In-situ and real-time measurements in water monitoring

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Chapter 3

In-situ and real-time measurements in water monitoring

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ABSTRACT

In situ and real-time measurements are increasingly used in environmental monitoring and control to enhance the efficacy of decision-making in environmental management. In situ measurement techniques enjoy high priority in surface water and wastewater management, particularly in contaminated or otherwise endangered water and land investigations. Real-time measurement methods in particular are employed in water management and global earth monitoring systems. The in situ measurements and associated telecommunication, along with the local and worldwide network technologies, make early warning and automation possible. Only in situ, real-time measuring methods can fulfill the requirements of environmental regulations worldwide to ensure good quality air and water, healthy agricultural products and a sustainable ecosystem.

In situ sensors that provide real-time output data are a rapidly developing field with hundreds of innovations and applications. The number and quality of the commercial products has also shown a significant increase in recent years.

This chapter categorizes in situ and real-time water monitoring measurement techniques as geophysical, geochemical, chemical analytical, biological, ecological and ecotoxicological methods. The fields of application cover surface waters and oceans, drinking water and wastewaters. Rapid methods, field equipment and portable devices are discussed starting from the simplest test papers and visual observations to the most advanced sensor techniques.

The soil and solid waste measurement and test methods are discussed in Chapter 4.

An automated continuous monitoring device for measuring toxic element concentrations in surface-, ground- and wastewaters is also introduced and its use demonstrated.

1 INTRODUCTION

Continuous real-time observations and reporting on the status of the environment is the only way to understand and manage regional and global environmental problems. Real-time information on the environment is essential in problem- and site-specific investigations for dynamic decision-making as well as in technology monitoring and process control.

Real-time information may originate from in situ detection – when the measurements are realized in the field –, or from remote sensing. On-site or near-site
measurements can provide almost real-time data with little delay. *In situ* measurements may be continuous or intermittent, depending on the time requirement of the measurement method and the type of the device used.

### 1.1 Regional and global monitoring

*Real-time serial data* show the changes and the trends which are especially useful in environmental monitoring both for endangered and contaminated land as well as for process control of environmental technologies. Information based on real-time measurements provides the best early warning solution and certainly constitutes the only viable approach to more efficient environmental management in the future. Atmosphere and land surface have been observed remotely for many decades, but waters need an urgent change from a data acquisition point of view. Conventional, laboratory-based water analytical techniques currently used hamper fast and effective interventions needed to supply sufficient quantities of high-quality water. There is a huge global interest in efficient water management which is also reflected by WHO regulations and recommendations (GLAAS, 2014), the European WFD (2000) or the CWA (2002) in the US. In addition, the time requirement and cost of the conventional high-sensitivity analytical methods represent obstacles: ever faster and more sensitive and precise methods are required and developed to detect the hundreds of contaminants in environmental samples, and these analytical methods are becoming more and more expensive. It is clear that the strategy of measuring extreme numbers of water samples with extremely sensitive (while still not measuring all of the risky contaminants) analysis methods cannot be followed. New thinking is necessary to optimize the use of measuring capacities: stepwise assessment, cheap, well-designed, selective or generic early warning systems and screening tools, exclusion of the negative samples as soon as possible, and ensuring a good choice of inexpensive, readily available, verified, rapid, and *in situ* analytical and test methods.

Figure 3.1 illustrates the main regional and global observation and monitoring concepts, highlighting the differences in the length of management paths between online/remote monitoring and laboratory-based methods. Other advantages of online/remote monitoring compared to the conventional procedure are summarized below:

- On-line remote monitoring: continuous sampling results in an unlimited sample size and immediate results, without delay (green line in Figure 3.1). The evaluated results can be used both for short-term forecasting and for long-term management, depending on the coupled models.
- Laboratory-based monitoring: intermittent manual sampling and a limited number of samples, labor-intensive laboratory analysis, delayed decision-making and interventions make the corrective actions inefficient (violet line in Figure 3.1).

### 1.2 Technology monitoring and process control

Technology monitoring and process control in terms of industrial technologies are real-time, online measuring methods applied over a long period of time. Some environmental technologies, e.g. wastewater treatment, have also moved in this
direction, and environmental monitoring stands just before this step. Technology monitoring is *ab ovo* closely linked to process control and regulation, however the basic industrial concepts have been created for closed reactors and physicochemical processes or biotechnologies, while environmental technologies are in use mainly in open reactors and in complex biological-ecological systems. Technology monitoring solutions may be based on online, ‘next-to-line’ or on off-line measurements. Please see Figure 3.2 for a comparison.

Environmental technology monitoring and process control may increase the efficiency of wastewater or groundwater treatment and soil remediation significantly. The delay in workflow may result in deviations from the optimum and thus result in poor performance. If an environmental technology placed into or in close contact with the environment exhibits poor performance, it endangers the environment, surface waters or groundwater directly.

### 1.3 Measurement concepts and definitions

A few measurement concepts are described in the following, bearing in mind that the meanings of the used terms are not exactly and consistently defined and applied in the professional literature.

*In-situ measurements* have specific characteristics such as the fact that the sample remains in its original place and in its natural state. It is not separated from the surrounding medium, thus the interactions with its environment are continuous. It also means that the measurement technology should either be employed in the field by placing it there stably or by using mobile, portable devices.
It may be extremely important in many environmental studies to keep the sample in continuous interaction with its environment, exposed to air, precipitation and the surrounding abiotic and biotic compartments. An uprooted sample is significantly different from the original one, especially in cases of low redox potential and anaerobic samples or solid matrices. The separation of the sample from the dynamic physical, chemical and biological context and from its natural habitat results in a one-off static situation at the moment of sampling and an entirely different ‘new life’ following sample removal. Preservation, packaging of samples and shipping into a laboratory cause further multiple uncertainties which cannot be compensated by accurate and costly analyses.

Invasive and non-invasive in situ measuring methods differ in terms of the type of signal and the scale of interaction during the placement and operation of the sensors/measuring devices. The scale of interaction of the sample with the measuring device may cover a wide range of

- analyzers working in deep boreholes – measuring gas, water or solid samples after an invasive preparatory phase;
- technologies and modern sensors based on in situ chemical reactions or very mild interactions;
- detection of the reflected electromagnetic radiation – a natural interaction without any additional interaction between the object and the sensor.

In situ measurements provide real-time information, making early warning, process control and long-term environmental management possible.
1.3.1 Some definitions

Real-time measurement means data acquisition and processing during a chemical, physical, biological or other process without delay or asynchronism. A real-time environmental signal is well defined spatially and temporally. It can be a single measurement point or several time points creating a series. Long-term data-series are usually what is meant when using the term real-time data. In the context of real-time measurements, all the steps of sensing, detection, data transfer, processing, and evaluation should also be ‘real-time’, with the acceptance of a minimal delay which is necessary for data transmission and processing.

Sensing and detection of environmental events and changes in quantities or qualities is the basis of environmental monitoring. The term sensor is used for a device that measures signals with little interaction and shows the scale of response in the form of an interpretable signal. Another term often used for the activity of placing the sensors or equipment containing sensors is probe. An environmental probe can be defined as the act of exploring or monitoring the environment with a device or instrument, e.g., a sensor or electrode that can be placed into environments to take and convey measurements.

A sensor is a transducer which detects signals from its environment. It detects quantities reflecting interactions, events or changes and provides an electrical or optical signal, e.g., electrical current or voltage. The signal may derive from chemical species present (chemical sensors) or from the activity of a living organism (biosensors). Biosensors can detect the biological response of indigenous organisms (e.g., the chlorophyll fluorescence excitation spectra of algae) or that of a test organism which is built into the ‘whole-cell biosensor’. Another way of sensing biological responses is the detection of chemical species which are the product of biological activities, for example switching on genes to adapt or to become resistant to toxicants. Using this sensor concept the biospecific chemical species (genes, enzymes and immunomolecules) can be measured directly as the product of the indigenous biota, or in a simulation test where the environmental sample and test organisms interact. The latter approach ensures uniform, repeatable and comparable results and avoids a negative result if the response of the natural ecosystem is weak or missing.

Sensors are able to detect physical, chemical and biological signals, as follows:

- Physical signals: light, temperature, magnetic fields, gravity, humidity, moisture, vibration, pressure, electrical fields, sound, motion, position, etc.;
- Chemical signals: nutrients and toxic chemical substances, indicator molecules, biomolecules;
- Biochemical and biological signals: metabolic indicators, signal molecules, e.g., hormones, neurotransmitters, specific indicators such as DNA, RNA and several omics (see details in Section 2).

Local and remote sensing is possible by placing the sensor into the location of interest, in other words, into the sample, or by remote application, without direct contact, at various distances (typically on-site, in air or in space), depending on the type and transmission of the emitted signal to be detected. Data may be collected in situ or on site, close to the monitoring point and accessed locally or remotely.
Remote sensing is the acquisition of information about the environmental object or process without making physical contact with it. Sensors can detect natural signals (passive systems) or the responses on artificially emitted signals.

Remote surveillance is the alternative to reading electronic measuring equipment and collecting the data of sensors in person. It requires access to the collected data from the remotely placed base station via telemetry. One single base station can receive a large amount of data from monitoring systems (Figure 3.3).

1.3.2 Data transmission and processing

Detection by sensors, data recording, transmission, data logging and evaluation can be done in situ, on-site or remotely, and all kinds of combinations may be feasible. Remote sensing is essential for non-accessible objects. In this case, primary signals should be able to reach the remotely placed sensor. In other cases, the sensor is placed in situ and the data logger and the following step of data processing is carried out remotely. As an alternative, more sensors and the data logger are assembled in a measuring station and the information is forwarded by telecommunication to a remote place for data processing and evaluation. Special combinations occur in professional practices, e.g., an oceanographic device with sensors and data logger can be operated (i) from a ship and the data read either after taking the sensor out or (ii) continuously through a wire, on the ship, or (iii) autonomously once programmed and placed into the proper place and communicate via satellite. Whichever method is applied, the real-time signal...
characterizes the momentary state of a system or a compartment of the system, and serial signals reflect how a process progresses in time; the only difference is in the mode and place of reading, processing and use of results.

A data logger or data recorder is a computer-based, static or programmable data acquisition system, an electronic device that records data over time or in relation to location. It can be either built into the sensor/instrument or external. Electronic data loggers have replaced the former chart recorders in many applications. They are generally deployed and left unattended to record real-time data from the environment or from technologies such as weather stations, water monitoring systems (water level, depth, flow, pH, conductivity, etc.), soil moisture recorders, flow meters, gas pressure, temperature, light intensity recorders, and several other environmental and process monitoring solutions.

The priority activities requiring real-time methods as concerns water bodies are the oceanographic and other surface water monitoring activities, including runoff and wastewater quality control. As regards soil, the groundwater and the natural- and agro-ecosystems are meant to be protected by the integration of these innovative measurement techniques. In addition to generic environmental monitoring, there is a great need for in situ and real-time methods for endangered and contaminated land. Real-time data may be decisive in risk management as they can characterize true actual risks and the change trends.

The signals of the sensors can be used not just for monitoring, but also for control and regulation. Several computer-based combined data acquisition and control systems are in operation for industrial, agricultural and environmental technologies as well as for early warning. The control of remote equipment is possible via communication channels with coded signals. The main types of monitoring and control systems are:

- ICS: Industrial Control System for technology control;
- SCADA: Supervisory control and data acquisition from large-scale processes that can include multiple sites and large distances;
- DCS: Distributed control system for a process, wherein control elements are distributed throughout the system. A hierarchy of controllers is connected by communication networks for command and monitoring;
- PLC: Programmable (logic) controller is a computer used for automation of processes.

To exploit the advantages of the in situ and real-time information and the connectable dynamic decision-making, the innovative approach is not enough, but innovative tools are also needed. In situ and real-time measuring tools are introduced in this chapter through their application. Their advantages and disadvantages will also be discussed to determine the most efficient application of these methodologies in environmental management.

2 IN SITU AND REAL-TIME MEASUREMENT TECHNIQUES FOR ASSESSMENT AND MONITORING

In situ environmental investigation and monitoring gives real-time and real-space information about the environment but—of course—it is still loaded with uncertainties based on spatial and seasonal heterogeneities, similarly to most of the techniques applied
directly on the environment. The uncertainties and the random errors can be reduced by larger sample sizes and by the elimination of the outliers in a time series. *In situ*, real-time measurements can be used at molecular, microscopic or macroscopic as well as global scales, by means of airborne or satellite coupled sensors in the latter case.

An optical sensor can measure and characterize:

- The DNA-protein interactions by detecting the kinetics of DNA conformational changes;
- The growth of microorganisms or the heart rate of a microscopic insect;
- The movement of an aquatic organism e.g., the opening frequency of a clam;
- The light absorption of local air or the global atmosphere, surface waters and oceans or the terrestrial surface using airborne or spaceborne sensors.

Other types of signals such as radar (synthetic aperture radar = SAR) or hyperspectral signals, can be logged and converted into images and treated in a similar way to optical sensor data. Computer programs evaluate the arriving data, so the logged number of signals can be increased to extreme scale. The evaluation and interpretation of the large amounts of data generally requires modeling and statistical tools.

*In situ* applicable non-invasive site assessment tools vary within a wide range from the visual observations of macromorphological characteristics of the ecosystem to the molecular-level biomarkers, and from sensors (including human eye) used in the close environment of the biomarkers to remote sensing with space satellites. A practical combination of *in situ* sensors with remote data collection and processing makes full automation possible: it may change the control and intervention in environmental management to become more efficient in the future.

The advantages and disadvantages of *in situ*, real-time measurements compared to laboratory-based assessments can be summarized as follows:

**Advantages**

- Data are gathered under ambient conditions;
- The sample is not separated from its environment;
- *In situ* data acquisition is extremely useful for exploratory studies and screening;
- Delineation of contaminated sites is possible;
- Sampling strategy can be modified during field work;
- Research/management strategy can be altered during field work;
- Samples for laboratory analyses can be selected;
- Samples for technological experiments can be selected;
- Real-time data acquisition shows the change trends and allows better estimates;
- Data series from frequent sampling decrease uncertainties and show the trends;
- Long-term data series can serve as basis for statistical evaluation and forecasting;
- The measured values can be supplemented by visual observations, taking photos or videos;
- Sensors and rapid methods may give immediate results on actual risk (bioavailable nutrients and contaminants, toxicity, presence of toxins and pathogens, etc.);
- *In situ* real-time information is directly related to environmental risk; it results in a shortcut in environmental management, and as such avoids (i) the reduction of
the environment to a chemical or biological model; (ii) the re-extrapolation from the results to the real environment – as illustrated by Figure 2.17 in Chapter 2;

- Supports a better understanding of ecosystem complexity;
- Combining in situ measurement with large distance data transmission and remote data access will ensure its widespread use.

Disadvantages:
- The sensitivity of the in situ methods is often lower compared to the sophisticated laboratory analytical methods;
- Not every type of measurement can be implemented in the field;
- Certain assessment tools are not available in an in situ applicable, e.g. portable form;
- Sensors sensitive to contact with solid and biological matter cannot be placed directly into surface waters or soil;
- Part of the in situ-applicable sensors should be in direct contact with the environment, which causes deterioration;
- Maintenance and regeneration of the sensors need to be improved.

High-frequency discrete or continuous signals of real-time measuring devices place data in a time dimension, which widens their applicability. Sensors which detect electric signals directly from the analyte have the best accuracy and precision. However, uncertainty due to environmental variability may override this benefit, and therefore harmonizing and optimizing the sampling plan and sensor accuracy is the best strategy. Real-time detection of light or electrons emitted by chemical or biological reactions, although it is less precise, provides more realistic results e.g. nutrient content in water. The latter can be determined by a color reaction with a reagent or microbiota respiration based on CO₂ production.

Types of in situ and real-time measurement methods show great variability: traditional and innovative ideas are used and combined for acquisition and transformation of data into environmental information. Some of the traditional methods such as geophysical assessments are ab ovo used in situ. Others such as geochemical methods and contaminant analyses, are traditionally carried out in laboratories. These results are loaded with high uncertainty due to sample collection, storage, transport, extraction or other sample preparation methods and with significant delay compared to the date of sampling.

It is important to emphasize that the monitoring itself is a management issue and that individual devices are merely the tools serving the design in line with the scope and concept of the monitoring. After the concept has been laid down, the best fitting tool battery should be assembled and the individual tools selected in harmony with the monitoring requirement and among the tools themselves. The following sections will introduce a number of commercially available measuring equipment and devices, and this overview can help practitioners to make the optimal choice.

2.1 Geochemical and chemical monitoring

Geophysics and hydrogeology traditionally apply in situ methods. The measuring devices applied for positioning and for invasive or non-invasive explorations,
are all portable devices, often supported by airborne or spaceborne technologies, telecommunication and electronic data storing and processing.

Geochemical and chemical analytical methods are used to describe the chemical composition of the earth’s gas, liquid and solid phase compartments and for the identification and measuring of contaminants in the environment.

The theoretical background of the environmental monitoring techniques as well as the methods and devices for general use are discussed in Chapter 4. The \textit{in situ} rapid technologies from the simplest colorimetric test kit applications to \textit{in situ} placed sensor techniques are discussed in this and the next chapter; the methods for water are presented in this Chapter and for soil in Chapter 4.

\subsection{2.2 Rapid test kits for \textit{in situ} water analysis}

A \textit{test kit} is a commercially packaged system of an analytical method’s key components used to determine the presence of a specific analyte in a given matrix. Test kits include instructions for their use and are often self-contained, complete analytical systems in easy-to-carry, lightweight boxes. They may require supporting supplies and equipment, which can be the part of the ‘box’ or these could be available separately. The key components frequently represent proprietary elements or reagents that may be readily prepared by the producer of the kit (AoAC, 1994). Many of these kits fulfill the requirements of relevant standard analytical methods.

Most of the rapid test methods in water analyses are chemical analytical methods, but some tests based on biochemical, enzymatic, immunochemical or DNA techniques are also available in the form of kits and usable in the field with or without mobile laboratories.

\subsection{2.2.1 Rapid chemical analytical methods and test kits}

The \textit{in situ} rapid versions of conventional chemical analyses based on colorimetry apply reagents and indicators for colorimetric evaluation. The simplest and least precise manual analyses apply visual evaluation with test strips or cuvette tests with color cards, cubes or wheels for comparison and reading of the results. More precise titration-based methods need mobile instruments or mobile laboratories. Transportable photometers allow running complex analysis. All the necessary chemicals and tools are assembled into an analytical kit or set available in a handy package. The methods follow international standards and easy-to-understand instructions are added to the kits. The verified products ensure adequate sensitivity and selectivity regarding the analyte, limit or exclude interferences successfully, and can compensate turbidity and color.

Colorimetric analytical kits for waters are provided by AppChem, CHEMetrics, Hach, Lovibond and the Tintometer Group, Macherey-Nagel, Merck Millipore, Systea, Wagtech, Waterworks and many other companies. Table 3.1 summarizes the types, methods, indicators and the detectable concentration ranges of some commercially available colorimetric rapid test kits.

Similar to other titrimetric/colorimetric analytical methods, the corresponding rapid kits are used for targeted assessment, i.e. only known, formerly identified, or otherwise predicted/expected parameters or contaminants.
Table 3.1 Rapid colorimetric test kits for water analysis.

<table>
<thead>
<tr>
<th>Measured chemical parameter</th>
<th>Method/indicator</th>
<th>Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity/base capacity</td>
<td>Litmus (pH 5–8), methyl orange (pH 3.1–4.4)</td>
<td>2–7.0 mmol/L H⁺</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Phenolphthalein (pH 8.3–10.0)</td>
<td>0.2–7.0 mmol/L OH⁻ or 0–240 CaCO₃</td>
</tr>
<tr>
<td>Alkalinity – total (pH 5.1; 4.8; 4.5 or 3.7)</td>
<td>Brom cresol green-methyl red and Brom phenol blue</td>
<td>0.2–7.0 mmol/L OH⁻</td>
</tr>
<tr>
<td>Aluminum</td>
<td>Eriochrome cyanine r</td>
<td>0.002–0.25 Al⁺</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Nessler or indophenol blue reagent</td>
<td>0.02–0.4 NH₄</td>
</tr>
<tr>
<td>Ammonium-N (more ranges)</td>
<td>Salicylate</td>
<td>0.02–50 NH₃-N</td>
</tr>
<tr>
<td>Anionic surfactants</td>
<td>Cristal violet</td>
<td>0.2–2 LAS</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Ag-DDTC = Ag-diethylthiocarbamate</td>
<td>2–400 ppb</td>
</tr>
<tr>
<td>Bromine</td>
<td>DPD = N,N-diethyl-p-phenylenediamine sulfate</td>
<td>0.05–4.5 Br₂</td>
</tr>
<tr>
<td>Calcium</td>
<td>Eriochrome blue, black r</td>
<td>1.5–5.0 Ca</td>
</tr>
<tr>
<td>Carbonate hardness</td>
<td>Eriochrome black t or calmagite</td>
<td>0.07–4.0 Ca-Mg</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>Phenolphthalein</td>
<td>0.1–25 &amp; 5–1000 Cl⁻</td>
</tr>
<tr>
<td>Chloride (more ranges)</td>
<td>Mercury thiocyanate</td>
<td>0.04–5.0 Cl₂</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>DPD indicator</td>
<td>0.02–10 Cl₂</td>
</tr>
<tr>
<td>Chlorine – free and total</td>
<td>DPD indicator</td>
<td>0.02–10 Cl₂</td>
</tr>
<tr>
<td>Chromate</td>
<td>Sodium thiosulfate or DPC reagent</td>
<td>0.1–25 CrO₄²⁻</td>
</tr>
<tr>
<td>Chromium – hexavalent</td>
<td>1,5-diphenylcarbazide (DPC)</td>
<td>0.01–0.7 Cr⁶⁺</td>
</tr>
<tr>
<td>Chromium – total</td>
<td>Ox-alkaline hypobromide</td>
<td>0.01–0.7 Cr</td>
</tr>
<tr>
<td>Cobalt</td>
<td>PAN indicator (=1-(2-pyridylazo)-2-naphthol)</td>
<td>0.01–2.0 Co</td>
</tr>
<tr>
<td>COD high (more ranges)</td>
<td>Reactor digestion, ferroin</td>
<td>100–1500–15,000 COD</td>
</tr>
<tr>
<td>COD low (more ranges)</td>
<td>Reactor digestion, ferroin</td>
<td>2.0–40 &amp; 10–150 COD</td>
</tr>
<tr>
<td>Copper</td>
<td>Bicinchoninate</td>
<td>0.04–5.0 Cu</td>
</tr>
<tr>
<td>Copper</td>
<td>Porphyrin</td>
<td>0.002–0.2 Cu</td>
</tr>
<tr>
<td>Cyanide</td>
<td>Pyridine pyrazalone, or p-dimethylamino-benzalrhodanine</td>
<td>0.001–0.25 CN⁻</td>
</tr>
<tr>
<td>Fluoride (SPADNS method)</td>
<td>SPADNS reagent (=4.5 dihydroxyl-3-(p-sulfophenylazo)-2,7-naphthalene-disulfonic acid-Na salt)</td>
<td>0.02–2.0 F⁻</td>
</tr>
<tr>
<td>Hardness</td>
<td>Calmagite colorimetric</td>
<td>0.07–4.0 Ca–Mg</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>p-dimethylaminobenzaldehyde</td>
<td>0.004–0.6 ppb</td>
</tr>
<tr>
<td>Iodine</td>
<td>DPD indicator t</td>
<td>0.07–7.0 I⁻</td>
</tr>
<tr>
<td>Iron</td>
<td>Ferrozine</td>
<td>0.009–1.4 Fe</td>
</tr>
<tr>
<td>Iron ferrous</td>
<td>Phenanthroline 20</td>
<td>0.02–3.0 Fe</td>
</tr>
<tr>
<td>Iron total</td>
<td>Phenanthroline 10</td>
<td>0.02–3.0 Fe</td>
</tr>
<tr>
<td>Manganese</td>
<td>Periodate</td>
<td>0.2–25 Mn</td>
</tr>
<tr>
<td>Manganese</td>
<td>PAN indicator</td>
<td>0.007–0.7 Mn</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Mercaptoacetic acid</td>
<td>0.3–40 Mo</td>
</tr>
<tr>
<td>Nitrogen (Ammonium-N, more ranges)</td>
<td>Salicylate</td>
<td>0.02–50 NH₃-N</td>
</tr>
<tr>
<td>Nickel</td>
<td>PAN indicator</td>
<td>0.007–1.0 Ni</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Diazotization</td>
<td>0.003–0.5 N-NO₂</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Ferrous sulfate</td>
<td>2–250 N-NO₂</td>
</tr>
<tr>
<td>Nitrate (chromotropic)</td>
<td>Chromotropic acid</td>
<td>0.2–50 N-NO₃</td>
</tr>
<tr>
<td>Oxygen (dissolved) (Winkler method)</td>
<td>Manganese sulfate and alkaline iodide-azide reagents and thiosulfate titration</td>
<td>1–12 O₂</td>
</tr>
</tbody>
</table>
Table 3.2  Rapid colorimetric test kits for water analysis.

<table>
<thead>
<tr>
<th>Measured chemical parameter</th>
<th>Method/indicator</th>
<th>Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (several ranges)</td>
<td>Test strips for different pH ranges</td>
<td>0–14 pH</td>
</tr>
<tr>
<td>Phenols (several ranges)</td>
<td>Sodium peroxidisulfate</td>
<td>0.5–50 phenols</td>
</tr>
<tr>
<td>Phenols</td>
<td>4-aminoantipyrine</td>
<td>0.1–3.0 phenols</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>Molybdovanate</td>
<td>1–1000 P-PO₄³⁻</td>
</tr>
<tr>
<td>Total phosphate</td>
<td>Acid persulfate</td>
<td>0–1.5 P-PO₄³⁻</td>
</tr>
<tr>
<td>Silica</td>
<td>Silicomolybdate</td>
<td>1–100 SiO₂⁴⁻</td>
</tr>
<tr>
<td>Silica ULR rapid</td>
<td>Heteropoly blue rapid liquid</td>
<td>0.003–1.0 SiO₂⁴⁻</td>
</tr>
<tr>
<td>Sulfate</td>
<td>Sulfate</td>
<td>100–1000 SO₄²⁻</td>
</tr>
<tr>
<td>Sulfate</td>
<td>Sulfate</td>
<td>2–70 SO₄²⁻</td>
</tr>
<tr>
<td>Sulfide</td>
<td>Methylene blue</td>
<td>0.005–1.0 S²⁻</td>
</tr>
<tr>
<td>Sulfite</td>
<td>Starch</td>
<td>0.1–0.8; 2–100 SO₄²⁻</td>
</tr>
<tr>
<td>Total nitrogen (more ranges)</td>
<td>Chromotropic acid</td>
<td>3–150 N</td>
</tr>
<tr>
<td>Total phosphorous</td>
<td>Molybdovanadate</td>
<td>0–3.5 P</td>
</tr>
<tr>
<td>Total phosphorous</td>
<td>Acid persulfate</td>
<td>1–100 P</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zincon</td>
<td>0.01–3.0 Zn</td>
</tr>
</tbody>
</table>

Figure 3.4  Test strips for sensitive pH measurement (Indigo, 2015), color chart for hydrogen sulfide analyses with reagent paper (Hach, 2015), comparator color discs (Comparator disc, 2015) and cube for iron (Comparator cube, 2015).

For arsenic, which is one of the main problems of drinking water supply in many areas of the world, several simple field technologies are available which do not need analytical instruments and results are obtained in 12–30 minutes. Quick test kits are available for average, low and ultra-low arsenic ranges from several companies (Hach, 2015, LaMotte, 2015, Merck, 2015, MN, 2105, ITS, 2015). The color reaction can be evaluated visually using comparative color charts, color discs or color cubes (see Figure 3.4 for some examples). More recent field kits include a digital display of arsenic levels to rule out subjective judgement by the professional who visually detects the difference between the color shades of the strip. One example for digital reading is the Arsenator from Wagtech (2015).

Comparative studies support the usability of test kits for field assessments of arsenic. The results suggest that the portable kits can be used to identify water sources with high arsenic concentrations and may provide an important tool for arsenic surveillance and remediation programs (Spear et al., 2006).

Test kits are typically organized into sets by the manufacturers, for example the ten-parameter test kit developed for aquacultures (Hach Aquaculture, 2015) to measure
the ten most important parameters for keeping aquacultures well-balanced: acidity, alkalinity, ammonia, carbon dioxide, chloride, dissolved oxygen hardness, nitrite, pH and temperature. Sets for drinking water chemical and microbiological quality are offered by Wagtech (2015) for field analysis. The microbiological set includes an incubator; the chemical set includes the photometer, a compact turbimeter and pocket sensors for pH and conductivity. The mentioned sets are shown in Figure 3.5.

2.2.2 Enzymatic test kits

Enzyme-analytical methods and test kits are customarily used in the food industry for food quality control and are becoming more and more widespread for environmental analytical purposes, too. Enzyme-analytical methods can be characterized by

- selectivity: enzymes find their target analytes even in complex mixtures;
- sensitivity: low detection limits;
- specificity: enzymes react with high certainty with their substrates, i.e. the target analytes (in its equilibrium state an enzyme binds to its substrate with an affinity of $10^5$–$10^8$ M, meaning that the associated complex is $10^5$–$10^8$ times that of the dissociated enzyme and substrate);
- safety: working under biological conditions, using non-toxic reagents.

Some examples of commercially available enzymatic test kits are introduced below:

- Organophosphate/Carbamate screen kit from Abraxis (Abraxis, Pesticides, 2015) is an in vitro enzymatic test used to detect organophosphate and carbamate (OP/C) type insecticides in water and other environmental matrices. It is a qualitative, colorimetric assay based on the inhibitory effect of OP/C on the acetylcholinesterase enzyme. In the case of enzyme inhibition, acetylcholine will not be hydrolyzed, it does not react with $5,5'$-dithio-bis(2-nitrobenzoic acid) (DTNB), and fails to produce a yellow color, which is the expected color when no inhibition occurs. The test kit is designed for field use.
– The organophosphate test kit, of OP-Stick Sensor is a largely simplified Japanese development for OP/C pesticides. On the stick, two spots can be seen after use, which indicate the presence of the insecticide (yellowish) by contrast with the reference (brown). If no insecticide is present, both spots are brown (OP-Stick, 2015).

– Rapid enzymatic methods with fluorescence detection can quantify fungi – Mycometer®-test and bacteria – Bactiquant®-test – present in water and air. The technology is based on fluorogenic detection of fungal/bacterial enzyme activities. The sample is contacted with a test solution containing a synthetic substrate, which can be hydrolyzed by the fungal/bacterial enzymes. The hydrolysis product fluoresces upon excitation with ultraviolet light. Fluorescence is measured by a handheld fluorometer after processing for a reaction time at the ambient temperature. Sample preparation and analysis is performed on site within one hour (Mycometer, 2015).

– Nitrate determination in wastewater is a newly developed enzymatic method approved by the US EPA (Campbell & Davidson, 2014). Nitrate reductase replaces the cadmium of the traditional nitrate determination method here. In this way cadmium can be eliminated and the substitute is a safe, biodegradable protein. NECi provides easy-to-use test kits for determining nitrate content in any water, soil, plant tissue or livestock feed sample (NECi, 2015).

2.2.3 Immunoanalytical test kits

Immunoanalytical test kits form a special group of rapid methods applicable in situ for toxic, mutagenic and reprotoxic contaminants in waters and soils. Immunoassays are based on the very selective and strong binding of an antibody to antigen, i.e. the analyte in this case. The affinity can be characterized by an equilibrium constant of $10^9 – 10^{12}$ M, meaning the rate of the associated and dissociated molecules under equilibrium conditions. Several technical solutions have been developed for qualitative and quantitative analyses, for rapid in situ/on site analyses such as test strips, tube kits or kits using microplates and readers.

Enzyme-linked immunosorbert assay – ELISA is the immunoanalytical technique on which most of the rapid immunological test kits are based. Its essence is that the analyte from the sample (this is the antigen in the immune reaction) is attached to a solid surface. The antigen-specific antibody is linked to an enzyme. The enzyme-antibody complex is contacted and reacted with the surface bound antigen, and the unbound surplus is washed out. The added substrate of the enzyme produces a measurable color change in proportion to the amount of the bound enzyme.

Magnetic Particle Enzyme Immunoassay (MPEIA) is a relatively new immunoassay method for isolating and measuring antigen-antibody complexes. In the simple magnetic immunoassays (MIA), the antigen is the analyte, and the antibody is labeled by magnetic beads. The complex is formed on the solid-phase surface of the magnetic microparticles. The magnetic bead-linked immunocomplex is then detected by a magnetic reader, measuring the magnetic field change induced by the beads.

In MPEIA, a competitive immunoassay is applied: an enzyme linked analyte-antibody complex is added to the above described reaction mixture. The competition
between the analyte in the sample and the enzyme labeled and affixed to the antibody binding sites on the magnetic particles results in an exchange of the labeled and unla-
beled analytes. A relatively long reaction time (typically 1–2 hours) is needed to reach equilibri-
um. At the end of the incubation period, a magnetic field is applied to immo-
bilize the magnetic particles in the test tube. To do so, a magnetic separation rack
is used, which allows the separation and immobilization of magnetic particles to the
side or the bottom of the test tubes. Racks for special tubes or for normal microtiter
plates are available. The unbound reagents can be washed out and the substrate of the
enzyme and the chromogen added. The measured color is inversely proportional to
the concentration of the analyte in this competitive assay.

Such technology is provided by the test kits of Abraxis (2015), Biosense (2015)
for several pesticides, industrial chemicals and estrogens, or the RaPID Assay® (2015)
by Modern Water for polycyclic aromatic hydrocarbons (PAHs). The latter is a rapid
field testing kit for water and soil, suitable for testing 50 samples at a time within 60
minutes (see also MPEIA Video, 2015).

**Immunoassay test kits** are provided among others by Hach for the semi-
quantitative determination of total petroleum hydrocarbons (TPHs) and polychlo-
rinated biphenyls (PCBs) in soil (see more in Chapter 4) or alachlor and atrazine
in water (Hach Immuno, 2015). The rapid test kits include a waterproof pocket
colorimeter. We introduce the commercially available products developed for water
contaminants:

- **Atrazine immunoassay** reagent set for the determination of atrazine in water.
The US EPA Environmental Technology Verification (ETV) Program’s Advanced
Monitoring Systems (AMS) Center has tested (EPA ETV Atrazine, 2007) the quan-
titative and the qualitative immunoassays: an ELISA kit (Abraxis Atrazine, 2015),
a tube kit (Beacon Atrazine, 2015), as well as the qualitative Watersafe® Pesticide
kit (Silver Lake, 2015).

- **Several other pesticides** can be detected by the immunoassay kits (Modern Water,
test kits, 2015) listed below:
  - RaPID Assay® tube kits for 2,4-D (2,4-dichlorophenoxyacetic acid), atrazine,
    triclopyr;
  - EnviroGard® tube kits for triazine;
  - EnviroGard® well kits for isoproturon and triazine;
  - QuickCheck® for chlordane, DDT, isoproturon and triazine (Modern Water,
    pesticides, 2015).

- Abraxis (Abraxis Pesticides, 2015) provides ELISA kits for the pesticides of 2,
  4-D, acetochlor, alachlor, atrazine, azoxystrobin, triazine, carbendazim/benomyl,
  DDE/DDT, diuron, fluridone, glyphosate, imidacloprid/clothianidin, metolachlor,
  organophosphate/carbamate (OP/C), penoxsulam, pyraclostrobin, pyrethroids,
  spinosyn and trifluralin.

- **Microcystins**, the toxic compounds produced by the cyanobacteria species within
  their cell wall, can also be detected by immunoassay test kits. When the cell
dies and disintegrates, microcystins are released into the water, where they
have the potential to cause skin rashes, eye irritations, respiratory symptoms,
and liver damage for humans, and toxic effects for cohabiting ecosystem mem-
bers. In 2010 and 2011, six microcystin test kits produced by Abraxis (Abraxis
Microcystin, 2015), Beacon (Beacon Microcystin, 2015) and Zeu-Immunotech (Zeu Microcystin, 2015) were evaluated by the US EPA ETV Program (EPA ETV Microcystins, 2012) in recreational waters. Modern Water, too, produces microcystin immunoassay test kits (EnviroGard® microcytins, 2015).

Several biotoxin-specific immunoassay test kits are available for algal toxins of saxitoxin, domoic acid or octanoic acid accumulated by shellfish. These test kits can also be used for the well-known pathogenic bacterial toxins of anthrax, botulinum, ricin, plague, brucella serving biosafety purposes. The immunoassay kits exist in the form of immunoassay test strips (ADVNT Biotechnologies, 2015, Tetracore, 2015, Zeulab, 2015, Abraxis, 2015), test kits with automated analyser (BioVeris, 2015) or immunoassay test cartridge (Response Biomedical, 2015). The biosensor developments are focused on hand-held tools for rapid and accurate detection of bacterial targets (such as Bacillus anthracis) and protein toxins (such as botulinum toxin).

Endocrine disrupting chemical compounds (EDC) are typical water contaminants, which are difficult to analyse and pose an increasing risk for humans and ecosystems. Estrogen ELISA kits have been developed for the detection of estrogenic chemical compounds in water, among others by the Japanese Tokiwa Company (Ecologiena, 2015) and distributed by Abraxis (2015) in the US and Biosense (2015) in Europe for:

- total estrogen;
- 17β-estradiol;
- estrone;
- ethinyl estradiol.

Industrial chemicals such as surfactants, bisphenol A, triclosan or PCBs can be detected by using rapid immunoassay kits in waters. Several distributors provide ELISA kits such as Abraxis, Biosense and Modern Water. Ecologiena (2015) for example produces kits for:

- anionic surfactants: linear alkylbenzene sulfonate (LAS) by ELISA kit;
- nonionic surfactants: alkylphenol ethoxylate (APE) ELISA kit;
- alkyl ethoxylate (AE) ELISA kit;
- alkylphenol (AP) ELISA kit;
- bisphenol A (BPA): super-sensitive ELISA kit.

Other available ELISA test kits for industrial chemicals:

- benzo(a)pyrene (B(a)P);
- coplanar polychlorinated biphenyls (PCBs);
- PCBs – high chlorination;
- PCBs – lower chlorination,
- polybrominated diphenyl ether (PBDE);
- triclosan.

Stress biomarkers play an important role in the detection of contaminants and early warning. Biosense (2015) has developed a range of new monoclonal and polyclonal antibodies against biomarkers for semi-quantitative rapid detection such as:

- Vitellogenin (Vtg) and vitellogenin standards from different fish species. Vtg is an egg yolk precursor protein in fish and other egg-laying species. In the presence of estrogenic endocrine disrupting chemicals (EDCs), male fish
express the vitellogenin gene in a dose manner, so it is a molecular marker of exposure to estrogenic EDCs.
- Fish zona radiata proteins (eggshell proteins, Zrp) are more responsive than vitellogenin for estrogenic effects, so Zrp may function as a more sensitive biomarker compared to vitellogenin.
- Cytochrome P450 1A1 protein is encoded by the CYP1A1 mammal/human gene of the enzyme aryl hydrocarbon hydroxylase (AHH). It is involved in phase I xenobiotic metabolism and is induced by PAHs. The increased amount of gene product indicates the presence of the inductor, the PAH.
- Spiggin, the glue protein of the three-spined stickleback fish species (Gasterosteus aculeatus) is produced by the male fish to construct a nest for the eggs. Spiggin production is regulated by androgens. Exposing female fish to androgenic contaminants, the female’s kidney also produces spiggin at a contaminant-proportional rate.

- Metallothioneins (MT) are specific proteins with the capacity to bind both physiological (zinc, copper, selenium) and xenobiotic (cadmium, mercury, silver, arsenic) metals. MT is a biological indicator for metal stress; its amount is proportional to bioavailable metal exposure.
- Other stress proteins such as the so-called heat shock proteins (HSPs) can also be used as indicators for environmental exposures. This family of proteins is produced by cells in response to exposure to stressful conditions. They were first described in relation to heat shock, but currently more proteins are listed which are expressed during other stresses including exposure to cold, UV light, toxic metals, metabolic inhibitors, amino acid analogs, chemotherapeutics, during diseases, or wound healing. The small-size protein ubiquitin which marks proteins for degradation is also classified as a HSP. Detection of the increased production of these HSPs by ELISA or other immunotechniques indicates stress.
- Gonadotropin-releasing hormone (GnRH) production and the consequent feminizing effects on male development may be the response to exposure to xenoestrogens such as atrazine, BPA, DDT, dioxin, endosulfan, PBB, PCBs, phthalates, or zeranol. The immunoanalytical detection of GnRH is a possible indication for the presence of xenoestrogens.

Mercury(II) immunoanalytical test strip can measure Hg(II) between 1–10 mg/L linearly, and its detection limit is 0.23 mg/L. Other metals had a negligible effect on the detection of Hg(II) (Xing et al., 2014). Test kits are mainly used in the initial environmental characterization phase of targeted assessments, but their application in later stages may also be justified, for example for monitoring groundwater or wastewater treatment conditions or for checking whether the treatment is effective. Test kits are not suitable for continuous monitoring; in situ or on-line applied sensor-based analytical techniques are recommended instead.

It is important to emphasize that such kits do not substitute the detailed chemical analysis in the positive cases but are able to select negative cases and save a lot of money spent on costly chemical analyses of negative samples. Another advantage is that the test kits provide quick and cost-effective results for the contaminant (e.g. atrazine) levels in positive cases, making the conventional laboratory analysis by gas chromatography/mass spectrometry (GC/MS) easier when the expected concentration range is known.
2.3 Biosensors

The term biosensor covers a wide range of equipment which applies biologically active molecules (nucleotides, enzymes, immunomolecules) or living organisms to detect a biologically relevant response to pollutants via electrochemical or optical signals.

One type of biosensor representing the molecular level responses of living systems is the highly selective sensor applied for targeted analyses, namely to find the target molecule in a complex mixture and/or matrix. These sensors follow the concept of traditional chemical analysis: highly selective and sensitive detection of target analytes in the sample. The interaction between the analyte and the sensor-fixed molecules is a pure chemical reaction. The traditional chemical analysis generally achieves selectivity not by selective detection, but rather by a selective enrichment of the target analyte during sample preparation. In contrast, biosensors ensure very selective biochemical binding without enrichment, just with the help of the built-in bioactive molecules which mimic biological responses. The built-in molecules may be nucleotides, enzyme-proteins, immunomolecules or engineered molecules with similar roles.

Other types of biosensors representing organism-level responses have broad-spectrum sensitivity, aggregating all exposures present in the sample while taking the biological availability of the analytes into consideration. These types of sensors work with built-in cells or organisms, or such components of the living systems which give the same response for several impacts (such as the luminol or chlorophyll a) so that not only the exposures are aggregated but also the compensatory response of the test organism. This means that the selection of the proper organism assumes the conscious integration of the rules of chemical analysis and ecotoxicology to serve the monitoring concept. Most of these sensors are used for toxicity testing or pathogen detection in waters.

3 IN SITU ECOTOXICOLOGY

In situ ecotoxicological methods can measure real-time adverse effects on living organisms in the real environment. They may give highly realistic results, although still loaded with spatial and seasonal heterogeneities and uncertainties. Whole-cell biosensors (see in Section 2.7), are the most advanced in situ ecotoxicity measuring devices, whose response is representative of the environment under assessment. Unfortunately, not all organisms can be integrated into sensors.

The toxicity of waters measured by the representative aquatic species is essential information for the regulation of discharges into surface waters from different facilities, e.g. industrial, mining, agricultural or urban areas as well as runoffs and storm sewer systems. Direct toxicity testing is necessary in every case when unknown substances or a mixture of substances are expected in the water, or in the case of a completely unknown situation when non-targeted toxicity screening is necessary. In the US EPA regulation, the National Pollutant Discharge Elimination System (NPDES) includes whole effluent toxicity (WET) testing as a monitoring requirement in the permits the facilities must obtain if they have direct discharge to surface waters (SETAC, 2004).

The test organisms can be the native inhabitants of the aquatic ecosystem or sound/controlled representative ecosystem members.
Direct toxicity testing or whole effluent toxicity testing is based on the actual toxicity of all toxic components in contrast with the chemical model-based approach, which measures the concentrations of some supposed contaminants and from these chemical concentrations tries to extrapolate the actual adverse effects and ecotoxicological risks. A whole effluent diluted with the water of the receiving body of water can simulate the real situation and may support the decision on whether or not to allow the discharge into the stream or lake. In the case of significant toxicity, chemical analysis is used for the identification of the responsible contaminants. Freshwater or marine fish, invertebrates, algae and/or macroplants can be used for studying water toxicity.

3.1 Mobile laboratories, rapid toxicity testing, and toxicity test kits

The mobilisation of laboratory bioassays is an innovation in environmental toxicology and monitoring. These bioassays can be transformed into rapid on-site test methods, meaning that a short contact of the test organisms with the material is sufficient. If the specific effects measured manifest themselves during the test organisms’ growth or propagation, the resulting lengthy time does not allow for the bioassay to be adapted to rapid on-site use. However, if the test is performed in the organisms’ non-reproductive phase, the bioassay has a good chance for rapid on-site use. It is worthwhile to work with preserved test organisms which are revitalized in the field as part of the test method. The use of color indicators or other easy-to-detect reagents to the test medium makes the test evaluation easy. The application of portable detectors is also a good option for in situ real-time measurements, e.g. colorimeter, densitometer, luminometer (see Figure 3.6). The most advanced solution is the construction of sensors which detect primary signals from selective reactions in a miniaturized system.

3.1.1 General and toxicant-specific testing

Toxicity screening of environmental samples, unlike the detection of particular targeted contaminants, aims to assess the sum of adverse effects. For this purpose, it uses sensitive and non-selective test organisms and test end points. Bioluminescent bacteria and their capability to emit light is such a sensitive and generic end point. When the living cell is exposed to toxic substances, the amount of light emitted decreases...
proportionally with the toxicity. Microtox® technology is based on this bacterial bioluminescent light emission. It serves as a basis for most of the commercialized whole-cell toxicity tests. Measuring the difference in light emission between bacterial cultures unexposed and exposed to toxic substances will therefore indicate the presence of toxicants in the water sample. The gene responsible for light emission can be a ‘naturally’ or an artificially (by genetic engineering) built-in element of the living test bacterium.

New developments are steering the engineering of semi-specific biosensors which contain fusions of stress-regulated promoters and reporter genes. These may have advantages over the generic biosensors due to higher sensitivity and specificity. Whole-organism-containing biosensors can be created by using the organism’s selective molecular response for a specific contaminant. Such molecular level responses may come from the genes of adaptive enzymes, stress proteins, immunomolecules or metabolites responsible for resistance, tolerance or biotransformation (read more in Charrier et al., 2010). The disadvantage of the limited duration of these kinds of sensors and their time-consuming development often makes their application unfeasible.

Bioluminescence was probed by a single-photon avalanche diode detector by Elad et al. (2011) for an agar gel immobilized recombinant luminobacterium, which is sensitive to water pollutants. A flow-through biosensor was constructed in this way for online continuous water toxicity monitoring.

Jouanneau et al. (2012) constructed bioluminescent bacterial biosensors for the online detection of metals in environmental water samples. They applied freeze-dried bacteria on a disposable card, which allowed stable detection for 10 days with 3% reproducibility of the bioluminescence signal both in laboratory conditions and in the environment. The application of an analytical software made multidetection of Cd, As, Hg, and Cu possible.

A contaminant-selective, in situ or remotely readable Hg biosensor was developed with a whole-cell system by Goddard et al. (2009) who have successfully built a biosensor containing intact cells to detect both inorganic Hg(II) and methyl-Hg(II). A hypersensitive Gram-negative mutant was created by directed evolution of MerR (mercury resistance operon repressor) with subsequent high throughput microplate screening to increase detection sensitivity. The MerR family is a group of transcriptional activators, activating the transcription through protein-dependent DNA distortion. The regulators of Gram-negative mercury resistance (mer) operons were found on transposable genetic elements (Lund et al., 1986). The Hg(II) biosensor with mutant MerR can detect Hg(II) at concentrations of 0.1 nM (20 ppt ≈ 0.02 µg/L). The developed Hg(II) biosensor has high specificity, gives a signal only to Hg(II) ions and no signal with other metals, and is stable for up to 7 days. The prototype has been completed in the form of a handheld portable detector.

3.1.2 Test kits for general and targeted toxicity

Test kits for on-site aquatic ecotoxicity testing are available with the Aliivibrio fischeri luminobacterium or crustaceans. Rapid on-site screening of water for the presence of specific bacteria or bacterial contamination can also be performed using field kits based on chemical ATP measurements and luminescence detection with a portable luminometer.
3.1.2.1 Chemiluminescence in a cell-free system

Chemiluminescence is applied as a test end point based on the inhibitory effect of pollutants on the oxidation reaction of luminol. Toxicants, being free radical scavengers, prevent the reaction leading to chemiluminescence and proportionally reduce the amount of light of the sample compared to the reference (pure water). The percentage inhibition of the light emission is the calculated end point of the test. Some of the products available on the market for rapid in situ toxicity assessment based on chemiluminescence are introduced shortly below.

- **Eclox™ Rapid Response test kit**: a qualitative chemiluminescence technology with a luminometer to test toxicity of trace contaminants in water, in the field. Eclox is the abbreviation of Enhanced ChemiLuminescence and OXyradical test. The free radical oxidation of luminol is enhanced by the presence of the oxygen source of horseradish peroxidase (HRP), and the enhancer of 4-iodophenol. Luminescence will be reduced in the presence of toxic substances inhibiting the oxygen-producing HRP enzyme reaction. The results of inhibition rates correlate with other established toxicity tests for several types of contaminant such as metals, antioxidant toxicants, phenols, cyanides, permanganates, pesticides, etc. The chemiluminescence-based rapid toxicity test is unable to identify specific contaminants or their concentrations; it functions instead as a screening tool to quickly determine whether water is potentially toxic (Eclox™, 2015).

- **Chlorophyll fluorescent signals** from photosynthetic enzyme complexes have become one of the most powerful indicators for ecophysologists in the last few decades. When samples are illuminated by UV light, the intensity of the resultant fluorescence is proportional to the chlorophyll concentration. The major part of the absorbed light energy is used to drive photochemical reactions during photosynthesis. A certain part of the absorbed light is emitted in the form of fluorescence. The presence of electron transport inhibitor toxicants modifies the ratio of absorbed and emitted light energy and the parameters of fluorescence (Boucher *et al.*, 2005). Commercially available apparatuses based on chlorophyll fluorescence are LuminoTox and Robot LuminoTox, produced by the Canadian laboratory Bell Incorporated.

- **LuminoTox** and **Robot LuminoTox** are rapid toxicity detection systems which work as easily as a chemical test: the toxicity result can be seen within less than 15 minutes. Both the handheld model for field analysis and the automated model for online monitoring are available and they measure photosynthetic efficiency by the fluorescence of chlorophyll a, an indicator of electron transport efficiency (LuminoTox, 2015; LBi, 2015).

3.1.2.2 Whole-cell toxicity measuring devices

The in situ applicable rapid versions of whole-cell toxicity methods are designed as portable monitors or are integrated into mobile labs. The whole cells can be single microorganisms (bacteria, algae), eggs of aquatic invertebrates or the mixed microbiota of activated sludge. Most of the methods are based on the luminescent marine bacterium, the *Aliivibrio fischeri* (formerly *Vibrio fischeri*) and the Microtox® technology applying it. These devices try to combine the advantages of whole organism
toxicity testing and instrumental precision. A few commercial products are listed below:

- **DeltaTox® II** portable toxicity monitor uses Microtox® technology for measuring bioluminescence. It is a simple, rapid, portable water quality test system combining Microtox for acute toxicity and another method for measuring the microbial pollution in the water. Applications include drinking water emergencies and detection of chemical spills entering water systems. Results are available within 5 minutes (DeltaTox II, 2015).

- **Microtox® CTM** is a site-based, broad range, continuous toxicity monitor (CTM). It continuously measures the chemical toxicity of a water source, giving an instant indication of water health. It is a fully automatic instrument that offers a 4-week, autonomous operating cycle and requires a low level of skill for both operation and maintenance (Microtox® CTM, 2015).

- **AppliTOX®** includes a fully automated batch bio-assay using freshly prepared luminescent bacteria based on the standardized laboratory luminescence inhibition test (ISO 11348 – Part 1, 2007) with *Aliivibrio fischeri*. The AppliTOX analyzer system is suitable for ensuring the security of drinking water (intake water, distribution systems), monitoring river water quality (monitoring stations), controlling water recycling of industrial technologies, and monitoring the effluent in wastewater treatment plants (WWTPs) (AppliTOX, 2015).

- **ToxBox** is a toxicity testing box for monitoring toxicity based on bacterial luminescence autonomously, allowing continuous monitoring of rivers, drinking water production or wastewater treatment. With the application of special bacterial strains mutagenicity, biocorrosion and metabolism inhibition can also be monitored. ToxBox is fully autonomous and does not require manual preincubation of the monitor microorganism. Depending on the analysis frequency, ToxBox will operate autonomously for up to four months (ToxBox, 2015).

- **Toxi-chromotest** is a bacterial assay based on the inhibition of the *de novo* synthesis of the inducible enzyme of beta-galactosidase in the *Escherichia coli* K12 OR85 strain. The test applies freeze-dried bacteria, and a rehydration cocktail containing the enzyme beta-galactosidase as inducer. The toxicant-containing sample is added to the revitalized bacterial culture. The toxicants penetrate the cell wall of the bacterium and inhibit the *de novo* synthesis of the beta-galactosidase. The produced amount of the induced enzyme is detected by its reaction with a chromogenic substrate. The greater the toxicity, the lesser the color intensity (Toxi-Chromotest, 2015).

- **Rapidtoxkit** is a rapid, 1-hour toxicity test with larvae of the anostracan crustacean *Thamnocephalus platyurus* for rapid detection of water contamination. The test organism is included in the kit as dormant eggs (cysts) which can easily be hatched on demand to supply the live biota for the assays. This very sensitive sublethal assay is based on the decrease or the absence of ingestion of red indicator microspheres by the test organisms exposed to contaminated waters (Rapidtoxkit, 2015). The colored particulate matter is added to the test after 15 minutes incubation of the test organism in the sample. The control is clean freshwater, wherein the healthy animals take up more microspheres than the stressed ones in the sample. The colored particles can be observed in the digestive tract under a low magnification microscope (e.g. a stereomicroscope).
- **ToxAlarm** toximeter is designed for continuous monitoring of toxicity in drinking or surface waters. It is based on assessing the inhibition of nitrification of activated sludge microorganisms. The conversion of ammonia to nitrate needs oxygen. When the process is inhibited by toxic substances, oxygen consumption decreases. ToxAlarm monitors this oxygen consumption and hence the toxicity. The highly sensitive self-reproducing nitrifying bacterial culture is constantly and independently producing biomass, so enough fresh bacteria are always available for the new measurement. It is characterized by low operational costs since no purchase or external cultivation of bacteria is necessary. The response time is 5–10 minutes (ToxAlarm, 2015).

- **NitriTox** is an online toximeter developed for wastewater treatment plants, especially for the protection of the biology of the nitrification process. Its operation is similar to the previous ToxAlarm equipment, but it is suitable for the continuous toxicity monitoring of wastewater treatment plants. The measurements follow at intervals of less than 5 minutes, thus allowing enough time to introduce countermeasures after the occurrence of pollution. NitriTox offers three warning levels which can be individually set (NitriTox, 2015).

- **TOXcontrol** is a completely automated online toxicity monitoring system. It uses the freshly cultivated test organism of the *Aliivibrio fischeri* bacterium. The luminescence is measured before and after exposure and the inhibition is calculated as a percentage. The automated cultivation of the bacteria occurs inside the instrument and the test method is the online version of the conventionally used ISO standard method. The toxicity information can be sent to a database that is accessible online. Its ability to give an online signal when the water quality has changed allows the operator to take immediate action, for instance to shut down the water intake or stop the water processing. Subsequently, the operator can start a more detailed analysis of the nature of the pollution. Its integration with the monitoring of pathogenic bacteria (BACTcontrol) and algae (ALGcontrol) as well with an optional online solid-phase extraction method makes it possible for the monitoring system to function as a monitoring station. This water quality monitoring system became known from its first application as a biological early warning system in 2004 at a Dutch water intake station along the Rhine River (TOXcontrol, 2015).

- **TOXmini** (2015) is a portable and easy-to-use device for lab and field toxicity testing. It uses *Aliivibrio fischeri* and the same reagents as TOXcontrol.

### 3.1.2.3 Detection of the presence of microorganisms in waters

A special *in situ* applicable, real-time microbiological monitor was developed for the detection of the metabolically active acidophilic microorganisms in bioleaching solutions by bioluminescence. The activity of the *sulfide-oxidizing bacteria* is responsible for the production of acidic mine drainage from mines or mine waste disposal sites. The same microbes are responsible for the efficiency of the heap or dump bioleaching technologies applied as metal ore processing (Viedma, 2010).

*Pathogenic microorganisms* in drinking waters, bathing waters and pools, in surface waters as well in some technological waters need time-consuming and costly inspection when using conventional cultivation-based laboratory methods. This
inefficiency is further increased by the probable high number of negative samples. The coliforms in drinking water, the algae in surface waters or the *Legionella* species in bathing water require rapid and automated measuring devices.

*Escherichia coli* (*E. coli*) and total coliform detection is essential for good quality drinking water supply all over the world.

- **TECTA™** is a polymer-based optical sensor built into an incubator-analyzer-data logger system. The test utilizes enzyme substrates: beta-galactosidase enzyme for total coliform and beta-glucuronidase enzyme for *E. coli*. The enzymes of the bacteria present in the water cleave the substrates, resulting in the release of fluorescent products. The fluorescent molecules are extracted and concentrated within the polymer of the optical sensor, facilitating early and rapid detection by a UV detector (TECTA™, 2015). The equipment can be operated in both manual or automatic modes. A 100–mL water sample is needed and 2–18 hours’ cultivation to reach the cell number causing the minimum measurable signal.

- **Colifast ALARM** is an at-line automated remote monitor. The technology uses fluorogenic substrates that are hydrolyzed by the enzymes of coliforms and *E. coli*. An increase in the cell concentration leads to an increase in the proportion of the fluorescent product which is measured by an internal spectrophotometer. It can be operated both manually using intermittent sampling and automatically using periodical or continuous sampling. The 100 mL water samples are automatically collected at programmed intervals. In addition, it measures the turbidity level of the water. The system can automatically send results to the control room or to any remote workplace (Colifast ALARM, 2015).

- **ColiPlate kit** (2015) is a prefabricated 96 well microplate for *E. coli* detection. A convenient test for the quantitative measurement of total coliforms and *E. Coli* bacteria from waters within 24 hours.

- **BACTcontrol** (2015) online monitor is also based on the measuring of fluorescence produced after the bacterial enzyme cleavage of the fluorogenic substrate.

- **ALGcontrol** (2015) equipment offers an online monitoring solution for different kinds of algae through fluorescence detection. It can identify different kinds of algae.

*Legionella species* are widespread pathogens in waters, which live primarily in cooling towers, swimming pools, domestic water systems and showers, ice making machines, refrigerated cabinets, whirlpool spas, hot springs, and fountains. It is transported by air and vapor from water into the respiratory system, where the bacteria can infect alveolar macrophages. Several rapid kits have been developed for the detection of *Legionella* in waters (Figure 3.7).

- **Legionella detection** (2015) is an on-site applicable rapid test kit using a lateral flow immunochromatographic assay to detect the presence of cell surface antigens from *Legionella pneumophila* serogroup 1 within 30 minutes. The presence of the antigen in the water causes the ‘test line’ to turn red in color. A ‘control line’ is included which should always turn red if the test has been performed correctly. It is developed for the rapid analysis of water systems such as cooling towers, hot
and cold water systems, showers or pools. Several companies produce and sell this test such as for example Accepta, Biotica, Lovibond, etc.

- **Legionella kit** (2015) from Drop Test Kits (DTK Water) is also a rapid immunoanalytical method developed for weekly and monthly analysis of water systems.

- **Legipid test** is a fast detection system with combined magnetic immunocapture and enzyme-immunoassay for the detection of *Legionella* in water. It can simultaneously process up to 40 tests in 1 hour. It is a low-cost mobile device. It detects the amount of free and intact *Legionella species* in water, based on the capture of the bacterium by an interaction that depends on the integrity of the cell envelope, because the recognized element is that in the cell envelope which regulates the infectivity of this bacterium (Legipid, 2015).

There are many other hazardous bacteria living in waters which represent a human health and ecological hazard or may cause technological problems. *AquaScope*® is a fully autonomous biosensor which can be applied for the rapid biomonitoring of specific microorganisms, both in the laboratory as well at the test site. It combines filter cytometry with fluorescence in situ hybridisation. It can quantitatively measure the total number of bacterial and yeast cells, numbers of *Escherichia coli*, *Enterococcus species*, *Legionella pneumophila*, aeromonads, pseudomonads, *Thiobacillus*
ferrooxidans and Desulfouibrio species. The analysis time is 20 to 45 minutes and the detection limit is up to 1 cell per mL sample volume. It can be applied in the laboratory or at test sites (AquaScope, 2015).

3.2 Biomonitoring tools and devices

**Active and passive biomonitoring** methods (introduced in Volume 2. Chapters 4 and 5) (Gruiz & Molnár, 2015; Gruiz et al., 2015b) may be based on the monitoring of abundance, morphology, behavior, activity, biochemistry or genetics of the ecosystem’s native species. Passive monitoring of the inhabiting organisms is loaded with high uncertainty as regards age, sex, size, antecedents, individual genetics, etc. The other approach is to apply test organisms of controlled, homogenous and synchronized cultures in cages or boxes permeable for the monitored air, water, sediment or soil moisture, but with no free passage for the test organisms.

Active biomonitoring uses test organisms placed into the real environment, exposed to variable environmental conditions. The organisms placed into real waters are cultured, prepared and selected to ensure as good statistics as possible in terms of their number, age, size, sex, health, sensitivity, adaptability, etc. The advantage of this approach is that besides ensuring a controlled population of the test organisms, it is realistic, able to represent a multicontaminant situation and include matrix effects. However, realism has its limitations, as the caged or otherwise fixed organisms are not able to avoid the polluted environment or demonstrate behavioral characteristics such as the burrowing of crustaceans, which is essential from the point of view of healthy food chains. In addition, the environment may also spoil the advantages by producing extreme conditions, differing greatly from a normal situation (e.g. high temperature, heavy rain, flood, storms, or other disasters).

Conventionally, the recollected test organisms (active biomonitoring) or the collected natural inhabitants (passive biomonitoring) are investigated in the lab. Some advanced methods provide continuous signals during the stay of the test organisms in the environment at sublethal contaminant concentration ranges. Rapid methods and mobile labs are becoming more and more available for in situ investigations of the sampled organisms’ morphology and biochemistry.

A Musselmonitor (Mosselmonitor®, 2015) is an in situ passive biomonitoring method with remote data processing, indicating the frequency of valve opening of mussels in a cage equipped with a motion detector. It works in most cases with the mussels Dreissena polymorpha or Mytilus edulis and is applied as an early warning tool for the continuous monitoring of drinking waters, surface waters or effluents. The observed and measured end point is the opening of the valve, whose frequency and duration depend on the type and level of contamination. The variations on the normal movement pattern include a more rapid opening and closing of the valves (flapping), keeping the shell closed for a fixed period or opening the shell for shorter time and to a lesser extent. Extreme contamination causes the death of the mussel.

Valve movement is detected and transformed into an electric signal by a microprocessor and the signals are processed by software to get the end point of the movement pattern, which unequivocally indicates the negative effect of the water on the mussel.

The mussel is glued to a platform and the sensors are fixed on each half of the shell (Figure 3.8). The sensors are small coils, one of which generates a magnetic field
when current passes through and the current induced in the other coil is measured. The magnitude of this electric current depends on the distance between the two coils. Current is continuously measured. The change in the current is converted into distance and the movement pattern is evaluated as a function of time. The Musselmonitor can be used as an *in situ* placed field unit deployed into surface waters with a locally or remotely arranged data logger, or it can be used in flow-through mode by placing the measurement chamber with the mussels into the side-flow of any water fluxes of a water treatment system (Mosselmonitor®, 2015).

The monitor was applied as far back as 1998 for the automatic water quality monitoring of the Danube River at Bad Abbach (near Regensburg) and Jochenstein (on the German/Austrian border). In this study, a surveillance system was used: when deviations from the normal behavior were recognized, an alarm was triggered and the water was sampled for detailed analysis (IAD, 1999). Several successful applications have been carried out since that time, e.g. for assessing offshore contamination in the Adriatic Sea (Gorbi *et al*., 2008; Gomiero *et al*., 2011; Pilot project Mosselmonitor, 2013).

Other caged animals, e.g. daphnia or fish, can be observed by digital video camera. The video image analysis indicates the probabilistic relationship between health and the adversely effected state of the test organism, e.g. abnormally rapid and slow motions or immobility.

A *daphnia toximeter* is an instrument to observe living daphnids in the targeted water from water bodies and water treatment plant intakes of sewers. Its predecessor is the Extended Dynamic Daphnia Test, the oldest *in situ* biomonitoring method, in use from the 1980s. The new development of the company bbe Moldaenke is DaphTox II for the detection of toxic substances in water via computer-assisted digital image analysis. If the change is statistically significant, an alarm is triggered (Figure 3.9). The image analysis covers speed parameters (average speed, speed distribution, distance between the animals), behavioral parameters (swimming height and location, turns and circling movements, curviness) and growth (daphnia size). The system triggers the alarm when more parameters at the same time give characteristic results within a certain period of time (DaphTox II, 2015).
Changes in ventilatory behavior and certain locomotor activities of fish can be detected by non-invasive electronic sensors in a tank. Fish signals are amplified, filtered, and interfaced to a computer. When a significant number of fish respond simultaneously in an abnormal manner, an alarm is initiated.

A *fish toximeter* can continuously analyze fish behavioral patterns for the detection of toxins in water. It observes fish under the influence of a ‘sample’ water stream (Figure 3.9). The technique is aided by a digital video camera and continuous computer-assisted image analysis. The measuring system, based on the videos, evaluates the speed, swimming depth, size and the number of fish, and indices are calculated based on the determined values. Animal avoidance behavior can also be observed. If the aggregated ‘Toxic Index’ exceeds a default criterion for a certain duration, an alarm is triggered (Fish Toximeter, 2015).

Similar measuring systems can be applied for drinking water supply protection, waterway quality analysis and assessment, dam monitoring and for general surveillance. The instrument *ToxProtect 64* has been created especially for drinking water. The evaluation is mainly based on fish activity, swimming on the surface and the escape reaction. In contrast to the more general fish toximeter, the evaluation here is based on interruptions in light barriers by the fish movement. Unacceptable toxicity is associated with a certain level of interruptions per minute and fish. The thresholds for the alarm triggers can be set individually (ToxProtect, 2015).

An *algae toximeter* (2015) continuously monitors water for the presence of toxicity with the help of sensitive green algae. The algal concentration and the photosynthetic activity are measured in the measuring chamber or, alternatively, in the flow-through sample loop. The fluorescence measurement is carried out by the coupled *AlgaeOnlineAnalyser* for online detection of chlorophyll concentration, algae classes and photosynthetic activity. Chlorophyll a is responsible for the fluorescence of algae via excitation by visible light. The presence of other pigments indicates different algae classes. The interaction of these different pigments with chlorophyll-a results in a special excitation spectrum for taxonomic algae classes. The AlgaeOnlineAnalyser (2015) can be switched from continuous to batch mode. The algae are continuously propagated in a separate turbidistatic reactor, producing a well-controlled standard
algae culture for the measurement. First the concentration and the activity of the naturally occurring algae are determined in the water, then the standard algal culture is added and the changes observed in the measurement chamber (Figure 3.9).

**Bioaccumulation** is a very plausible end point for chronic exposures to persistent organics and toxic metals. Some of the accumulator organisms may collect significant amounts from the environment or from food, often without visible health effects. Filter feeders such as bivalves (clams and mussels) tend to concentrate metals in their gills or other organs and tissues. This is because mollusks can limitedly excrete or metabolize pollutants directly and therefore attain higher bioaccumulation or bioconcentration factors compared to other taxonomic groups.

**Active biomonitoring** with caged mollusks has long been known and practiced (Mussel Watch from the 1970s), however the acquired information is in most cases not proportionate with the extensive workload, the number of problems to be solved and the analysis cost. On the other hand, for some purposes such as long-term monitoring is ideal, as the contamination levels in the mollusks reflect a time-integrated amount and the ecologically relevant bioavailable fraction. The accumulation of filter feeders characterizes water pollution, whereas the sediment-living deposit feeders characterize sediment pollution (Oehlmann & Schulte-Oehlman, 2002).

The separately grown and then translocated animals are exposed to the contaminated natural waters. They are left unattended in the cages for a certain time. The conventional monitoring method is to chemically analyze the accumulated toxicant in the tissue after retrieval of the mollusks, and the body burden is calculated. A more promising and less time-consuming biomonitoring solution is the investigation of bioaccumulation-specific biomarkers in an animal exposed for a relatively short time. The biomarkers could be metallothioneins, stress/heat shock proteins, several oxidative enzymes including the cytochrome P-450-dependent monooxygenase (MFO) and the flavine-dependent monooxygenase (FMO), monoamine oxidase (MAO), dehydrogenases, peroxidases, etc. The lysosomal stability and membrane integrity may also be characteristic of bioaccumulation. DNA damage in mollusks is detected by the comet assay already after a short time exposure (Steinert *et al.*, 1998).

4 APPLICATION OF IN SITU AND REAL-TIME METHODS FOR SURFACE WATERS AND OCEANS

Ensuring water quality, primarily drinking water quality, requires urgent action around the world. Millions of people, mainly children, die every year due to the lack of clean drinking water. Thousands of chemical substances and aggressive microorganisms are contaminating our waters. In order to improve the situation, an exponentially increasing number of measurements would be necessary, which is not feasible due to the lack of the enormous equipment capacity needed for the analysis. The conventional, laboratory-based chemical analytical and microbiological tools are unable to fulfill the requirements of low cost, speed and precision necessary to deal with the large number of samples required. Regulators encourage the development of innovative methods and instruments, aided by frequent data acquisition and getting rapid analytical responses. There is great demand for miniaturized and automated systems,
which can function in the long term without significant human workload and costly laboratories.

4.1 Real-time water quality monitoring

Real-time water quality monitoring is an essential need to reduce health and environmental risk. In situ, real-time methods are needed in surface water monitoring activities, in oceanography, as well as for runoff and wastewater management. Both research and practice require real-time information on the qualitative and quantitative characteristics of our waters. Changes in flow rates and water levels, temperature, pH, redox potential, nutrient and contaminant concentration may have significant impacts on the aquatic ecosystem. They also largely influence human water uses and health risks. Early warning is essential to prevent damage due to delayed risk reduction measures. Acquiring continuous real-time data may increase the efficiency of risk management of physical, chemical and biological hazards such as algal blooms, which are typically easy to monitor with in situ sensors. Most of the in situ, real-time measurements and devices have their conventional counterparts for measuring depth, flows, water chemistry, and biology-based physical or chemical signals, but the conventional ones cannot compete with the benefits of the in situ placement, the high measurement frequency and the programmable and autonomous versions. Some in situ and real-time instruments used in water monitoring are introduced in the following.

A Conductivity-Temperature-Depth recorder (CTD) is the basic instrument of all practitioners working in marine and freshwater environments. It may be equipped with sampling rosettes, an additional oxygen sensor, transmissometer and fluorescence detector. A CTD recorder is typically placed by ships into the sea/ocean or other surface waters and is connected by cables to transmit real-time data to the data logging system on board the ship. It is continuously let further down in the water. Depths and intervals of measurements are programmable by the user. Modern equipment has an internal memory and can be powered both by batteries or externally. Designs differ for 600 m depth use with a plastic housing, and at 7,000 or 10,500 m, with a titanium housing (SBE, 2015a).

Submersible multi-channel data loggers, recorders, versatile probes, controllers and sensors are produced for water quality measurement by RBR (2015). The high-precision instruments are recommended for oceanographic, freshwater, groundwater and cryospheric research. The standard data logging instruments range from one to 24 channels, configured as a CTD, conductivity, temperature, depth (pressure) or multi-parameter sensors/recorders.

Real-time water quality assessment of pipe discharges, streams and rivers, lakes, estuaries and other shallow waters can be implemented by the YSI multiprobe instruments, for example the 6600 V2-4. It measures dissolved oxygen, pH and redox potential, turbidity, chlorophyll and blue-green algae. Additional calculated parameters include total dissolved solids, resistivity, and specific conductance. Self-cleaning optical sensors with integrated wipers remove biofouling and maintain high data accuracy. The fluorescence-based blue-green algae sensor enables monitoring of blue-green algae populations where their presence is a concern. The sensors provide early warning of algal bloom, track taste and odor-causing species in drinking water supplies,
or conduct ecosystem research (YSI, 2015). The experience of users is that YSI sensors – similar to all water-placed sensors – require rigorous maintenance and frequent calibration. The performance of sensors begins to deteriorate after 2–3 weeks.

Real-time ocean observing systems provide critical information for the study of ecosystems, water quality, and fisheries, as well as data for long-term climate change studies. The Inductive Modem (IM) system for moorings provides reliable, real-time data transmission for up to 100 instruments that can be positioned or repositioned at any depth, in wireless mode. The Inductive Modem Module (IMM) communicates with the buoy controller and with the underwater instruments measuring various combinations of temperature, conductivity, pressure, dissolved oxygen, and data from integrated auxiliary sensors. The data are transmitted from the buoy to the remote receiver (SBE, 2015b).

A Sea Tramp profiling system is – similar to the previous SBE system – an autonomous, multi-cycling, data collecting platform designed for unattended marine monitoring and research. It profiles along a guiding wire and performs well also in stratified waters and when equipped with a non-stream-lined payload. Sensors are selected by the operator and may be installed on site (Ocean Origo, 2015). The buoy system (surface and sub-surface buoys) includes data logger, controller and the remote communication unit of SeaMoose, which is a flexible ‘meteorological and oceanographic observation system for the environment’. These parts of the system are shown in Figure 3.10 and the complete system in Figure 3.11. The buoy system also includes a bottom-mounted acoustic doppler current profiler (ADCP) (Teledyne RDI, 2015) with integrated wave measurements for real-time monitoring of coastal currents.

### 4.1.1 Surface water and oceanographic sensors

Chelsea sensor technology has built several surface water and oceanographic sensors (CTG, 2015) for environmental monitoring of rivers, reservoirs, lakes, and groundwater. Sensors are provided for *in situ* chlorophyll and algae class studies, dye tracing, oil spill monitoring, airport runoff or water abstraction management and effluent detection. The priority technologies are i) fluorometers for water quality monitoring, ii) compact, multi-parameter monitoring system for oceanography and limnology, iii) sensitive digital infrared turbidity sensor designed for compliancy with ISO 7027:1999 standard iv) submersible bioluminescence sensor which monitors the visible emissions...
from bioluminescent organisms in seawater, and v) sensors for the measurement of photosynthetically active radiation (PAR). This company offers a plankton sampler for automated towed and shipborne use, too.

The *autonomous profiling nutrient analyzer* (APNA) is designed to be adaptable for deployment on a wide variety of ocean observation platforms including: shipboard profiling or towed sensor array; fixed-depth or vertical profiling moorings; autonomous underwater vehicles and gliders. The commercially available analyzers can monitor and establish the concentrations and distributions of nutrients and other chemicals – nitrate, nitrite, phosphate, ammonium, silicate and iron – in fresh and marine waters (SubChem, 2015). They are equipped with a 4- or 6-channel analyzer with multichemical capability. It is able to conduct autonomous vertical profiling, continuous underway surveying and intermittent long-term sampling (APNA, 2015).

The *flow-through analyzer system* of NAS-3X (2015) is a robotic analyzer. It is the latest development for high-frequency, time-series determination of nutrient concentrations (nitrate, phosphate, silicate and ammonia) in marine and fresh waters. The NAS-3X is typically deployed unattended for periods of 1 to 3 months, although longer deployments can also be achieved. It has been used near surface in many buoy and riverine applications or moored at depths down to 250 m. It can be applied for early warning of phytoplankton blooms, eutrophication and for the identification of episodic events. Suitable for run-off monitoring and changes in nutrient concentrations.

Satlantic (2015) together with SBE (2015a) developed a wide range of sensors and measuring systems for the study of aquatic environments. They offer real-time *in situ* i) nutrient sensors such as the SUNA V2, Deep SUNA and ISUS V3, ii) *in situ* fluorometric analysis for chlorophyll fluorescence in photosynthetic organisms, iii) radiometers for optical profiling, water color and PAR, and iv) hyperspectral and multispectral radiometers, etc. The company also develops large-scale ocean observatory systems and data extraction tools.
In-situ and real-time measurements in water monitoring

The LISST-100X instrument from Sequoia (2015) is a multi-parameter system for in situ observations of particle size distribution and volume concentration. Additionally, it records optical transmission, pressure and temperature.

4.1.2 Global monitoring

Argo is a global array of more than 3,000 free-drifting profiling floats that measures the temperature and salinity of the upper 2000 m of the ocean. This allows systematic, continuous monitoring of the temperature, salinity, and velocity of the upper ocean. Measured data are assimilated in near real-time into computer models and made publicly available within hours after collection. Compared to the traditional ship-based measurements, Argo covers the oceans in their entirety (not only shipping routes), summer and winter period equally, and with a much larger number of real-time measurements (Argo, 2015). It is part of an integrated global ocean observing system (GOOS, 2015), within the global earth observation system of systems (GEOSS, 2015). Figure 3.12 shows the concept and operation of Argo. The floats weigh 25 kg, their operating depth is 2000 m and comprise three subsystems: (i) the hydraulics controlling buoyancy adjustment by an inflatable external bladder so the float can surface and dive, (ii) microprocessors dealing with function control and scheduling, and (iii) a data transmission system controlling communication with a satellite. Several types of floats are used in the ARGO project such as:

1. PROVOR and ARVOR floats built by nke (2015) and IFREMER (2015);
2. APEX, produced by Teledyne Webb Research Corporation (TWR, 2015);
3. SOLO float designed and built by Scripps Institution of Oceanography (Scripps, 2015) and the SOLO-II float built by MRV Systems (MRV, 2015).

The early projects, such as MAST I and MAST III – the European contribution to the GOOS (2015) – can be considered the predecessors of Argo. An autonomous in situ multidisciplinary ocean observatory was developed within MAST I (BABAS project from 1990 to 1994) and MAST III (YOYO 2001 – Ocean ODYSSEY project from 1998 to 2001). The YOYO is a Eulerian (starts and ends on the same vertex) autonomous multisensor profiler providing time series of parameters continuously over the water column. It is intended for long-term in situ monitoring of the ocean, opening a wide range of possible scientific applications ranging from specific process studies to climate monitoring. YOYO was equipped with the Autonomous Nutrient Analyser in Situ (ANAlS), a spectrophotometrical instrument providing real-time data on the nutrient status of the ocean water by measuring nitrate, phosphate and silicate. The analyzer is a set of three chemical sensors, a manifold where the reaction takes place, a colorimeter for the analyses, and two clamping plates for fixing the pump and sealing the manifold. The set is placed in a container together with the bags for the reagents. An IT card system was built in to control the sensors and for data storage and transmission (Joća et al., 2013).

Advanced sensing for ocean observing systems and the projects of O-SCOPE – Ocean-Systems for Chemical, Optical, and Physical Experiments and MOSEAN – Multi-disciplinary Ocean Sensors for Environmental Analyses and Networks, were carried out from 1998 to 2008, sponsored by the National Oceanographic Partnership Program (NOPP, 2015). The projects focused on developing and testing...
new sensors and systems for autonomous, concurrent measurements of biological, chemical, optical, and physical variables from a diverse suite of stationary and mobile ocean platforms. Design considerations encompassed extended open-ocean and coastal deployments, instrument durability, biofouling mitigation, data accuracy and precision, real-time data telemetry, and economy (Dickey et al., 2009a).

O-SCOPE aimed to measure pH, CO$_2$, partial pressure ($p_{CO_2}$), dissolved inorganic carbon, total alkalinity, dissolved oxygen, water turbidity, chlorophyll, and optical absorption and scattering for the applications of reflectance models for remote sensing of ocean color (Dickey et al., 2009a). O-SCOPE sensors were tested on three deep-sea moorings: (i) about 80 km southeast of Bermuda, (ii) Monterey Bay, California, and (iii) the NOAA Tsunami warning buoy at Ocean Weather Station Papa in the North Pacific.

The in situ instrument of a spectrophotometric elemental analysis system (SEAS) autonomously mixes seawater and reagents, and records absorbance at user-defined wavelengths. The precision of the spectrophotometric pH measurement is $\pm 0.001$ pH units. A non-dispersive infrared spectrometer has also been developed for measuring the difference in CO$_2$ partial pressure ($\Delta p_{CO_2}$) across the air-sea interface (Friedrich et al., 1995). Further developments in this area resulted in a sensor suite for measuring absolute air and sea surface $\Delta p_{CO_2}$ (with an accuracy of $\pm 3$ µbars), dissolved oxygen and nitrate concentrations (Johnson & Coletti, 2002).

The O-SCOPE project also focused on the application of bio-optical sensors with improved stability and endurance for operational monitoring. A chlorophyll...
fluorometer and a multi-angle scattering sensor for measuring the volume scattering function (VSF) were developed for phytoplankton biomass monitoring (Moore et al., 2000). A new modular servo-controlled anti-biofouling shutter system for open-faced optical sensors was tested on the O-SCOPE optical systems (Manov et al., 2004).

The main goal of the MOSEAN project was to test small, lightweight optical and chemical sensors for autonomous deployment on a variety of stationary and mobile platforms. The MOSEAN mooring sites – using the channel relocatable mooring (CHARM) – are located on the Hawaiian HALE-ALOHA, in an open ocean oligotrophic environment ca. 100 km north of Oahu, HI and on the coast in the Santa Barbara Channel. The project was also aimed to develop a near real-time data telemetry system and the mitigation of biofouling.

In summary, the two projects of O-SCOPE and MOSEAN were used to successfully develop and test new, compact, energy-efficient sensors and systems for the autonomous measurement of biological, chemical and optical parameters, in particular chemical sensors, water samplers, and spectrophotometric elemental, pulsed-membrane, colorimetric and microfluidic/fluorometric technologies. Optical technologies such as fluorescence and turbidity meters, multispectral and multi-angle scattering and backscattering sensors, a hyperspectral absorption-attenuation meter, and spectral fluorescence sensors have also been developed. These advances, along with improved water storage and validation techniques, enable accurate in situ and remote observations and estimates of a wide variety of biogeochemical parameters, for example inorganic and organic particles such as phytoplankton and hazardous algal blooms (HABs) (Dickey et al., 2009b).

5 APPLICATION OF IN SITU REAL-TIME MEASUREMENTS FOR WASTEWATER TREATMENT AND QUALITY CONTROL

The risk of wastewaters on surface waters can be lowered both by process control of the wastewater treatment technology to ensure its optimal functioning, and by product control of the treated wastewater to stop its release when quality does not fulfill the quality objectives.

Monitoring for the purpose of process control in wastewater treatment and for quality control of the inflowing and treated wastewaters still has several shortcomings such as limitations in accurate and frequent sampling and analyses by conventional, laboratory-based measuring methods. Innovative, rapid and cheap in situ and real-time methods and devices are needed to drive the monitoring of wastewater treatment efficiency and product quality.

Conventional end points based on standardized methods to follow microbial activity in wastewater treatment plants are dissolved oxygen, oxidation reduction potential (ORP), and solids retention time (SRT) to control the sludge: sludge blanket level and total suspended solids. To control treated water quality the following variables are generally measured: biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), total suspended solids (TSS), specific organic compounds, e.g. phenols; mineral compounds, e.g. total nitrogen and total phosphorus; pH, residual Cl₂ (after treatment with chlorine chemicals), toxicity and pathogenic microorganisms. These conventional methods are extremely time-consuming and cannot be
used for real-time/online monitoring and automation. The measurements are loaded with uncertainties due to sampling problems, conservation, storage, transportation and laboratory analysis – immediate or postponed – depending on capacity.

Water quality is a huge world-wide problem. Residual BOD and nutrients load cause eutrophication in receiving surface waters. The problem of pathogenic microorganisms and micropollutants of emerging concern, typically with long-term human health effects such as endocrine and immune system disruption must be solved.

To improve the situation, innovative methods should be developed and introduced into practice. For example, instead of the 5-day long BOD₅, several rapid methods have been developed and demonstrated such as rapid BOD determination based on respirometry, COD, TOC, fluorescence and UV absorbance (Guwy et al., 1999), or biosensor-based methods (Liu & Mattiasson, 2002). Several online methods have already been applied for controlling the wastewater treatment process such as photometric, colorimetric, or titrimetric methods, ion-specific electrodes, UV spectrometry for organic contaminants, etc. (Vanrolleghem & Lee, 2003). Qualitative or semi-quantitative colorimetric test kits are also available for rapid, on-site (ready-to-use) application. The ISO 17381 (2003) standard establishes criteria for the selection and application of test kit methods in water analysis. Specific sensors, DNA- and immunotechniques are increasingly being developed and routinely applied also in the management of wastewater treatment.

The application of online working sensors would make efficient control of wastewater quality possible and of the treatment process itself. For the efficient utilization of the real-time online signals of the sensors, the whole wastewater treatment and control strategy should be harmonized with a high-level online monitoring. Monitoring should be linked to an automatic control system which is coupled to several technological options. The control system can process the output of the measuring device, select and carry out the proper countermeasure.

Online sensors for temperature, pressure, liquid level, flow of liquid/gas, pH, and conductivity are commonly applied devices in several technologies and these are not discussed here in detail. Biological activities, biodegradation rate, suspended solid, gaseous biodegradation products, dissolved metabolites, and the microorganisms present are typical variables for wastewater treatment, and their conventional laboratory-based analysis methods are extremely time-consuming and costly. A detailed discussion of the innovative methods focuses on the latter subjects.

### 5.1 Innovative analytical tools for wastewater management

The easiest way of innovation is to modify a conventional method e.g. by size reduction or portable design for on-site applications. A more advanced type of innovative method is based on a new principle, e.g. applying optical sensors or biosensors. Many of these sensors do not give a correct absolute value; they can be used after site-specific calibration of the local wastewater. The best known optical method in this group is the estimation of total suspended solids (TSS) from the results of turbidity or nephelometry, detecting transmission and scattering of light.

Exploitation of the whole UV spectrum and analysis of the spectrum makes it possible to do parallel analyses of several organic pollutants (total organic carbon,
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phenols, surfactants, etc.). Biosensors are increasingly available and accepted; some are validated for treated wastewater quality monitoring as will be shown in Section 5.3. Immunoenzymatic test kits are available for several micropollutants and electrochemical measurement systems for metals.

Toxicity monitoring is essential both for the undisturbed operation of wastewater treatment plants and the acceptable quality of the product, the treated wastewater. Handheld instruments are in widespread use for measuring the main physico-chemical parameters such as temperature, conductivity, pH, and dissolved oxygen and are often integrated into a multiparameter device. Handheld and portable designs are available for most of the conventional parameters measured. Passive samplers represent a new way and new concept in sampling. The specific materials of the different samplers ensure selective adsorption of specific molecules and micropollutants when the sampler is immersed into a stream. The application of cyclodextrins for selective sorption of certain contaminants is detailed as an example in Chapter 7 Section 3. Short- and long-term applications may refer to instantaneous and aggregated loadings. Their calibration may still be problematic.

The application of models may be a good solution in those cases when the mathematical function between online measurable variables and a difficult-to-measure quality indicator (BOD or nitrifiable nitrogen) is known.

Another conceptual innovation could be (as in other environmental management tasks too) tiered monitoring: the frequent or online monitoring by a qualitative or semiquantitative method as a first tier, and the quantitative analysis of the positive or borderline samples as the second tier.

Emerging measurement techniques have also approved also for online monitoring of wastewater during and after treatment. ISO 15839 (2003) prescribes the test procedures to be used to evaluate the performance of online sensors and devices.

Biomonitoring, early warning systems, bioassays using intact organisms and biomarkers are emerging surface water analytical tools (Allan et al., 2006a and 2006b) which can be, and partly have been, adapted to wastewaters (see the Volume 2 of this series) (Gruiz et al., 2015). The quality of, and the risk due to, the wastewater can be characterized using these biomonitoring results, e.g. the ‘no effect dilution’ can easily be determined (see Chapter 9 in Volume 2 – Gruiz et al., 2015a).

Online biomonitoring is considered rapid and inexpensive, but currently it has significant limitations (lifetime, reproducibility, etc.) and only a few parameters can be measured online.

Biosensors, introduced in Sections 2.3, can detect chemical substances based on a very selective biocatalytic or bioaffinity response or on the more general, integrating response of living organisms. Many of the techniques developed for surface waters or drinking waters can be applied for wastewaters, and conversely, the methods and tools developed for wastewater can also be applied for contaminated groundwater, leachates and soil waters as well as for the pore water of sediments and saturated soils. The additional problem in their use for soil and sediment is their deployment and protection from the impact of the solid matrix.

Some DNA- and immunotechniques, microanalyzers, online respiration and toxicity measuring methods used for wastewater analysis are introduced briefly in the following section.
5.2 Measuring microbial quality and activity in wastewater treatment plants

Biological wastewater treatment is one of the best known biotechnologies and as biotechnologies in general, it can properly work if both the ‘catalyst’, the living biomass, and the ‘technological parameters’, ensuring the conditions for the best functioning of the biomass, are kept at an optimum. Optimization needs continuous feedback on the quality of the wastewater, both the quality (composition) and quantity of the biomass and the value of the technological parameters, e.g. temperature, O₂ supply as well as the level of toxicants which have a potential inhibitory effect on the biomass. The efficiency of the technology determines the quality of the product, i.e. the treated wastewater.

Hundreds of publications deal with the developments of, and the experiences with, the developed innovative wastewater monitoring methods and devices. A practical summary of the applied technologies was published in the form of a guidance document by US EPA in 2013. All the emerging (less than three years after demonstration), innovative (less than five years’ application) and established (more than five years’ application) methods, including adapted uses, are briefly described (US EPA, 2013). Quevauviller et al. (2006) reflect the global concepts on wastewater treatment-related monitoring and control, giving an overview on policies, standard methodologies, reference materials and discussing biosensors and alternative methods.

The innovative measuring methods and devices introduced in the following section are applicable online in wastewater treatment plants and can fulfill the previously listed requirements of biomass and technology. Some of them are already validated and accepted methods, whereas others are still under development.

5.2.1 Whole-cell biosensors for measuring biodegradable organic material content

Biosensors that are able to continuously measure the BOD value are highly desired devices for wastewater treatment. Such equipment is commercially available and a method has recently been standardized in Japan. The whole-cell sensor did not measure the BOD directly, but the end points of respiration and biodegradation rate from which the BOD can be estimated. The same sensors may also function as an alarm system for toxicity based on a massive decrease or full termination of respiration. The measurement is done in a small volume reactor, where the biodegrading biomass is generally fixed on a solid carrier. When the wastewater containing biodegradable organic material meets the metabolically activated microorganisms under aerobic conditions, oxidation-based biodegradation begins. The active biomass in the equipment can be the separately propagated artificial mixture of microorganisms or the local sludge. The microorganisms degrade the nutrient content of the wastewater at the expense of the dissolved oxygen (DO) and produce CO₂ in parallel; both DO and CO₂ can easily be monitored. The cell based BOD-sensors generally consist of more reactors and are equipped with a dissolved oxygen sensor and a thermometer and can be mixed, pumped, heated or cooled. Constant activity of the biomass is critical. Diez-Caballero (2002) successfully applied an independent chemostat (a flow through bioreactor to which a fresh medium is continuously added, in order to keep constant chemical
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composition and nutrient supply) for propagating and maintaining the activated cells for continuous application in the BOD sensor.

BOD measurement with constant activity biomass can be applied both to characterize the wastewater’s biodegradable material content, or the toxicity of the influent waste into the wastewater treatment plant. The conventional BOD<sub>5</sub> testing is not enough for the simulation of the wastewater treatment plants’ operation; instead it is total BOD which should be measured, including both BOD<sub>5</sub> and the nitrification step. An online total BOD and a rapid BOD analyzer are introduced below:

- **BioMonitor (2015)** is an online BOD analyzer for the determination of biological oxygen demand (total BOD), respiration and toxicity in wastewater. It is a miniature wastewater treatment plant, using its own activated sludge. Measurement of BOD takes place in less than 4 minutes. This speed guarantees that very short peaks can be determined during a daily cycle. Its measurement range is 1–200,000 mg/L BOD, 0–100% toxicity and respiration O<sub>2</sub> mg/L x min.

- **Quick Scan** BOD analyzer is a small size respirometer that has 4 reactors with a magnetic stirring base. It is ideal for toxicity screening and short-term BOD assessment. This same system can be used for soil and compost testing with the use of special soil/compost columns in place of standard glass reactors (Challenge Technology, 2015).

5.2.2 Online respirometry

Respirometry to obtain estimates of the rates of metabolism of the microorganisms during biodegradation, is a basic measurement in the process control of wastewater treatment. By measuring the rate of respiration (amount of oxygen/volume × time), a measure of the rate of biodegradation can be obtained. It makes respiration rate an important end point both in process control and toxicity management (Spanjers et al., 1998).

The traditional means of measuring respiration rate is a laboratory method in a closed system based on the decrease of dissolved oxygen content directly in the liquid phase (oxygen electrode) or indirectly in the air space above the liquid (manometric method). Rapid methods need a very small amount of biomass/activated sludge and respond within 5–10 minutes. Multiple electrodes and specific software support the evaluation. Rapid laboratory measurements are useful in influent toxicity control in order to protect biomass from toxic wastes. The results can be used for the determination of the maximal inflow rate of the toxic waste, in other words, the necessary dilution of the waste before releasing it into the wastewater treatment plant.

Laboratory measurements are not suitable for getting real-time feedback from the continuously working biomass and the dynamic wastewater treatment process. Online respirometry applied for process control should be continuous and carried out in real-time. Open respirometry measures dissolved oxygen (DO) in an open flow system. The sensor can be immersed into the main reactor/flow; however, in most cases the location of the sensor is a separate reactor or side flow established for measurement purposes.

The respiration rate measurement in addition to process control can also determine residual BOD in treated wastewaters, the toxicity (Geenens & Thoeye, 1998;
Davies & Murdoch, 2002) and the shock load (Henriques et al., 2007) in a system, as long as the baseline rate has been set for that system. Rapid laboratory and online respirometry are complementary in toxicity management.

Sensors of a respirometer can also be calibrated to measure other gases of concern like carbon monoxide, hydrogen sulfide, and methane.

A classification of online respirometers may occur according to the phase where oxygen is measured, i.e. liquid or gas and the reactor, which can be static or flow-through. In the flow-through system, the flowing phase can be either gas, liquid or both (Vanrolleghem, 2002). Examples of online monitors:

- **The Ra-BOD** is an online process analyzer for determining short-term BOD in wastewater and surface water and fits into a control strategy for activated sludge plants. **Ra-COMBO** is both for determining BOD or toxicity (AppliTek, 2015).
- **The Rodtox NG** has been designed for determining BOD and toxicity and to detect acute and chronic toxic effects of incoming wastewater streams of wastewater treatment plants (Kelma, 2015).
- **STIP TOX** a toxicity monitor which has been designed for continuous toxicity measurement for protection of biological processes from toxic substances (Axon Automation, 2015).
- **Amtox** uses an immobilized culture of nitrifying bacteria to produce a fast and reliable result. Inhibition is measured by comparing feed and effluent ammonia using a probe technology. Results are displayed continuously via a graphic interface (PPM, 2015).
- **Online Oxygen Demand Monitor** model ODM–100 is a portable unit for measuring real-time oxygen demand (oxygen uptake rate = OUR) at any point in a wastewater treatment process. It can be used for monitoring in continuous, batch or sequential batch modes. It is supplied with a submersible sewage pump to drop in at any point along the treatment process to allow real-time data at any given location (Challenge Technology, 2015).
- **Strathtox respirometer** is a 6-cell OUR measurement system for rapid measurement of activated sludge bacterial performance. The corresponding software provides a real-time display of bacterial respiration. Automatic report generation and the calculation of EC10, EC20 or EC50 values (Sensara, 2015). The software can calculate the following end points: respiration inhibition; nitrification inhibition; short-term BOD, nitrification status, sludge health, OUR and SOUR (specific OUR), and critical oxygen concentration point.
- **Bio-Scope** is an immersible sensor to see how the bacteria are performing in the wastewater treatment plant in real-time. It gives information on the biodegradation rate profile under real-time conditions and on bacterial health by comparing the current sample with the last ten measurements at a specified point. It also provides information on the critical oxygen point for energy optimization, DO and temperature (Sensara, 2015).

### 5.2.3 Fluorescence in situ hybridization

**Fluorescence in situ hybridization** (FISH) can be applied for identifying any kind of microorganism or group of microorganisms, even in the smallest amount of a complex
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mixture. This is why it is ideal for the study of wastewater biomass. Based on the knowledge of concrete DNA or RNA sequences exclusively occurring in the targeted microorganism, genetic engineers are able to prepare a probe. The probe is a polynucleotide sequence complementary to the targeted sequence in the microorganism to be detected or identified. Complementary nucleotides link to each other with high affinity and form a double helix. To find and detect these hybrid nucleotide pairs in a complex mixture, the artificially prepared component of the ‘hybrid’ nucleotide is labeled (marked). In the case of FISH the label is a fluorescent marker, a covalently attached fluorophore which fluoresces after staining with a specific dye. The fluorescently labeled 16S rRNA (ribosomal RNA) probes are hybridized, stained and observed under an epifluorescent microscope. A validated method exists for filamentous and nitrifying bacteria and for phosphorus accumulating organisms (PAOs).

5.2.4 Quantitative polymerase chain reaction for the quantification of microorganisms

Quantitative polymerase chain reaction (qPCR) technology is able to quantify microorganisms based on their DNA, both in influents and effluents as well as in the sludge of the wastewater treatment plants. PCR is used to amplify specific regions of DNA to be able to selectively detect microorganisms of small copy numbers in mixtures. To use the technique for the identification and quantification of Escherichia coli and enterococci in wastewaters, – similar to hybridization – the species-specific DNA sequence of the 16S ribosomal region of the targeted microorganism should be known to be able to synthesize the primer. Real-time quantitative PCR (qPCR) is a relatively rapid method (2–4 hours) for simultaneous DNA quantification and amplification. It uses a hybridization probe and a PCR primer together, to measure the amount of the product in real time. The probe containing a fluorophore is attached to its template in the specific region of the targeted cell, close to the location of the primer linkage. When the polymerase enzyme moves the template DNA forward, it encounters the probe and degrades it. The released fluorophore from the degraded probe is quantifiable by measuring fluorescence, and is proportional with the amount of the target microorganism. A calibration with known cell count of the target microorganism is needed to determine the absolute value of the cell count.

5.2.5 Nicotinamide adenine dinucleotide probes

The presence of the reduced form of nicotinamide adenine dinucleotide (NADH or NADPH) is proportional with the reduction potential of the biomass. Light of 340 nm wavelength induces fluorescence in NADH, and the emitted fluorescence light is detectable at 460 nm; therefore monitoring the level of reducing power is possible by measuring fluorescence at 460 nm. The measurement is done using immersed probes with no sampling or subsequent analysis.

Different NADH probes (molecules emitting light at 340 nm), fluorescent sensors and reporters (manifesting a large change in fluorescence upon NADH binding) have been developed and applied for measuring NAD and NADH ratios as well as NADH outside and inside the cells (Lemke & Schultz, 2011, Zhao et al., 2011 Zhao et al., 2014). By means of the SymBio process (SymBio, 2015, Spellman, 2014) nitrification and denitrification can be quantitatively characterized in one step. NADH is measured
in the intracellular pool to quantify the real-time biological activity. Dissolved oxygen (DO) is measured in parallel to control aeration and to precisely adjust the relatively low DO level that is necessary for the simultaneous nitrification in the outer aerobic zone and denitrification in the inner, anoxic zone of the activated sludge flocs (Trivedi, 2009).

5.2.6 **Immunosensors and immunoassays**

The application of antigen-antibody interaction to detect the presence of toxins in wastewater is based on the specific recognition and binding of a bacterial antigen by antibodies. Immunosensors detect the signals of immunoassays. The artificially labeled antibody is responsible for the detectable signal. Both direct and competitive assays are used, and the label in the practice of wastewater management can be

- an enzyme such as alkaline phosphatase and horseradish peroxidase in enzyme immunoassay or enzyme-linked immunosorbent assay i.e. ELISA;
- chemiluminescent (e.g., acridinium ester), or
- fluorescent (e.g., fluorescein) agents.

Detection of the signal is carried out by spectrophotometric or colorimetric measurement in the enzyme linked immunoassays: the enzyme label on the unbound antibody produces a signal in the presence of a specified substrate that is added to develop the color. When using luminescent or fluorescent labels, luminescence or fluorescence detectors should be applied.

5.2.7 **Biological microelectromechanical systems for characterizing microbial activity**

Biological micro-electro-mechanical systems (bioMEMS) are miniaturized biosensors used for rapid testing of biomolecules that are indicative of an upset process due to bulking, foaming or disadvantageous changes in the microbial population of the activated sludge. BioMEMS are very similar to lab-on-a-chip (LOC) and micro total analysis systems (µTAS), but strictly for biological applications. The mini mechanical sensors mechanically detect stress or mass through micro- and nano-scale cantilevers or micro- and nano-scale plates or membranes. Bending of the cantilever is caused by the biological process on one side of the cantilever and is measurable either optically or electrically. Electrical and electrochemical detection is possible by amperometric, potentiometric or conductometric sensors, based on the changes in redox, or electric potential as well as impedance caused by the biochemical reactions.

5.2.8 **Handheld advanced nucleic acid analyzer for detecting pathogens**

The handheld advanced nucleic acid analyzer (HANAA) can be used for real-time detection of pathogens in water and wastewater by relying on a polymerase chain reaction (PCR). This technique allows for a small amount of DNA to be amplified exponentially. Commercially available HANAA are Bio-Seeq (2015) and RAZOR (2015) (originally developed for bioterrorism monitoring purposes). HANAA is a portable/handheld design, otherwise equivalent with a laboratory thermal cycler.
5.3 Toxicity measuring biosensors

The majority of the possible toxicants in wastewaters are not included in monitoring programs so they remain unknown and uncontrolled in many cases, causing significant long-term health and environmental risk due to discharging treated wastewaters into surface waters. Toxicity affects not only receiving waters but the wastewater treatment plants themselves. Incoming toxic wastes lower the treatment capacity of the microbes and to compensate it, the treatment plants must either be larger or use more energy. A rapid, reliable test method for incoming waste increases the stability and efficiency of the wastewater treatment technology. For toxicity measurement some of the equipment uses pure microorganism cultures, activated sludge from a wastewater treatment plant or even GEMs, which recognize the presence of specific environmental pollutants.

5.3.1 Respirometry based toxicity measuring methods

Respirometry is the primary method for the detection of toxicity, as toxicants inhibit the biodegradation capacity of the sludge microorganisms, which is indicated by reduced respiration rate. The oxygen consumption can be measured electrochemically by an oxygen electrode or optically with an optrode, using optical fibers as signal transducer (read more in Quevauviller et al., 2006). Online applicable respirometers have already been introduced in Section 5.2.2. Toxiguard is a commercially available automated respirometer for toxicity evaluation which sounds an alarm when the oxygen concentration is higher (not consumed by the micro-organisms) than a preset value. Toxalarm (2015) uses standard bacterial culture to produce a toxicity result in the water within minutes. Rapid oxygen demand for toxicity (RODTOX) assessment from Kelma (2015) is recommended both for rapid BOD and toxicity.

5.3.2 Microtox and online Microtox

Microtox is based on a bacterial whole-cell biosensor. The measured end point in the patented Microtox® method is the luminescence of the marine bacterium *Aliivibrio fischeri* (strain, NRRL B-11177). Indigenous bioluminescence significantly decreases due to the effect of toxicants. The water samples are added to the standardized bacterial culture and the decrease in light intensity is compared to the negative control. The test is standardized in many countries and also by ISO (2007a,b,c). The ASTM D5660 96 (2014) standard was withdrawn in 2014. The measurement can be carried out online or offline. It can provide near real-time information on water and wastewater toxicity. Microtox CTM (Continuous Toxicity Monitor) is a fully automatic instrument that offers a four-week, autonomous operating cycle and requires a low level of skill for both operation and maintenance. It produces real-time toxicity results without manual intervention except for monthly maintenance (Modern Water, Microtox®, 2015). Laboratory and portable versions are available.

5.3.3 Toxicity testing methods and equipment – commercially available devices

Microtox (2015) is not the only commercially available rapid toxicity test based on *Aliivibrio fischeri* luminescence: ToxAlert® 101 (Merck) and LUMIStox (1999 and 2010) from Hach-Lange are similar in applicability to Microtox as published by
Figure 3.13 The SciTOX electrochemical sensor detects the signal of an artificial electron-acceptor which captures the electrons from the energy-producing process of the microorganisms (SciTox, 2015).


Toxicity monitoring in wastewater treatment plants primarily serves the protection of the activated sludge microbes. For the purpose of measuring toxicity, the respiration or other activities of the local sludge microbiota can be used. Alternatively, stable quality pure or mixed microbial cultures can also be applied, similar to those which are used for normal chromotests or luminotests. An abridged list is as follows:

- The Eclox™ luminometer – a handheld design – applicable both for the chemiluminescence toxicity test and the luminescent bacteria test to measure luminescent light inhibition of water samples. The Eclox luminometer is designed for field use. Together with the LUMIStherm thermoblock and the corresponding software, in situ toxicity measurements are possible (Eclox handheld luminometer, 2015).
- SciTOX™ ALPHA is a patented technology for the measurement of toxicity in sewage and in wastewater treatment plants. The complete assay requires only fifteen minutes including sample incubation. It is effective for a wide range of inorganic and organic toxicants. The system uses indigenous bacteria with no importation requirements, and the reagents are simple to prepare (see Figure 3.13). The respiration of the indigenous bacteria is not measured via oxygen consumption, but rather the wireless electrochemical (amperometric) sensor detects the signal of the reduced electron-acceptor of potassium ferricyanide. The artificial electron acceptor, added to the reaction mixture as an indicator, captures the electrons produced by the microorganisms. When the catabolic activity of the microorganism is inhibited by toxicants, the signal of the reduced electron-acceptor is smaller. Due to the high solubility of potassium ferricyanide, the toxicity analysis can be completed in less than 20 minutes, assuming that the inoculum is ready to use and checked. The inoculum is checked by a toxicant of known effect (3,5-dichlorophenol) on wastewater treatment plant microorganisms (SciTox™ ALPHA, 2015).
- **POLYTOX-RES** (2015) is an automated real-time monitoring of the overall toxicity present in water in one hour’s time. It works based on respirometry with a dissolved oxygen sensor. The programmable frequency of analysis covers 10 to 20 tests per day. The equipment can be applied as an early warning system and for capturing samples exceeding certain limits for later analysis. Data logging at, and transmission to, remote locations is possible by telemetry.

### 5.4 Online analyzers and electrodes for the water phase in wastewater treatment

The practice requires reliable, simple and low-maintenance sensors for continuous monitoring and control of the wastewater treatment in order to meet effluent quality objectives. The routine online monitoring of several parameters of the water phase has already been solved and applied in wastewater treatment plants. The parameters monitored are the temperature, pressure, pH and redox potential, conductivity, dissolved oxygen concentration, liquid level, flow rate, nutrients such as $\text{NH}_4^+$, $\text{NO}_3^-$, TOC, BOD, respiration rate, and toxicity. Some other sensors for measuring wastewater parameters or components are still under development or need further development, for example the measurement of chemical oxygen demand (COD) or phosphate concentration. The online applications are based on membrane technologies, UV spectrometry or fluorescence detection and ion-selective sensor techniques. The measuring techniques may be intermittent, continuous flow-through systems (e.g. flow injection analysis, FIA), or sequential injection analysis (SIA). Compared to batch, FIA and SIA have the advantage of small sample size, low reagent use and high sample throughput (Vanrolleghem & Lee, 2003). The processes of nitrification/denitrification and phosphorus removal are critical in wastewater treatment and require intensive and thorough monitoring and control. These innovative methods provide real-time or near real-time monitoring data in the wastewater treatment system, making immediate feedback, timely process adjustments and corrective actions possible if a shock or toxic load occurs.

The most important applications of the online measuring methods and instruments are the monitoring and control of nitrification, denitrification and phosphorus removal. The target molecules of ammonium (converted to ammonia), nitrate, orthophosphate and total phosphorus are analyzed based on colorimetry or ion-selective electrodes. Some commercially available *in situ* real-time measuring devices are briefly introduced below. See references for detailed information.

#### 5.4.1 Real-time measurements based on colorimetry

Colorimetry, the most conventional analysis method, has been developed to make it suitable for real-time application. Some of the commercially available analyzers are listed below:

- **Alert colorimeter** is an analyzer for ammonia, nitrate, nitrite, or phosphorus. A differential technique is applied for compensating fouling and initial sample color (Metrohm-Applikon, 2015a).
- **Trescon analyzer** measures orthophosphate by colorimetry using the vanadate/molybdate method (yellow product), and total phosphorus after a chemical-thermal digestion with the molybdenum blue method (WTW, 2015a).

- **ChemScan** UV process analyzers are online, single or multiple parameter analyzers using full-spectrum, UV-visible detection with chemometric analysis of spectral data. Multiple sample lines allow sampling from several locations to the same analyzer. Nitrate and nitrite are directly analyzed from the spectra of the sample. Ammonia analysis is reagent-assisted using bleach and hydroxide reagents (ASA Analytics, 2015).

- **AMTAX** ammonia analyzer takes samples automatically in every 5 to 120 minutes (arbitrary set). The sample is mixed with sodium hydroxide to convert all ammonia to free ammonia; ammonia gas is then expelled from the sample, redissolved in the indicator reagent and the color measured with a colorimeter (Hach AMTAX, 2015).

- **PHOSPHAX** is a continuous flow analyzer with a five-minute cycle time for ortho-phosphate using the photometric methods with vanado-molydan (Hach PHOPHAX, 2015).

- **NitraVis® system** is a UV/VIS spectrometer probe for in situ, real-time spectral measurement of nitrate concentration without filtering. Turbidity is detected and compensated for. Automatic cleaning is solved with compressed air before each measurement (WTW, 2015b).

5.4.2 **Real-time measurements based on ion-selective electrodes (ISE)**

In wastewater treatment, ion-selective electrodes are most frequently used for ammonia and nitrate analysis, but electrodes are also available for sodium, potassium, calcium, chloride and fluoride.

- **Direct immersion ISE sensors:**
  - for ammonium and nitrate: Varion® Plus 700 IQ;
  - for ammonium with potassium compensation: AmmoLyt® Plus;

- **Myratek Sentry C-2 electrode**, based on ISE technology, continually measures ammonia and nitrate levels in the treatment process. Calibration is performed automatically at user-set intervals (Biochem, 2015).

- **AISE sc ISE ammonium probe** provides continuous measurement by direct immersion. Potassium interference is compensated by including a potassium ISE. Optional air cleaning ammonium, nitratesystem may reduce maintenance frequency (Hach ISE, 2015a).

- **AN-ISE sc combination sensor** for ammonium and nitrate provides reliable measured values and considerably reduces maintenance time and costs compared to conventional ISE probes. It is equipped with a cartridge sensor cartridge and automatic cleaning unit with a compressor (Hach ISE, 2015b).
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- **ISEMax CAS40** is a potentiometric ion-selective electrode system for the continuous measurement of ammonium and nitrate (Endress, 2015).
- **Alert Ion Analyzer ADI 2003** is a potentiometric ISE either for ammonium, calcium, chloride, fluoride, nitrate, potassium or sodium (Metrohm, 2015b).

### 5.4.3 Voltammetry for trace metal monitoring

In voltammetry, information about an analyte (toxic metals) is obtained by measuring the current as the potential is varied. The metal ions are drawn onto the working electrode when a specific voltage is applied to the water sample under test. In stripping voltammetry for the plating step, the potential is held at an oxidizing potential, and the oxidized species are stripped from the electrode by sweeping the potential positively. When the stripping voltage is applied, the metals return to the sample solution, generating a small current. Each metal has a specific voltage at which it returns to solution. So the metal is identified by its stripping voltage, and the current generated indicates the concentration of metal in the sample. The stripping step can be either linear, staircase, square wave, or pulse. Some equipment useful in wastewater treatment based on voltammetry are introduced below:

- **PDV 6000 portable analyzer** measures trace metals in water, soil and food. Voltammetry offers a generally accepted alternative to laboratory analysis or automatic samplers in dissolved metal analysis. The sensors provide an easy way to generate and store real-time data, which in turn allows real-time decision-making. The sensor can be used as a stand-alone device or logged to a computer with the VAS software according to purpose. The PDV6000 ultra version is equipped with a standard analytical cell, which can detect a wide range of different metals. An extra accessory is the SV LabCell that allows cathodic stripping voltammetry (CSV) in the same PDV analyzer. The SV LabCell extends the PDV’s range of metals to include molybdenum and uranium, and it also gives a better response for nickel, cobalt and chromium at low concentrations. Color or turbidity does not affect the method. Dirty water and soil samples may require simple on-site sample preparations to prevent interferences. Further advantages are: multiple, sequential metal analysis is possible and the detection limits may be below 1 ppb (PDV 6000, 2015).

- **TraceDetect Nano-Band™ explorer** is a metal analyzing system which works with stripping voltammetry and includes the Nano-Band instrument and Explorer software to operate the instrument. It measures trace metals in aqueous solutions: ppm measurements instantly, ppb measurements in seconds, and ppt measurements in minutes. The same equipment can be used for anodic and cathodic stripping voltammetry, cyclic and square wave voltammetry, potentiometric stripping analysis, amperometry, chronocoulometry. The improved version of Explorer II is an on-site applicable system with TriTrode™ electrode technology. This unites all three system electrodes into one piece: the Nano-Band working electrode, the reference electrode and the auxiliary electrode, resulting in simpler maintenance (Nano-Band, 2015).

- **Lead SA1100** (2015) scanning analyzer based on voltammetry, represents a step forward in real-time testing of lead and copper in water. It is a robust, portable instrument, including a disposable electrode which can quickly and accurately detect the presence and concentration of lead and copper.
5.4.4 Real-time measurement of chemical oxygen demand (COD)

Photoelectrochemical oxygen demand (PeCOD™) technology can measure photocurrent charge originating from the oxidative degradation of soluble organic substances. The extent of electron transfer at a TiO₂ nanoporous film electrode is measured during the exhaustive photoelectrocatalytic degradation of organic matter in a thin layer photoelectrochemical cell. It overcomes the problems of other rapid COD determinations, i.e. partial oxidation due to matrix effect. The sensor produces an objective value and no calibration is necessary. The PeCOD method has been validated by comparing it to the standard dichromate method: good agreement was achieved. It is robust, rapid (0.5-5 min/measurement), simple to use, and easily automated. Long sensor life, high sensitivity and a wide linear range characterize the sensor. Real-time soluble COD monitoring enables efficient process control and waste management (Zhao et al., 2004). Some products are commercially available, such as the CONDIACELL.

CONDIACELL (2015) for industrial wastewater treatment uses an electrochemical advanced oxidation process (EAOP®), a technology which is nowadays used for wastewater treatment, too. EAOP® reduces all organic water components by approximately 99%. The high oxidation potential of hydroxyl radicals ensures good efficiency by non-selective oxidation of any kind of organic substances to carbon dioxide. Hydroxyl radicals produce a ‘cold incineration’ of the organic components. DIACHEM diamond electrodes are used for this rapid electrochemical COD/TOC determination.

5.5 Real-time and online methods for controlling the solid phase in wastewater treatment

In wastewater treatment the most important phase is the solid phase, the activated sludge. Total suspended solids, sludge volume, settling velocity, sludge blanket level, and sludge density, are important technological parameters in controlling the performance of a plant. Laboratory-based methods are extremely time-consuming and thus cause long time delays. Innovative methods are based on in situ sensors establishing the way to automation.

A sludge blanket level detector integrates ultrasonic absorption and turbidity devices to detect the suspended solids interface as a result of the sudden change in sludge concentration when penetrating into the sludge blanket.

Settling velocity measurement applies a similar approach. In the settlometer of Vanrolleghem et al. (1996) the evolution of the blanket height is recorded with a moving optical detection system. From the resulting sludge sedimentation curve, the maximum sedimentation velocity and the sludge volume index can be obtained.

Sludge density can be monitored online by a microwave density analyzer. Solids flowing through pipes cause a phase lag of the microwave. The difference in microwave phase lag between the control wave and the one that passed through the fluid containing sludge is proportional to sludge density. The density meter measures density in electric current, so the signal can be directly applied for process monitoring and control. The measured phase difference is not affected by flow velocity and is resistant to the effects of contamination, scaling, fouling, and gas bubbles (US EPA, 2013).
**Floc size and size distribution** are the result of a dynamic equilibrium state between formation, transformation and breakage of the microbial aggregates. Floc parameters represent one of the most important parameters for characterization of the process performance and the influence of technological parameters such as substrate loading, sludge age or dissolved oxygen concentration (see more in Govoreanu et al., 2009). To model sludge dynamics and control nitrification/denitrification and settling of the sludge, *in situ* measurement of floc size and distribution is essential. A laser light diffraction technique has been developed for on-line monitoring of the changes in floc structure expressed as a fractal dimension (Guan et al. 1998). Biggs & Lant (2000) applied the laser light diffraction technique for direct observation of size distribution. De Clerq et al. (2004) successfully applied the focused beam reflectance to measure the floc chord length distribution *in situ* in a secondary clarifier of a wastewater treatment for a wide range of solids concentrations, up to 50 g/l.

### 6 AUTOMATED INSTRUMENTS FOR CONTINUOUS MONITORING OF TOXIC ELEMENTS IN SURFACE, GROUND- AND WASTEWATER

The assessment of surface waters and effluents of municipal landfills and of abandoned mining sites with associated waste dumps requires continuous control of their toxic metal contents. The most widespread method for determination of metal concentrations in surface, ground and wastewater is via grab sampling and subsequent laboratory analysis. This method is both costly and typically involves a 24 hour turnaround time, which means that pollution events might be missed or detected too late.

Electrolyte-cathode discharge (ELCAD) spectrometry was invented as a direct analytical detection method for dissolved metals in aqueous solutions (Cserfalvi et al., 1993). This analytical method was used to develop an automatic instrument for monitoring heavy metals in wastewaters loaded with high fat emulsion and suspended solid contents like municipal sewage waters mixed with industrial wastewaters (GREENWW1, 2014). Further developments have improved the sensitivity to make the method useful for the measurement of toxic metals in groundwater, contaminated surface water and sediment.

#### 6.1 Principle of ELCAD

The measuring principle is cathode sputtering wherein the cathode is the electrolyte solution (the water sample) to be measured. The main unit is a flow-through cell in which atmospheric direct-current glow discharge plasma is generated emitting light with specific wavelengths which are characteristic of heavy metal contamination. The emitted light is processed by a built-in spectrometer unit.

The resulting optical emission spectrum of this plasma is very simple, containing only the basic atomic lines of the metals and background molecular emissions from the water matrix and the air atmosphere. The atomic lines of metals dissolved in the solution appear immediately in the spectrum emitted by the ELCAD. In this way the concentrations of metals in a sample can be determined within a few minutes. The plasma receives only the cathode-sputtered components and is therefore not
disturbed or interfered with the non-sputtered components such as suspended solids and emulsions.

The different systems and designs developed in the past 20 years have recently been reviewed (Jamroz et al., 2012).

Time-programmed sampling of the sewerage water is done by a submerged pump through a coarse pre-filtering (5x5 mm) net. An approximately 20 L sample is pumped to a raw water vessel in the monitor device. An innovative self-cleaning rotating-slit filter (Cserfalvi, 2011) operates in this vessel which removes particles that are larger than 200–300 µm from the analytical sample stream of 10 mL/min pumped in by the monitor unit. The only sample treatment is a controlled acid addition to the sample to solubilize metals present in the form of suspended particles of hydroxides, sulfides and carbonates, or they are partly complexed and bound to the suspended organic particles. The sampling (= measuring) frequency can be as high as 3–6 measurements/hour. It depends mostly on the level of the suspended and organic load of the water stream (flushing of the sample lines).

6.2 In situ applications

The ELCAD instrument can be operated on-site, installed in a measuring station or in mobile laboratories. An early industrial demonstration of the performance of the ELCAD method was done on the inflow stream in the North-Pest Wastewater Treatment Plant. It revealed sporadic but high metal pollution peaks occurring at midnight and weekends on mornings (Mezei & Cserfalvi, 2007). After several demonstrations (Figure 3.14) the recent field test now runs on the inlet flow stream of the Municipal Wastewater Treatment Plant of Székesfehérvár, Hungary. ELCAD is able to detect illegal industrial metal releases into the municipal sewerage as well as fluctuations in the metal content of effluents from landfills, abandoned industrial and mining sites. It is capable of monitoring around and above the regulatory limits. It can trigger a regular sampler device for the standard laboratory measurement providing an early warning.

Figure 3.14 Testing the ELCAD instrument in Malta and its application in Bohumin Steelworks (CZ) (Cserfalvi, 2014).
Recent developments (introduction of the capillary technique) resulted in highly improved detection limits (LOD). LOD depends on the chemical nature of the metal. It is as low as 28, 14, 22, 34 and 28 g/L for Zn, Cd, Cu, Ni, Pb, respectively (Cserfalvi & Mezei, 2003). The LOD for some other toxic metals such as Cs, Sr, Ag, Au and Hg 211, 49, 5, 78 and 349 µg/L, respectively, were also published (Webb et al., 2005). By applying additives (surfactants or organic acids), further improvement in sensitivity for various metals, for example mercury (2 µg/L), was achieved (Shekhar, 2012; Zhang et al., 2014). These developments are the basis for the construction of a metal monitor for ground and surface waters.

6.3 Advantages and disadvantages

The high-sensitivity laboratory methods (Inductively Coupled Plasma spectrometry – ICP and Atomic Absorption Spectrometry – AAS) can be applied for measuring concentration values of low ppb levels, but they require thorough sample preparation. In most cases the sample has to be colloid-filtered and heavily acidified. Some type of nebulization technology (pneumatic, electrospray, ultrasonic, etc.) is necessary for the introduction of the sample. In addition, these instruments require auxiliary gases (ICP: argon, AAS: acetylene) for the operation. ICP has very significant energy demand with its 1–3 kW high-energy excitation unit. The most important distinction is that these methods cannot be applied for in situ monitoring.

Advantages of ELCAD:

– in situ/on-site, continuous monitoring technology;
– long-term stability of autonomous operation;
– no specific reagents or rare gases are used, only HCl is added;
– high suspended solid content and even oil and fat emulsion contents are allowed in the sample, because it only measures components dissolved at pH 1.5–1.7;
– low environmental footprint (low power consumption);
– low investment/maintenance cost.

The disadvantages of lower sensitivity compared to the high-sensitivity laboratory methods (ICP and AAS) are overcome by simpler operation, and the provision of real-time results.

7 CONCLUSION

This chapter provided an overview of the in situ and real-time monitoring methods useful for the characterization of surface and groundwater as well as of wastewater. The main advantages of the in situ real-time measurements, the fast response and undisturbed sample compensate for the usually lower sensitivity compared to the laboratory-based water analytical techniques in assessment, technology monitoring, and in decision-making both at global and regional level. The online real-time analysis in process control, e.g. in wastewater treatment, increases the efficiency of the technology.
The principles of local and remote sensing are discussed showing several examples of geophysical, hydrogeological, geochemical, biochemical, biological, ecological, ecotoxicological, etc. sensing tools.

Real-time water quality monitoring of surface waters and oceans is useful as early warning and makes possible immediate intervention and avoidance of damage to the aquatic ecosystem. The recently developed sensors autonomously measure some biological, chemical and optical parameters. Innovative analytical tools, bioassays, and biosensors are used for measuring the microbial quality and activity, the biodegradable organic compounds, and toxicity in wastewater. The online analyzers based on colorimetry and the ion-selective electrodes provide data for continuous monitoring and control. A recent innovation applying electrolyte cathode discharge (ELCAD) spectrometry is used for continuous control of toxic metal content even in municipal sewage water mixed with industrial wastewaters.

Many of the methodological developments have achieved practical implementation in the form of commercially available mobile equipment, portable or handheld devices. The mobilization of the environmental analytical tool system enables on-site environmental assessment and decision making, which, in turn, results in dynamic and efficient environmental risk management.

In addition to this review of monitoring tools for the aquatic domain, the next chapter covers the methods and tools applicable for soils, primarily for subsurface soils.

REFERENCES


In-situ and real-time measurements in water monitoring


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**Photos:**


