]. A method to differentiate TOCl and TOBr was developed [204]. In this method, HBr and HCl, contained in the off-gas from the TOX combustion furnace and equivalent to TOBr and TOCl in samples, are collected in water with a bubble diffuser. Then, Br⁻ and Cl⁻ concentrations are determined by ion chromatography. Interference with Cl⁻ quantification by CO₂ gas (an auxiliary gas for the combustion furnace) can be eliminated by sparging the solutions with N₂ gas. A modification of this method supplies simultaneously the three TOX concentrations (TOCl, TOBr, TOI) [205,206]. This analytical method has recently been used by Li et al. [203,207] to study the reduction of halogenated DBPs to halide during the adsorption step by the AC used in the TOX standard method. These halides will be removed from the AC during the rinse step with nitrate, leading to an underestimation of the amount of TOX present. Brominated DBPs reduction was lower than that of the chlorinated DBPs and the reduction of the TOI was negligible. Therefore, the reduction of halogenated organic compounds by AC could lead to measurement errors, and TOX measurements may be improved by using ozonated AC that can minimize the reduction of halogenated DBPs during the adsorption step.

EPA Method 9022 (Total Organic Halides (TOX) by Neutron Activation Analysis) [208] has the advantage of determining the individual concentrations of halogen chlorine, bromine, and iodine in addition to TOX. The method uses a carbon adsorption procedure identical to the Method 9020, irradiation by neutron bombardment, and then detection using a gamma-ray detector. Nevertheless, this method needs expensive facilities and skilled analysts in the operation of neutron activation analysis.

As in TOC determination, when samples are initially purged with inert gas, nonpurgeable dissolved organic halide (NPDOX) (i.e., TOX of sample that has been purged of volatiles) is determined. The purgeable fraction is named purgeable organic halide (POX) [196]. The POX fraction may be estimated by subtracting the NPDOX value from the DOX value. Alternatively, the POX fraction may be determined directly by Method 9021 [209]. Method 9021 determines organically bound halides (chloride, bromide, and iodide) purged from a sample of drinking water or groundwater. The volatile organic halides are purged into a pyrolysis furnace using a stream of CO₂ and the HX pyrolysis product is trapped and titrated electrolytically using a microcoulometric detector. The method detects purgeable organically bound chlorine, bromine, and iodine but it does not measure fluorine-containing species. It measures POX concentrations ranging from 5 to 1000 μg/L. Thus, depending on the approach, the analysis of POX, DOX, and NPDOX may be determined directly or by difference. Recently, Davlin et al. [210] described a system that employs an on-line membrane introduction interface coupled with parallel flame ionization and electron capture detectors (FID/ECD) to detect total volatile organic halides (ΣVOCs) and volatile organic compounds (ΣVOCs) as aggregate parameters in environmental water samples at sub-parts-per-billion levels without the need for sample handling or analyte preconcentration. The instrument provides rapid screening and real-time monitoring of formation of volatile disinfection by-products in the chlorination of natural waters.
Since only adsorbable organic compounds can be analyzed by the adsorption–pyrolysis–titrimetric method, TOX is sometimes referred to as adsorbable organic halide (AOX). The determination of AOX [211–214] is also very useful for samples with high levels of solids, since the columns employed in the TOX test can be blocked. The AOX could be determined by the conventional adsorption–pyrolysis–microcoulometric method [214]. The AOX also includes some specific fractions (e.g., adsorbable organic chlorine (AOCl), adsorbable organic bromine (AOBr), adsorbable organic iodine (AOI)) [212]. Twiehaus et al. have developed a fully automated quasi-continuously operating monitor for element-selective analysis of AOX in water [215]. The new instrument is based on the element-selective analysis of halogens by means of a spectroscopic detection system consisting of a microwave-induced helium plasma excitation source and the plasma emission detector, which operates with oscillating narrow-band interference filters. However, bromine and iodine could not be detected with satisfactory interelement selectivity because of spectral interferences caused by matrix elements. But these fractions have been determined by coupling the AOX analyzer with an ionic chromatograph [216].

Finally, the sum of those organic halides that are extracted from solids is defined as extractable organic halide (EOX) [217]. Extractable organic halides containing chlorine, bromine, or iodine are detected. However, fluorine-containing species are not detected. The method has been evaluated for solid wastes, soils, and suspended solids isolated from industrial wastewater. A 1 g aliquot of solid sample is extracted with ethyl acetate by sonification to isolate organic halides. A 25 μL aliquot of the extract is either injected or delivered by boat inlet into a pyrolysis furnace using a stream of CO2/O2 (or appropriate alternate gas mixture) and the HX pyrolysis product is determined by microcoulometric titration.

### 17.5.2 Direct Measurement of Absorbance

This method is based on the fact that NOM exhibits an aromatic character that produces a strong radiation absorption in the UV spectrum region. When these compounds react with halogens, a sharp decrease in absorbance is observed. Therefore, TOX can be correlated with this absorbance drop, obtaining very good results [218]. In this study, changes in UV absorbance of NOM caused by chlorination were monitored at a wavelength of 272 nm, as opposed to 254 nm, which is more commonly used to monitor NOM content of natural waters [219]. A colorimetric screening procedure may be used to analyze water samples for total volatile organic halides [220]. The method consists of two steps: (i) extraction of the water sample and (ii) the reaction of the sample extract under UV light to produce a colored product, the absorbance of which is measured by a dedicated colorimeter. The color that is produced from the reaction is not stable and will decay after exposure. Given the potential loss of volatile constituents, samples should generally be analyzed as soon as it is practicable after collection, and always within 1 h. Usually, calibration is performed by analyzing three standards of trichloroethylene. However, if tetrachloroethylene, carbon tetrachloride, or chloroform is the predominant contaminant at a given site, a site-specific calibration can be generated, using the predominant contaminant.

### 17.5.3 Total Organic Halides as a Part of Disinfection By-Products

In drinking water, TOX is used as a surrogate measurement for the total halogenated DBPs formed from the reaction between chemical disinfectants and NOM. However, TOX represent only a portion of the types of DBPs formed. For example, the TOX measurement for chlorinated drinking water would not include contributions from nonhalogenated DBPs, such as formaldehyde or carboxylic acids. Treating surface waters with chlorine leads to the formation of numerous volatile and nonvolatile organic halogen compounds. Among them, the two largest classes of DBPs in drinking water are THMs and HAAs. The Environmental Protection Agency (EPA) especially promulgates those methods that meet agency requirements for monitoring drinking water contaminants. Currently, only these approved methods may be used for regulatory compliance monitoring. Approved methods generally include information on the collection, transport, and storage of samples, and they define procedures to concentrate, separate, identify, and quantify sample components. The regulated THMs are chloroform, bromodichloromethane, dibromochloromethane, and bromoform. Several methods are available for the measurement of the THMs. Some of these are specific for these compounds and others have a much broader spectrum.
Each test method purposely uses different techniques and equipments to accommodate the various skills and instruments available in compliance monitoring labs. Each method has been rigorously analyzed to meet the requirements for compliance monitoring. These techniques are revised and updated as new technology becomes available. There are six main EPA methods for regulated THMs. EPA method 501.2 is applicable only to the determination of four THMs, and it is based on a liquid–liquid extraction followed by GC–ECD for separation and analysis [221]. Method 551.1 refers to the liquid–liquid extraction GC–ECD method that can detect not only THMs but also other chlorinated disinfection by-products, chlorinated organic solvents, and halogenated pesticides and herbicides [222]. The developments of purge-and-trap led to EPA Methods 501.1, 502.2, 501.3, and 524.2 for the THMs [223–226]. Methods 501.1 and 501.3 are applicable to the determination of four regulated THMs, whereas Methods 502.2 and 524.2 are applicable to a wide range of organic compounds, including the four THMs. The detectors used are halide-specific detector (i.e., electrolytic conductivity or microcoulometric titration), photoionization and halogen-specific detector in series, mass spectrometry, and mass spectrometry in Methods 501.1, 502.2, 501.3, and 524.2, respectively.

There are nine species of HAAs (HAA9), of which five species are currently regulated (HAA5). These are monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid, as HAA5, and HAA5 plus bromochloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid, and tribromoacetic acid, as HAA9. HAAs are mostly deprotonated under drinking water conditions, so they cannot be directly injected onto a column. Methylating the carboxylate moiety is the standard approach to making these species amenable to GC. In Method 552 [227], hazardous diazomethane was used for methylation, and in the more recent Method 552.2, acidified methanol was used for methylation. EPA Method 552 has been improved to enable the analysis of all HAA9 [228]. The new method, EPA Method 552.3, improves the detection of the trihalogenated brominated DBPs by increasing the amount of methanol to improve methylation efficiency, incorporating tert-amyl methyl ether (TAME) as an optional extraction solvent to allow higher methylating temperatures (and better methylation efficiencies), and discontinuing the use of copper sulfate to prevent degradation of some HAAs. Recently, the EPA Method 557 for the determination of nine haloacetic acids in finished drinking water [229] has been published. It is a direct-inject, ion chromatography, negative-ion electrospray ionization, tandem mass spectrometry (IC–ESI–MS/MS) method. LODs for the method analytes fortified into reagent water ranged from 0.015 to 0.20 μg/L. New analytical methods for HAAs have also been developed. Jia et al. [230] created a GC/electron capture negative ionization (ECNI, also called negative ion chemical ionization)–MS/MS method for determining nine HAAs in water, plasma, and urine at 25–1000 pg/mL detection limits. Rather than using methylation derivatization, as most HAA methods do, this method utilizes derivatization with pentafluorobenzyl bromide, which allows increased sensitivity for ECNI–MS. One of the more unusual developed methods involved the creation of a molecularly imprinted sensor for screening HAAs in drinking water. The sensor calibrations were linear over a range of 25–1000 μg/L, with the detection limit of each HAA in the range of 0.2–5.0 μg/L. This simple method appears to be promising for the rapid and sensitive detection of HAAs in drinking water [231]. Chellam et al. [232] have presented the first results for speciation of DBPs resulting from chlorination in nanofilter permeates, without the artificial addition of bromide. Both DOC concentration and UV absorbance at 254 nm were found to correlate strongly with THM, HAA, and TOX concentrations in chlorinated nanofiltered permeates.

Kulkarni and Chellam [233] have used artificial neural network (ANN) to quantify the formation of total THMs, sum of six HAAs, TOX, as well as individual THM and HAA species upon chlorination of untreated water, and after conventional treatment, granular AC treatment, and nanofiltration. Highly accurate predictions of DBP concentrations were possible using physically meaningful water quality parameters as ANN inputs, including DOC concentration, UV absorbance at 254 nm, bromide ion concentration (Br−), chlorine dose, chlorination pH, contact time, and reaction temperature. This highlights the ability of ANNs to reduce the number of experiments to assess water quality for process control and optimization, comparison of treatment alternatives for DBP control prior to piloting, and when operating conditions are changed.

With stricter regulations for THMs and new regulations for HAAs, many drinking water utilities have to change from chlorine to alternative disinfectants (including ozone, chlorine dioxide, and chloramines) to meet the new regulations. However, new issues and problems can result. Ozone is the most efficient...
chemical disinfectant currently applied in drinking water treatment. Even for microorganisms such as protozoa, which are difficult to inactivate with other disinfectants (chlorine, chlorine dioxide), ozone provides adequate inactivation with reasonable doses and contact times. Ozone (with chloramines or chlorine) can significantly reduce (or eliminate) the formation of THMs and HAAs, but can result in the formation of bromate, especially when elevated levels of bromide are present in the source water. Bromate is currently the by-product that causes most concern due to its potential carcinogenicity. Once formed, the best bromate minimization strategies appear to be lowering of the pH or ammonia addition. Iodate is the main by-product formed during ozonation of iodide-containing waters. Iodate, quantitatively formed by oxidation of naturally occurring iodide by ozone, is of no toxicological concern and it is rapidly metabolized after ingestion. Chlorate, whose toxicological impact is unclear, is only formed during ozonation if a preoxidation of the water with chlorine and/or chlorine dioxide is applied [234]. Recent studies about the formation of halogen-specific TOX upon several disinfectants of NOM isolates in the presence of bromide and iodide ions concluded that free chlorine formed more THMs, HAAs, and TOX, and among them produces a much higher level of TOCl and TOBr, than chloramines and chlorine dioxide. In both chlorination and chloramination, TOCl is the dominant TOX species formed. TOI is always produced with chloramine and chlorine dioxide, and a suitable chlorine dose should be utilized to reduce the formation of TOI [206,235].

More than 50% of the halogenated DBPs in drinking water are still not accounted for [236,237]. Advanced instrumentation, especially mass spectrometry (LC/Q-TOF–MS, LC/Orbitrap-MS, or LC/Qq-linear ion trap–MS), has enabled progress in the detection of halogenated compounds not previously detected. For example, haloquinones are highly toxic and may be formed during drinking water treatment. Some haloquinones (2,6-dichloro-1,4-benzoquinone, 2,6-dichloro-3-methyl-1,4-benzoquinone, 2,3,6-trichloro-1,4-benzoquinone, and 2,6-dibromo-1,4-benzoquinone) such as DBP have been identified for the first time in drinking water using LC/ESI–MS/MS [238,239]. Iodinated DBPs are generally more toxic than their chlorinated and brominated analogs. Ding and Zhang [240] have developed a method for fast selective detection of polar iodinated DBPs using an electrospray ionization–triple quadrupole mass spectrometer (ESI–trqMS). By coupling state-of-the-art ultraperformance liquid chromatography (UPLC) to the ESI–tqMS, structures of 17 iodinated DBPs were tentatively proposed [240]. Zhai and Zhang [241] developed a new ESI/MS/MS method for differentiating ESI adducts of common drinking water DBPs, among them brominated DBPs, from higher-molecular-weight DBPs. Using this method, they also proposed structures for several new brominated DBPs in simulated drinking water [241]. Iodoacetic acids (IAAs) are the emerging DBPs that have been recently found in disinfected drinking waters with higher toxicity than their corresponding chloro- and bromoacetic acids. Shi and Adams developed a rapid and sensitive ion chromatography (IC)/ICP–MS method for simultaneously measuring four iodoacetic acids, six bromoacetic acids, iodate, bromate, iodide, and bromide [242]. However, mono-, di-, and trichlorinated species could not be detected because the sensitivity of ICP–MS for chlorine is poor. Method detection limits range from 0.33 to 0.72 μg/L for iodinated DBPs, and from 1.36 to 3.28 μg/L for brominated DBPs. This method is successfully applied to measuring brominated and iodinated DBPs in drinking water, groundwater, surface water, and swimming pool water [242].

Recommended reviews about the state of the art of water analysis, drinking water contaminants of emerging concern, and the analytical methods currently being used for their determination are Refs. [202,236,237,243], and for more water analysis methods, see Ref. [244].

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Spanish Ministry of Economy and Competitiveness (Project ref. CTQ2011-23968) for the financial support.

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