A set up of a modern analytical laboratory for wastewaters from pulp and paper industry

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The introduction of analytical techniques allowing rapid, selective, sensitive, and reliable determination of aqueous pollutants is of crucial importance for the protection of the environment. This *critical review* summarizes the advanced analytical techniques suggested over the last ten years together with already established methods, and evaluates whether they are fit for wastewater quality assessment considering the area of application, interferences, limit of detection, calibration function, and precision. The key parameters of wastewater quality assessment are: total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), organochlorines (AOX), nitrogen, phosphorus, sulfur, and toxicity. Chromatography and capillary electrophoresis, photocatalytic oxidation with semiconductor nanofilms and atomic emission spectrometry, optical fibre sensors and chemiluminescence, amperometric mediated biosensors and microbial fuel cells, respirometry and bioluminescence measurements are just part of the proposed wastewater analyst's toolkit. The diversity of fundamental phenomena and the captivating elegance of interdisciplinary applications involved in the development of wastewater analytical techniques should attract the interest of a wide scientific audience including analytical chemists, chemical physicists, microbiologists and environmentalists. To conclude, we suggest a laboratory set up for the analysis of wastewaters from the pulp and paper industry.

1 Introduction

Adequate control and monitoring of the pollution level in industrial wastewaters that are going to be discharged into receiving water bodies is a matter of supreme environmental importance. The responsibility for carrying out adequate wastewater quality assessments is laid on the industrial wastewater analytical laboratories which have to choose the methodologies and analytical equipment to be able to provide reliable, selective, sensitive, rapid and cost-efficient determination and quantitation of aqueous pollutants. This choice is an extensive task because of the diversity of chemical, physicochemical and microbiological phenomena, and interdisciplinary applications involved, which makes wastewater analysis an extraordinarily complex discipline for those trying to acquire a coherent understanding. To decide whether the considered instrumentation will be suitable for such a laboratory, a comparison of the analytic performance on certain analytes and a cost analysis of the equipment provided by the various manufacturers do not suffice alone, but an assessment based on adequate understanding of the underlying

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analytic principles and on the properties of wastewater samples is required.

The goal of this review is to give guidelines for setting up a modern analytical laboratory for wastewaters from pulp and paper industry. This task incorporates defining the priority analytes, obtaining the necessary analytic literature, providing a clear and simple picture of methods that should and could be used, and suggesting the instrumentation set up.

Our intention is to draw attention to the variety of methods and problems of industrial wastewater analysis in all their complexity, and to awaken interest in this most challenging and interdisciplinary area of research—as well as in its importance for environmental protection. The diversity of fundamental phenomena and the captivating elegance of interdisciplinary applications involved in the development of wastewater analytical techniques should attract the attention of a wide scientific audience including analytical chemists, chemical physicists microbiologists and environmentalists.

1.1 Scope

The period surveyed is the last 10 years. The focus is on the determination of total organic carbon (TOC), chemical oxygen demand (COD), chlorinated organic matter measured as adsorbable organic halides (AOX), nutrients, sulfur, biochemical oxygen demand (BOD) and toxicity. When possible, we concentrate on the analytic methods tested on pulp and paper wastewaters, but consider also some promising techniques applied to other industrial wastewaters and municipal wastewaters. To avoid endorsing any commercially available analysers and protocols, neither comparisons of analytic performance nor cost analyses of equipment by different manufacturers are given; the provided information is limited to the analytic principles, general schemes of the instruments and analytic performance reported in the scientific literature. Commercial brands mentioned in this review as well as comparisons between their performances refer only to the equipment specified in the literature.

1.2 Pulp and paper industry wastewaters: origin and composition

Pulp and paper production requires large amounts of water, but most of the water used in pulp mills conforming to modern standards is recycled. A typical figure for wastewater quantity from a kraft pulp mill is 30–50 m^3 water per tonne of pulp, or about 70000 m^3 of effluent per day.[1]

The main sources of water pollution from pulp and paper industry are wood debarking and pulp bleaching. The major water pollutants contain wood polymers, fillers, process and auxiliary chemicals and their reaction products in the form of suspended solids, colloidal and dissolved matter. Some compounds discharged from mills show toxic effects on aquatic organisms. Toxicity is principally caused by chlorinated lignin-based compounds such as phenols, catechols, guaiacols and aromatic hydrocarbons, and by tannins, resin and fatty acids from wood.

At a discharge point, total concentrations of resin acids may vary between 2–30 $\mu g\ l^{-1}$ and of sterols between 11–45 $\mu g\ l^{-1}$. A debarking plant consumes water and creates effluents containing sand, pieces of bark and wood fibres. Water-soluble components of wood bark consisting of polymeric tannins, tannic and non-tannic phenol monomers, alkaloids, carbohydrates and resin compounds which include long chain fatty acids, resin acids, apolar phenols and volatile terpenes, and phosphorous and nitrous compounds are released to water.[3] Concentrations of wood extractives in discharged wastewaters from kraft pulp mills and mechanical pulp mills vary between 0.4–11 kg per tonne of pulp and are removed with 95% efficiency by biotreatment.[2] The major part of these compounds is discharged as particulate matter. Organochlorine discharges from pulp and paper mills have been significantly decreasing over the last 10–15 years, owing to the withdrawal of elemental chlorine and to the development of bleaching technology. However, effluents from a bleach plant where chlorine dioxide is used (elemental chlorine-free bleaching) still contain some organically bound chlorine compounds.

The discharge of coloured substances dims the light coming into water thus causing an adverse effect on aquatic life. The organic matter dissolved from pulp in the refining stages of paper production is referred to as "generated in a paper mill" and its amount corresponds to about 2–10 kg per tonne of paper produced. Organic chemicals applied as additives or auxiliaries in papermaking, *e.g.* cationic starches, contribute significantly to the organic load. Calcium carbonates and kaoline used as coating additives contribute to the inorganic pollution. Metals such as calcium, sodium, magnesium, and small amounts of copper extracted from the wood are discharged in low concentrations, but due to high flows, the load can be of significance. The total solid content of dissolved matter in the effluent is usually 0.2–0.6%. Phosphorus and nitrogen are the nutrients that cause eutrophication when discharged in excess into aquatic ecosystem. Nitrogen and phosphorus compounds mainly originate from their necessary addition for ensuring the efficient operation of a biological treatment plant. Moreover, optical brighteners may contain organic-bound nitrogen and thus contribute to the nitrous water pollution. Reduced sulfur compounds (sulfides, thiosulfites and thiols) are formed in the kraft pulping process and come to water from process vents and from tanks with spills, and may contribute to oxygen depletion of receiving waters.

To conclude, wastewaters, especially those originated in pulp and paper industry, are of the most heterogeneous nature. Being a blend of hundreds of different compounds—organic and inorganic, neutral, acidic and basic, oxidisable and non-oxidisable, stable and unstable, toxic and non-toxic, in suspended, colloidal and dissolved forms, where species can react with each other and compete for added analytical reagents thereby causing interference matrix effects—they present considerable difficulties for analysis.

As the speciation and quantitation of all individual compounds found in wastewaters would be neither feasible nor reasonable in a daily routine, various summation parameters of water quality are used. In the analysis of final effluent discharges from paper mills, the most common quality parameters include total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), chlorinated organic matter measured as adsorbable organic halogen (AOX), total nitrogen and phosphorus (total N and

Table 1 Typical pollutant concentration ranges in effluents from modern pulp and paper mills (recalculated from ref. 1)

	COD ($mg\ l^{-1}$)	BOD ($mg\ l^{-1}$)	TSS[a] ($mg\ l^{-1}$)	AOX ($mg\ l^{-1}$)	Total N ($mg\ l^{-1}$)	Total P ($mg\ l^{-1}$)
Bleached pulp mills	200–575	7–40	15–40	<7	2–7	0.2–0.8
Recovered fibre paper mills with de-inking	166–333	4–16	8–25	<0.4	4–8	0.4–0.8
Integrated mechanical pulp and paper mills	125–3125	12–31	12–38	<0.07	2–7	0.2–0.7

[a] Total suspended solids

P), and biotoxicity. Dissolved salts are measured by electrical conductivity. Because the summation parameters do not provide information about the nature of individual compounds, chromatographic methods such as high-performance liquid chromatography and gas chromatography are used when the speciation of *e.g.* chlorinated organic matter or toxic wood extractives is required.[4–7] Typical values of the main summation parameters contained in effluents from modern pulp and paper mills are presented in Table 1.

1.3 Analytical meaning of the key wastewater parameters

Total organic carbon (TOC). Measuring TOC content is the most straightforward and rapid way to assess the content of organic matter in a wastewater sample. TOC correlates directly to the carbonaceous concentration regardless of the oxidation state of the organic compounds or the presence of reducing chemicals. At the same time, this non-selectivity towards oxidizability of organic matter becomes a weakness of the TOC parameter as it provides no information on the nature of organic pollutants whatsoever.

Chemical oxygen demand (COD). COD is defined as the amount of specific oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of oxygen equivalence. The principal idea of the COD test is that virtually all organic matter can be chemically oxidized to carbon dioxide and water. In a typical case, the purpose of this test is to assess the amount of organic pollutants in a sample, but it also measures the amount of oxidizable inorganic matter at the same time. For example, reduced sulfurous, nitrous and phosphorous species and iron(II) contribute to COD. If measuring either organic or inorganic COD alone is desired, additional procedures are required. From the ecological point of view, COD is a measure of the oxygen-depletion effect of waste contaminants on the water body and it indicates the content of both rapidly and slowly degradable organic matter present.

Chlorinate organic matter (AOX, EOX and TOCl). Total organic chlorine (TOCl) is the sum of all chlorinated organic compounds. Traditionally, TOCl is the result obtained by a procedure including sorption on XAD resin, Schöniger flash combustion of the eluate, and potentiometric titration of the resultant chloride.[8] AOX is an analytic convention representing the sum of organically bound chlorine (also bromine and iodine) which can be adsorbed on activated carbon under specified conditions; expressed as chloride. AOX yields no information about the structure or nature of the organic compounds to which the chlorine is bound, or about the degree of substitution (number of chlorine atoms per organic molecule). High AOX values suggest the need to identify and quantify the specific compounds. Solvent extractable organic halogens (EOX) represent mainly the non-polar fraction of the organochlorine. Purgeable organic halogens can be measured separately in the AOX test and thus quantify the volatile chlorinate organic fraction. Currently, in the pulp and paper industry, the total content of chlorinated organic matter in the waste water is typically measured by AOX analyses. However, the adequacy of AOX, EOX and TOCl in the assessment of chlorinated organic content in effluents is the subject of an ongoing discussion.

Total nitrogen and phosphorus. In wastewaters the forms of nitrogen of greatest interest are, in the order of decreasing oxidation state: nitrate, nitrite, ammonia, and organic nitrogen. Organic nitrogen is defined functionally as organically bound nitrogen in the trinegative oxidation state. Kjeldahl nitrogen is a sum of organic nitrogen and ammonia and can be determined by the Kjeldahl analytic method. Total N is a sum of nitrite, nitrate, ammonia and organic nitrogen. Phosphorus occurs in wastewaters in the form of phosphates. These are classified as orthophosphates, condensed phosphates, and organically bound phosphates. Organic phosphates may be formed from orthophosphates in biotreatment processes, or by the receiving water biota.

Sulfur. Hydrogen sulfide, thiols and thiosulfites are reduced forms of sulfur in wastewaters. Sulfur dioxide and sulfate ions are oxidized forms of sulfur. Apart from being odorous pollutants, reduced sulfur compounds interfere in the BOD and COD tests and thus their determination could be required. The measurement of total sulfur in a liquid sample is used in *e.g.* the petroleum industry and, in principle, could be applied to wastewaters. However, no summation parameter is defined any longer for sulfurous compounds in wastewaters.

Biochemical oxygen demand (BOD). Bacteria inhale oxygen and exhale carbon dioxide. BOD measures the rate of O_2 uptake by micro-organisms in a sample of water at a fixed temperature and over a given period of time that is needed to break down the organic matter present in the sample, thus assessing the concentration of biodegradable organic matter. The quantity of the respirated oxygen is expressed in terms of oxygen equivalence ($mg\ O_2\ l^{-1}$). There is no absolute BOD value for a sample; BOD results are defined by the test conditions. It is possible to determine BOD either directly by measuring O_2 or indirectly by measuring CO_2, since a molecule

of O_2 is converted into a molecule of CO_2. Dilution methods measure dissolved oxygen initially and after the incubation, and BOD is computed from the difference between the initial and final dissolved oxygen. Respirometric methods use CO_2 and measure the change in pressure. Because one mol of O_2 has the same volume as one mol of CO_2, no change in pressure occurs in the process unless the CO_2 is removed from the gas-phase resulting in a measurable negative pressure. Resembling COD, BOD indicates the oxygen depletion effect of wastewater with the difference that BOD reflects only the rapidly degradable organic matter present and nitrogenous demand unless nitrification is suppressed in the BOD test. In addition, reduced sulfur and phosphorus could contribute to BOD values. For a specific wastewater stream, the correlation between these parameters can be established so that *e.g.* TOC or COD could predict biodegradability of wastewater. In addition, biodegradable dissolved organic carbon (BDOC) measures the dissolved organic carbon decrease after incubation with micro-organisms for a period of 10–30 days.[9] A TOC analyser is generally used.[10]

Toxicity. Acute or chronic toxicity of wastewaters to aquatic biota or to activated sludge is assessed by a variety of bioassays and it is expressed in IC_{50} (or EC_{50}) or in LC_{50} units. IC_{50} is the half maximum efficiency inhibitory concentration, representing the concentration of a toxin that is required for the 50% inhibition of certain vital activities, *e.g.* the inhibition of bioluminescence. LC_{50} is the measure of concentration for a toxin at which 50% of the members of an exposed population die from exposure. Acute toxicity is related to a single exposure to a toxic substance, whereas chronic toxicity is related to a continuous exposure to a toxin over an extended period of time.

2. Analytical methods

The current legislation in Europe requires that pulp and paper mills monitor their emissions to water and air. The number of existing national and international standards for wastewater analysis is vast. To harmonise the wastewater monitoring practices in Europe, the Technical Committee 147 Water quality of the International Standardisation Organisation ISO and the Technical Committee 230 of the European Standardization Organisation CEN have already published appropriate standards or are working at it, respectively. An overview of the wastewater analytic methods in use within the European Union is given in the *IPPC Reference Document*.[1] Selected standard methods that should be consulted in Europe and in the USA are listed in Table 2. This chapter summarizes and discusses the already established and recently suggested analytical methods aimed at the determination of TOC, COD, organochlorines, sulfur, nitrogen and phosphorus, BOD and toxicity.

Sample handling and processing. The way in which the handling and processing of wastewater samples is carried out is very important. Sampling is done according to the intended analysis. Some methods allow direct injection of the sample, others require elaborate preparation.

Such important sample preparation procedures as *e.g.* solid phase preconcentration, digestion in a microwave oven, and removal of inorganic carbon or inorganic chlorine by acidification are described as part of the analytic procedure where required in the corresponding sections. Unless otherwise mentioned, the samples are handled according to the general rules of wastewater sample storage and filtration, and any further details are described as necessary in the original papers.

2.1 TOC determination

2.1.1 Established methods. In TOC analysis, the following fractions of total carbon (TC) in water are distinguished: total organic carbon and total inorganic carbon (TIC). TIC needs to be removed before TOC is measured, or subtracted from TOC after being measured separately. TOC, *i.e.* all the carbon atoms covalently bonded in organic molecules, comprises dissolved organic carbon and suspended organic carbon. TOC can also be divided further into purgeable (also volatile) organic carbon and non-purgeable organic carbon. These are, respectively, the fractions of TOC removed and not removed from a water sample by gas stripping under specified conditions. TOC analysis is based on the conversion of all organically bound carbon to the low molecular form of carbon dioxide (or methane), which is then quantified. An overview of current TOC measurement methods can be found in ref. 11,12. There are two principal oxidation techniques in TOC analysis: high-temperature combustion (600–1200 °C) and low temperature oxidation (below 100 °C) with UV-irradiation, heated persulfate or UV/persulfate.

Quantification of the resultant CO_2 can be carried out by a variety of methods: *e.g.* infrared spectrometry, thermal conductivity, acid–base titration, CO_2-sensitive electrode, and flame ionisation after reduction of CO_2 to methane; infrared detectors are mainly used. In most modern high temperature combustion analysers, the sample is homogenized and diluted as necessary and a micro portion is injected into a heated reactor packed with an oxidative catalyst such as platinum, cobalt oxide or barium chromate. The water is vaporized and the organic carbon is oxidized to carbon dioxide which is transported in the carrier gas stream and measured by a non-dispersive infrared analyser specifically tuned to the absorptive wavelength of CO_2. The instrument calculates the area of the peaks produced by the analyser, compares them to the peak area of the calibration standard stored in its memory, and prints out a calculated organic carbon value in mg l^{-1}. Inorganic carbon is separated by acidification. Typical combustion temperatures are 680–950 °C. Heated-persulfate instruments utilize a digestion vessel heated to 95–100 °C. Samples are added by direct injection, loop injection, line injection, or an auto sampler. After the inorganic carbon is removed by acidification and sparging, a measured amount of persulfate solution is added to the sample. After an oxidation period, the resulting CO_2 is sparged from the solution and carried to an infrared analyser. The instruments converts the detector signal to organic carbon concentrations in mg l^{-1} based on the stored calibration data. In the analytic scheme of some TOC analysers, the formed carbon dioxide is then

Table 2 Selected standard methods for water quality determination

Parameter	ISO standard	Standard methods[14] by APHA[a], AWWA[b] and WEF[c]
TSS	**ISO 11923:1997** Water quality—Determination of suspended solids by filtration through glass-fibre filters	**2540 D** Total suspended solids dried at 103–105 °C
Electrical conductivity	**ISO 7888:1985** Water quality—Determination of electrical conductivity	**2520 B** Electrical conductivity method
TOC	**ISO 8245:1999** Water quality—Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)	**5310 B** High-temperature combustion method **5310 C** Persulfate–ultraviolet or heated-persulfate oxidation method
COD	**ISO 6060:1989** Water quality—Determination of the chemical oxygen demand **ISO 15705:2002** Water quality—Determination of the chemical oxygen demand index (ST-COD)—Small-scale sealed-tube method	**5220 B** Open reflux method **5220 C** Closed reflux, titrimetric method **5220 D** Closed reflux, colorimetric method
AOX	**ISO 9562:2004** Water quality—Determination of adsorbable organically bound halogens (AOX)	**5320 B** Adsorption–pyrolysis–titrimetric method
Sulfur	**ISO 10530:1992** Water quality—Determination of dissolved sulfide—Photometric method using methylene blue	**4500-S^{2-} D** Methylene blue method
	ISO 13358:1997 Water quality—Determination of easily released sulfide	**4500-S^{2-} E** Gas dialysis, automated methylene blue method **4500-S^{2-} F** Iodometric method **4500-S^{2-} G** Ion-selective electrode method **4500-S^{2-} I** Distillation, methylene blue flow injection analysis **4500-SO_3^{2-} B** Iodometric method
Nitrogen and Phosphorus	**ISO 10695:2000** Water quality—Determination of selected organic nitrogen and phosphorus compounds—Gas chromatographic methods	**4500-P J** Persulfate method for simultaneous determination of total nitrogen and total phosphorus
Kjeldahl nitrogen	**ISO 5663:1984** Water quality—Determination of Kjeldahl nitrogen—Method after mineralization with selenium	**4500-N_{org} B** Macro-Kjeldahl method **4500-N_{org} C** Semi-micro Kjeldahl method **4500-N_{org} D** Block digestion and flow injection analysis
Total nitrogen	**ISO 11905-1:1997** Water quality—Determination of nitrogen—Part 1: Method using oxidative digestion with peroxodisulfate	**4500-N B** In-line UV/persulfate digestion and oxidation with flow injection analysis
	ISO/TR 11905-2:1997 Water quality—Determination of nitrogen—Part 2: Determination of bound nitrogen, after combustion and oxidation to nitrogen dioxide, chemiluminescence detection	**4500-N C** Persulfate method
Total phosphorus	**ISO 15681-1:2003** Water quality—Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA)—Part 1: Method by flow injection analysis (FIA)	**4500-P E** Ascorbic acid method
	ISO 15681-2:2003 Water quality—Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA)—Part 2: Method by continuous flow analysis (CFA)	**4500-P F** Automated ascorbic acid reduction method **4500-P H** Manual digestion and flow injection analysis for total phosphorus **4500-P I** In-line UV/persulfate digestion and flow injection analysis for total phosphorus
BOD	**ISO 5815-1:2003** Water quality—Determination of biochemical oxygen demand after *n* days (BOD_n)—Part 1: Dilution and seeding method with allylthiourea addition	**5210 B** 5-day BOD test
	ISO 5815-2:2003 Water quality—Determination of biochemical oxygen demand after *n* days (BOD_n)—Part 2: Method for undiluted samples	**5210 C** Ultimate BOD test
	ISO 10707:1994 Water quality—Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds—Method by analysis of biochemical oxygen demand (closed bottle test)	**5210 D** Respirometric method
	ISO 10708:1997 Water quality—Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds—Determination of biochemical oxygen demand in a two-phase closed bottle test	
Toxicity	**ISO 11348-2:1998** Water quality—Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test)—Part 2: Method using liquid-dried bacteria	**8050** Bacterial bioluminescence
	ISO 9509:2006 Water quality—Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms	**8712** Ceriodaphnia
	ISO/CD 20665 Water quality—Determination of chronic toxicity to *Ceriodaphnia dubia* toxicity test.	

[a] American Public Health Association. [b] American Water Works Association. [c] Water Environmental Federation.

diffused through a silicone membrane and absorbed by a weakly buffered phenolphthalein indicator. The decrease in the colour of the indicator measured as light absorbance at 550 nm is proportional to the carbon concentration. Some instruments utilize an UV lamp submerged in a continuously gas-purged reactor that is filled with a constant feed persulfate solution. The samples are introduced serially into the reactor by an auto sampler or they are injected manually. The produced CO_2 is sparged continuously from the solution and carried in the gas stream to an infrared analyser. Other UV-persulfate instruments use continuous-flow injection of the sample into the instrument. Removal of inorganic carbon by vacuum degassing is provided optionally. The sample is acidified and persulfate added. The sample flow is split; one channel passes to a delay coil while the other passes through the UV reactor. Wallace *et al.*[13] discuss the instrumentation of high temperature combustion and low temperature persulfate oxidation techniques, compared the analytic performance of the Phoenix 8000 and the Apollo 9000HS analysers and provide the flow path charts of the instruments.

Interlaboratory studies have shown biases of the order of 1 mg l^{-1} when using high-temperature combustion instruments.[14] Detection limits as low as 10 μg l^{-1} have been reported with more recently produced instruments. Some high-temperature combustion instruments are not designed for levels below 1 mg l^{-1}.

Solid particles, chlorides and organic compounds that are hard to oxidize interfere with UV/persulfate TOC methods. Low-temperature persulfate and UV/persulfate oxidation based methods are recommended for wastewaters with low organic content up to 10–50 g l^{-1}, whereas high-temperature combustion is targeted for more concentrated wastewaters containing these kinds of interference matrices.[14,15] Wallace and Furlong[16] point out the applicability of an improved UV/persulfate approach even to such difficult samples as those containing tannic acids and lignosulfonic acids. In their test, a modern UV/persulfate analyser yielded the recovery of lignosulfonic acid 96–98% at concentration 1–50 mg l^{-1}, which was as good as the recovery obtained with a combustion analyser. However, these low-concentration results are insufficient for extrapolating the obtained recoveries values to wastewaters rich in ligneous and tannic compounds.

We conclude that the choice of TOC method for pulp and paper industry wastewaters is high-temperature combustion.

2.1.2 Suggested methods. Kraatz and Farjam[17] compare the performances of a photometric cuvette-test and Shimadzu TOC-5050 catalytic combustion analyser. They conclude that TOC results obtained with the cuvette-test system are comparable with the results of the analyser. Simple instrumentation using a photometer and a dry thermostat included in the TOC cuvette-test system is illustrated therein.

Flow injection turbidimetric determination of TOC with a gas–liquid transfer microreactor[18] and with a gas-diffusion/flow injection system coupled with a bulk acoustic wave impedance sensor (GD-FIA/BAW)[19] have been suggested. In the first procedure,[18] the sample is decomposed in a microwave oven at 850 W. The sample aliquot (CO_2) is injected into the flow injection instrument with distilled water as the carrier solution and barium hydroxide as the absorbing solution. In the extraction side chamber there are three exits: gas purge, liquid drainage, and sampler, where a part of the formed barium carbonate suspension is taken and conducted to the flow cell for the turbidimetric determination (at 410 nm) in a UV-VIS spectrophotometer equipped with a quartz flow cell. In the other procedure,[19] a sample pre-treated by acidification with HCl, undergoes a wet chemical oxidation, and is then purged with nitrogen under heating to remove CO_2. The CO_2 diffuses across a gas-permeable membrane from a stream of sulfuric acid and water merging into a stream of tris(hydroxymethylamino)methane containing KCl. The CO_2 trapped in the acceptor solution is determined on-line by a bulk acoustic wave impedance sensor and the signal is proportional to the TOC content.

In the measurement of carbon atomic emission intensity by inductively coupled plasma atomic emission spectrometry (ICP-AES), the organic matter is not preoxidised.[20] A semi-automatic accessory connected to the spectrometer separates the different carbon fractions (*i.e.* organic and inorganic). A schematic diagram for TOC measurements with ICP-AES is shown in Fig. 1. The detection limit is 0.07 mg l^{-1} TOC and the maximum sample throughput is about 50 determinations per hour with a precision of about 1–10%. The linear calibration range is up to 1000 mg l^{-1} C. This method features the possibility of obtaining information about TOC and the concentration of heavy metals simultaneously. However, the method applies only to non-volatile organic compounds.

2.2. COD determination

2.2.1 Established methods. In most standard procedures, potassium dichromate in acidic solution is used as the oxidant. A sample is refluxed for 2 hours in strong sulfuric acid solution with a known excess of potassium dichromate. In the

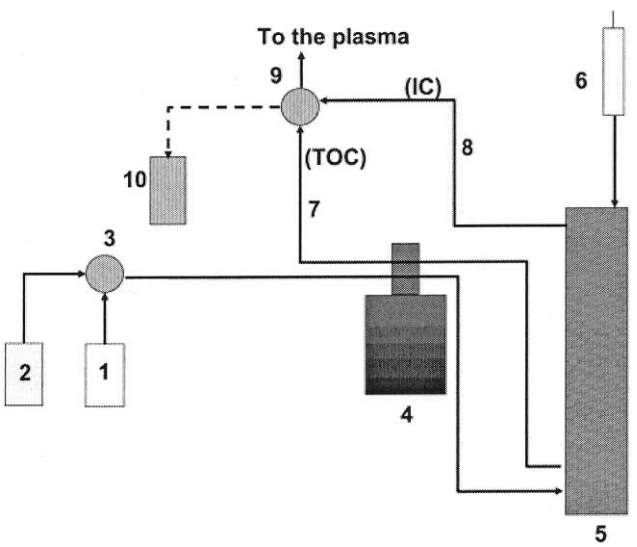

Fig. 1 A scheme of the ICP-AES for TOC analysis: (1) sample reservoir; (2) hydrochloric acid reservoir; (3) valve; (4) peristaltic pump; (5) reactor; (6) rotameter; (7) liquid conduction; (8) gas conduction; (9) valve, and (10) waste reservoir. (Reproduced with permission from ref. 20. Copyright 2003 American Chemical Society.)

oxidation of the sample, dichromate is reduced to a chromic ion state. After the oxidation, the remaining unreduced dichromate, or the reduced Cr^{3+}, is measured either by titration or spectrophotometrically. The oxidation is catalysed with silver nitrate. Because of its high concentration in most wastewaters, chloride is often the most significant source of interference. The reaction with potassium dichromate follows the equation:

$$6Cl^- + Cr_2O_7^{2-} + 14H^+ \rightarrow 3Cl_2 + 2Cr^{3+} + 7H_2O$$

In addition, chloride precipitates the silver catalyst.

Mercury sulfate is currently used to eliminate chloride interference by formation of mercuric chloride complex. Nitrite, sulfides and ferrous ions also cause interference. Thus, hazardous wastes of mercury, hexavalent chromium, silver, and acids are generated in the standard COD analyses.

Kumaresan and Riyazuddin[21] present an overview of the COD determination methods including interference arising from chlorides, non-oxidized volatile aliphatic compounds, nitrites, hydrogen peroxide, reduced inorganic species (such as ferrous iron, sulfides, manganous manganese and ammonia), titrimetric and spectrophotometric detection in dichromate reflux methods, electroanalytical methods, flow injection analysis and COD by empirical relation to suspended solids.

The measurement uncertainty of COD determinations in wastewater with the standard reflux method is discussed by Drolc *et al.*[22] They conclude that volumetric operations are the prevailing source of uncertainty at low concentration, while the measurement procedure is the dominant source of uncertainty in the COD results in wastewater samples at high concentration levels.

2.2.2 Suggested dichromate methods. A miniaturized closed reflux colorimetric method allowing reagents savings of 80% is proposed by LaPara *et al.*[23] The linearity range is 0–900 mg l^{-1}, and precision 10%. The possibility of a closed tube COD test without mercury is pointed out by Morrison.[24] Moreover, it is suggested that chloride interference could be suppressed without added mercury by utilizing the readily present species of silver and Cr^{3+} in the mixture. Originally, silver sulfate was added to the COD test to catalyse the oxidation of volatile carboxylic acids. Soon it was noticed that excess silver suppresses the chloride interference. Silver nitrate could be added to samples to precipitate chloride prior to COD digestion and a close agreement to the standard reflux method using mercury was obtained.[25] A small addition of Cr^{3+} could suppress chloride interference *via* the complexation of free chloride ion by Cr^{3+}.[26]

Jianhui *et al.*[27] propose a modified dichromate reflux method using sulfuric acid and phosphoric acid mixed solution with $Mn(H_2PO_4)$ as catalyst. The reflux time is 5 min. No silver catalyst is needed and the amount of mercury is 25% of the standard method. The cost of test decreases by 85%. The relative error for the samples of chemical fibre mill cotton pulp effluents is in a good agreement with the standard method.

Dan *et al.*[28] suggest a modified dichromate test based on an indirect determination of the excess Cr^{6+} by single sweep polarography. Digestion is done in a mixed acid; neither mercury nor silver is used. The reflux time is 15 min. After cooling, gelatine is added and the sample placed into an electrolytic cell. The derivative linear seep polarogram is recorded and the COD calculated from the peak height. For analytic performance details of the method, refer to Table 3.

Microwave radiation is another trend towards optimising the dichromate reflux procedure.[29–32] Cuesta[29] applied microwave radiation to assist the dichromate–sulfuric acid digestion in the flow injection method. Anionic exchange resin retains the excess dichromate, which, upon elution, is determined by atomic absorption spectrometry. The details are presented in Table 3.

Beltra *et al.*[30] developed an automated device utilizing microwave assistance of digestion which reduces the digestion time to 8 min. After digestion, the reaction mixture is pumped out of the microwave oven, cooled and filtered. A spectrophotometer measured the absorbance of generated Cr^{3+} at 590 nm. The device features a specific clean-up programme using 3 M ammonia. The analytic performance of method is described in Table 3. The automatic device is applicable to wastewaters with great variability of COD values, chloride concentrations and hard-to-oxidize organic substances.

Ramon *et al.*[31] compares the results of standard COD methods with the microwave assisted digestion. A constant power strategy and a constant temperature approach is applied. The digestion time is 3–9 min and temperature control recommended. The results are comparable with the standard reflux method.

Dharmadhikari *et al.*[32] propose microwave digestion in completely closed, transparent Teflon vessels. The samples are exposed to microwave power of 600 W for 15 min. After digestion, the excess dichromate is titrated with standard ferrous ammonium sulfate using ferroin (1,10-phenanthroline) as the indicator. The details are presented in Table 3.

Canals *et al.*[33] propose that ultrasound assists the digestion process in the conventional semi-micro method. The mixture for sonication is made by mixing the sample, a digestion reagent (dichromate in sulfuric acid) and an acid reagent (sulfuric acid and silver sulfate). After the 2 min sonication, the remaining dichromate is titrated with ammonium sulfate using ferroin sulfate as the indicator. In comparison, the obtained COD values lie between 50% and 60% of values obtained by the conventional semi-micro method and the precisions are comparable. Due to the simplicity of instrumentation, the sonochemical approach has promising potential to become the direction for optimization of digestion in COD analysis.

The closed microwave, open microwave and ultrasound assisted digestion methods have been recently compared by Domini.[34] The duration of ultrasound was 1 min using a glass sonotrode of special design. The recoveries were close to the values obtained by the open reflux method. Despite the advantages of the microwave and ultrasound-assisted digestion over the conventional COD methods, mercury and silver are still needed in all cases. Relative standard deviations of COD measurements are within 4%. In all cases, interference concentrations that produce a deviation of 10% in COD values are 13 mg sulfide l^{-1}, 23 mg nitrite l^{-1}, 21 mg Fe^{2+} l^{-1}, and 2819 mg chloride l^{-1}.

Table 3 Analytical performance of some methods suggested for TOC, COD, AOX, reduced sulfur and BOD

	Method	Detection level [mg l^{-1}]	Linearity range [mg l^{-1}]	Precision [std. deviation, %]	Sample throughput [h^{-1}]	Single analysis time [min]
TOC	Flow injection turbidimetric method with a gas-liquid transfer microreactor[18]	17	20–80	5	120	
	Gas-diffusion-FIA[a] with a bulk acoustic wave impedance sensor[19]	0.12	0.6–240	0.95	45	
COD	Closed microwave digestion procedure[32]	5	5–1000	0.61–8.33		15 (for digestion)
	Automatic microwave assisted device[30]		0–15000	0.4–8.3	5	
	Mixed-acid digestion and single sweep polarography[28]		20–1100	1.5		15 (for digestion)
	Direct photoelectrochemical method with a TiO_2 nanoparticle film electrode[40]	0.20	0.20–200	0.87		15
	Photocatalytic oxidation method using nano-TiO_2 film in the presence of dichromate[39,41]	20	20–500	1.0–3.3–8.8		40 (for irradiation)
	FIA with permanganate cation-exchange and chemiluminescence system[56]	2	4–4000	4.4–4.8	40	
	Dichromate chemiluminescence method[55]	2.1	2.1–700	6		90 (for digestion)
	FIA with microwave digestion and chromium speciation with FAAS[b29]	7–13.5	25–170, 100–850, 430–1900, 1700–4700, 2100–12000	4.1–8.6–13.8	50	
	Amperometric determination of chemical oxygen demand with FIA using F–PbO_2 modified electrode[52]	15	100–1200	5	—	seconds
	Amperometric method using a rotating Pt ring–Pt/PbO_2 disc electrode[53]	15	20–25000	1		seconds
AOX	Glow discharge atomic emission spectrometry with on-line continuous generation of chlorine[67]	0.00014	up to 5	1.0–4.3	—	
Reduced sulfur	Headspace solid phase microextraction followed by gas chromatography/pulsed flame photometric detection[76]	0.000012	up to 0.002	10		5 or 60 (for exposure)
	Automated flow-through method with Fischer's coupling and optode detection of generated methylene blue[87]	0.0029	0.002–0.2	0.7	8	
BOD	Respirometric method with manometric pressure sensor OxiTop®		0–4000	12	6–12 samples in parallel	5 days (incubation time)
	Micredox®120		19–150	15		60 (incubation time)

[a] Flow injection analysis, [b] Flame atomic absorption spectrometry

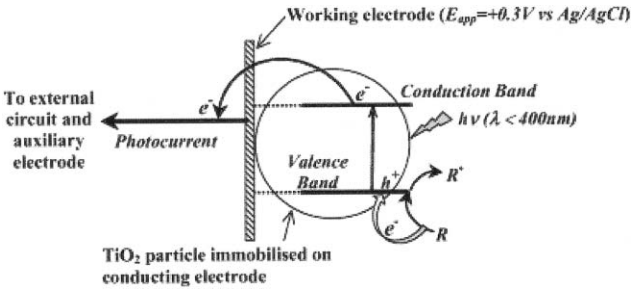

Fig. 2 The principle of photocurrent generation with TiO_2. (Reproduced with permission from ref. 37. Copyright 2004 ELSEVIER.)

According to Vaidya,[35] chloride interference could be removed by bismuth-based adsorbents.

2.2.3 Suggested photoelectrochemical methods. Due to their special photocatalytic, optical and electronic properties, nanostructured semiconductor oxides are attracting increasing attention of scientists working in different areas. In our opinion, oxidation using semiconductors is the most interesting approach to take in the development of COD analysis. Below we summarize the main results obtained with this approach.

The principle of photocurrent generation on TiO_2 film is schematically presented by Chen[36] and Zhang[37] (Fig. 2). When TiO_2 nanoparticles are irradiated by UV light, they generate h^+/e^- pairs that migrate to the solid surface. The valence band holes are powerful oxidants. For information on the morphology and surface properties of such films, the reader is directed to ref. 33,36,38.

A set up of a flow injection analysis system for COD determination with TiO_2 photocatalytic electrode and Ag/AgCl reference electrode is descibed by Chen[36] (Fig. 3). The linear working range 0.5–235 mg O_2 l^{-1} may not be sufficient for the pulp and paper industry wastewaters. The liability to chloride, sulfide and oxygen interferences is yet to be overcome.

Photocatalytic oxidation in a nano-TiO_2–$K_2Cr_2O_7$ system and the direct determination of Cr^{3+} resulting from the

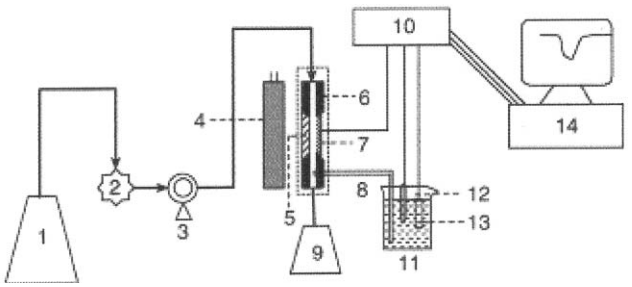

Fig. 3 A schematic diagram of the FIA system for COD determination with TiO_2 photocatalytic electrode: (1) flow carrier reservoir; (2) peristaltic pump; (3) injector; (4) UV lamp; (5) quartz window; (6) photocatalytic cell; (7) photocatalytic electrode; (8) salt bridge; (9) wastewater tank; (10) CHI660A electrochemical station; (11) $Fe(NO_3)_3$ solution; (12) Ag/AgCl reference electrode; (13) Pt counter electrode; (14) computer. (Reproduced with permission from ref. 36. Copyright 2004 ELSEVIER.)

photocatalytic oxidation of organic substances and simultaneous photocatalytic reduction of dichromate in the solution by absorbance measurements has been studied.[38] If a dichromate is present in the solution, it acts as an acceptor of photogenerated electrons and is reduced to chromic ion (Cr^{3+}).

The analytic performance of several photocatalytic oxidation methods featuring TiO_2[39–41] is presented in Table 3.

To enhance the oxidation efficiency and to minimize the degradation time, photoelectrochemical catalytic degradation of organic matter is carried out in a thin-layer photoelectrochemical cell.[40] This process is analogous to bulk electrolysis in which all of the analytes are electrolysed and the measured net charge becomes a direct measure of the total amount of electrons resulting from the complete photocatalytic mineralization of all compounds in the sample. This method is simple, direct and provides an absolute COD quantification.[49] It also avoids the problems associated with the use of oxygen as electron scavenger in the recent COD methods based on TiO_2. The analytical linear range of 0.2–360 mg O_2 l^{-1} with the practical detection limit of 0.2 mg l^{-1} is achieved using the photoelectrochemical method with TiO_2 film electrode and a thin-layer photoelectrochemical cell.[37] The COD results are also in good agreement with those of the standard COD method, which could allow the application of this system to treated wastewaters.

Kim[42] presents a flow method using photocatalytic oxidation in a solid-phase TiO_2 reactor with amperometric detection of the consumed oxygen. This sensor has a detection limit of 0.5 mg O_2 l^{-1} for COD_{Cr}.

Our attention was caught by the mentioning of an ongoing work on the modification of this sensor with Fenton's reagent.[42] Fenton's reagent is a solution of hydrogen peroxide and an iron catalyst that is used to oxidize difficult organics and chlorinated organics in wastewaters.[43–46] The idea of Fenton's reagent is based on the generation of free hydroxyl radicals. In acidic medium ferrous iron(II) is oxidized to ferric iron(III) by hydrogen peroxide to a hydroxyl radical and a hydroxyl anion. Iron(III) is then reduced back to iron(II) by the same hydrogen peroxide disproportionation to a peroxide radical and a proton:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\bullet} + H^+$$

The presence of iron is truly catalytic in the net reaction and two molecules of hydrogen peroxide are converted into two hydroxyl radicals and water. This makes Fenton's reagent environmentally acceptable as no hazardous side products are formed. The hydroxyl radicals may react with organics starting a chain reaction:[47]

$$OH^{\bullet} + RH \rightarrow H_2O + R^{\bullet}, \text{ RH-organic pollutant}$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \rightarrow \text{organic degradation products}$$

The oxidation potential of hydroxyl radical exceeds even the oxidation potential of ozone, as pointed out by

Beltran-Heredia,[48] who studied the kinetics of Fenton oxidation of phenolic compounds in pulping wastewaters.

In our opinion, since Fenton's reagent is already used for the oxidation of organic and chlorinate organic pollutants in pulp and paper mills' effluents, and because it is environmentally acceptable, it has potential to become an oxidant or to assist the oxidation in the COD test.

2.2.4 Suggested electrochemical methods. Electrochemical complexometric titration using differential pulse anodic stripping voltammetry by estimating complexing capacity of organo-Cu^{2+} complexes in terms of COD is reported by Mohan.[49] This method features a deposition time of 5 min, pulse height of 50 mV, pulse duration of 57 ms, and time 0.5 s.

The differential pulse anodic stripping voltammetric technique consists of adding aliquots of Cu^{2+} solution to the sample and measuring the peak until the slope of the peak current increases.[50] Samples with COD ranging from 10 to 1500 mg O_2 l^{-1} have been measured with both the complexing capacity and standard COD methods, yielding 98–99.5% of standard COD values on industrial wastewater samples.

A trial with a surface-oxidized copper electrode for coulometric determination of electrochemical oxygen demand is reported by Lee.[51] A single measurement takes 30 min. The net Faradic charges obtained by exhaustive electrolysis in a cell with the electrode agree with COD values obtained by the standard methods. However, humic substances present in natural waters interfere and cannot be oxidized fully by this electrode. Presumably the hard-to-oxidize organic matter present in pulp and paper industry effluents would destabilize the work of this electrode.

An electrochemical detection system with an F-PbO_2-modified electrode for flow injection analysis has been suggested.[52] When the F-PbO_2 electrode generates $HO^{\bullet}$ radicals, organic compounds are electrocatalytically oxidized by the unoccupied surface sites and the electric signals are produced. The COD value is proportional to the current response of the working electrode. The analytic performance is detailed in Table 3. The working range makes this method also applicable to untreated effluents.

The application of multipulse amperometry at the rotating Pt ring–Pt/PbO_2 electrode to COD measurements is listed in Table 3, and the electrode scheme is shown in Fig. 4.[53] The calibration plot of the COD signal of the electrodes obtained on different organic compounds shows two different linear ranges: up to 500 mg O_2 l^{-1} and 500–5000 mg O_2 l^{-1}. The broadest working range of 20–25000 mg O_2 l^{-1} with precision of 1%, and measurement time 1 min (after the calibration) are the strongest characteristics of this method.

Gutes[54] applied a flow injection voltammetric electronic tongue specifically to wastewaters coming from a paper mill. The obtained results do not allow using the method as it stands for COD measurements, but it seems a promising approach to a quick and simultaneous assessment of COD, conductivity and pH.

2.2.5 Suggested chemiluminescence methods. A chemiluminescence method based on luminol–H_2O_2–Cr^{3+} reaction and emitted light detection by the photodiode has been proposed.[55]

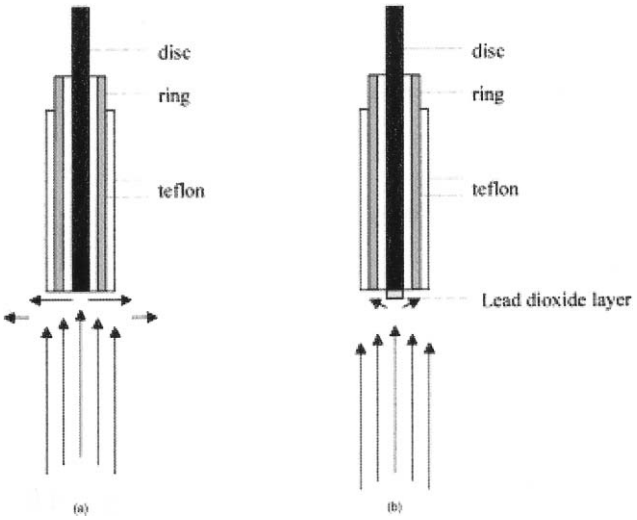

Fig. 4 A schematic view of the Pt-ring + Pt-disc electrode (a) and the Pt-ring + Pt/PbO_2-disc electrode (b) for COD determination. (Reproduced with permission from ref. 53. Copyright 2001 ELSEVIER.)

After oxidation, the appearance of Cr(III) proportional to the COD is determined by a photodiode luminometer system:

$$Cr^{3+} + \text{luminol} + H_2O_2 \rightarrow hv^{+} \text{ production}$$

The signal of chemiluminescence output of the photodiode is amplified and recorded. Analytic performance is given in Table 3. The detection time is several seconds, accuracy and precision are acceptable as compared to the standard reflux method with titrimeric determination of chromium. The linear dynamic range is suitable for wastewaters.

In another, automated, method permanganate reduced to Mn^{2+} is measured by a chemiluminescence system in conjunction with the flow injection analysis.[56] First, Mn^{2+} is adsorbed on a cation-exchange resin mini-column to be concentrated during chemical oxidation of the organic compounds at room temperature, while the excess permanganate passes through the mini-column to become waste. Then the concentrated Mn^{2+} is reverse eluted and measured by the luminol–hydrogen peroxide chemiluminescence system. The analysis time is 1.5 min. The analytic performance is given in Table 3. The linear working range is well suited for pulp and paper mill wastewaters, even prior to biotreatment.

2.2.6 Other methods. Zuev[57] suggests a rapid method for the determination of total organic content in water by an automated solid electrolyte analyzer measuring thermal oxygen demand based on high-temperature oxidation in a flow of a binary mixture of oxygen and argon. The working range is 10–500 mg O_2 l^{-1} with the RSD between 1–15% for different organic compounds. Single analysis time is 5 min.

A patent by Kalia *et al.*[58] suggests COD determination with the help of a colour chart. One ml of sample is mixed sequentially with three reagents, consisting of mercuric sulfate, potassium dichromate and sulfuric acid–silver sulfate. The reaction mixture develops a colour (from yellowish to blackish

brown) which can be read and distinguished. Various diluted samples can be tested in increasing order of their dilution. A properly diluted mixture will show a colour between yellow and sea green, indicating the COD value in the range of 300 to 500 mg l^{-1}. Absorbance is measured at 585 nm and 635 nm, and correlated to the exact COD value.

For in-line monitoring, COD analysers using ozone as oxidant are commercially available. The dilution water is enriched with ozone, and both dilution water and wastewater enter the reaction chamber where oxidation occurs. The initial and residual concentrations of dissolved ozone are measured. The working ranges of such analysers are 10–1500 mg l^{-1} and 10–100000 mg l^{-1}, featuring measurement times of 3–15 min, and a reproducibility of 5%. For an analytical laboratory with low sample throughput, this analyser could not be recommended as a cost-efficient choice.

Storage techniques for COD are discussed by Nivens.[59]

2.3 Organochlorines

Lopez[60] and Odendahl[61] have reviewed the methodology for the determination of potentially bioaccumulating complex mixtures of organochlorine compounds in wastewater.

2.3.1 AOX. The standard method for AOX is the adsorption–pyrolysis–titrimetric method which includes the following steps:[14]

1. Inorganic chlorine is removed from the sample by acidifying and adsorbing the dissolved organic material onto activated carbon.

2. Inorganic chlorine present on the activated carbon is removed by displacement by nitrite ions.

3. The activated carbon and adsorbed organic compounds are burnt in an oxygen flow in a sealed environment at temperatures ranging from 800 to 1000 °C. Combustion yields carbon dioxide, water and hydrogen chloride.

4. The hydrogen chloride is transported in a carrier gas stream to a microcoulometric titration cell where it is absorbed by sulfuric acid solution and the amount of chloride is quantified by argentometric titration (*i.e. via* measuring the current produced by silver-ion precipitation of the chloride).

2.3.2 AOX analysers. All AOX analysers are based on the same basic principle of adsorption, oxidative pyrolysis and microcoulometric titration, but utilize different carrier gases and temperature, and have different working ranges. The adsorption is done either by adsorption column method or by shaker procedure.

Sullivan and Douek[62] discuss the methods and sample related problems in the AOX test. The effect of various analytical parameters on the AOX results was studied: shaking time, number of adsorptions, quantities of granular activated carbon and NO^{3-}, and the total volume of water used during adsorption of effluents from bleaching with elemental chlorine. AOX fractions were conducted on the effluents from bleaching with elemental chlorine and from alkali extraction stage in bleaching as well as on several model compounds. The results show that the shaker method recovers quantitatively high molecular weight AOX, but recovers poorly low molecular weight AOX. Considerably better recoveries of low molecular weight AOX can be obtained using the column method.

Torrades[63,64] discusses the detection and elimination of constant error component and interactive matrix interference in the AOX determination of adsorbable organic in bleached kraft paper pulp mill effluents and points out the need to use the total Youden blank test. Measurements were carried out in the presence of the matrix at two different levels of the test portion to avoid interactive interference.

Because the main interaction between the carbon surfaces and organic compounds are polar–non-polar interactions, polar compounds are weakly adsorbed onto activated carbon. For example, only 20% of chloroethanol is recovered in AOX analysis.

2.3.3 Organochlorines with other methods. Hemming and Holmbom[65] developed a TOCl method based on resin sorption and particle-induced X-ray emission for kraft pulp bleach plant effluents. The detection limit is 10 μg l^{-1} and the measured range is 20–1160 μg l^{-1} of TOCl.

Shintani[66] compares the measurements of chlorinated organics in bleaching effluents by the AOX and TOCl methods. AOX was measured with a Mitsubishi-Kasei TOX-10 analyser. In TOCl measurements, the chloride present in a sample was absorbed by a KBr solution and determined by ion chromatography. Another aliquot underwent adsorption on a filter paper and Schöniger combustion: the organic chlorine was converted into inorganic chloride, and the formed chloride was determined by ion chromatography using bromide as internal standard. TOCl was calculated as the difference between total chlorine and inorganic chloride from absorption. The AOX values are much lower than TOCl values, which is interpreted as unsuitability of the AOX parameter for the determination of chlorinated organic in elemental chlorine bleaching effluents. Nevertheless, AOX and TOCl methods give almost the same values for alkali extraction effluents, from which unstable organic chlorine has been already removed by the hot alkali treatment. Acid treatment in AOX procedure is named as another possible reason for discrepancy between TOCl and AOX results.

The need has pointed out to replace AOX test by the new EOXfob test which measures the most hydrophobic part of the extractable organic halogen (EOX) for the determination of organochlorine compounds in wastewaters, such as pulp mill effluents. The methodologies for EOX determination are thoroughly reviewed by Lopez.[60] The following advantages of the EOXfob test have been reported: no cleanup needed for the sample, direct contact to particulate matter, the ability to measure the potential bioaccumulation of unknown organic chlorine matter, directly releasable in effluents, originated from diverse chemical/biological degradation mechanisms, quickness, simplicity, sensitivity and low cost. The standard deviation of the method is 7% for laboratory samples and 30% for real effluents. The detection limit is 0.6 μg chloride l^{-1}.

A sensitive method using radio frequency glow discharge atomic emission spectrometry (rf GD-AES) with the detection in the near IR based on the evolution of molecular chlorine from chloride in an aquatic solution is suggested for the

determination of AOX in wastewater after the preconcentration and combustion steps to yield chloride ions from the organochlorides.[67] The detection limit is 0.5 ng ml^{-1}.

Oleksy-Frenzel[68] describes a method of differential AOX-analysis, *i.e.* the simultaneous determination of AOCl, AOBr and AOI by adsorption, combustion and ion-chromatography. The method shows a minimum detection level of 0.03 mg l^{-1}, with the recovery of 4-chlorophenol: working range of 5–100 μg l^{-1}, RSD 1.7%; with the ion chromatography calibration: working range of 0.1–2.0 mg l^{-1}, RSD 0.7%

Muna[69] analysed chlorinated phenols in river water using solid-phase extraction with a styrene–divinylbenzene adsorbent, capillary electrophoresis coupled with amperometric detection using a three-electrode system consisting of a diamond working electrode, a Ag/AgCl reference electrode, and a carbon rode auxiliary electrode. The linear dynamic range of the method is 0.02–150 μg l^{-1} for most chlorinated phenols. Diamond exhibits good electroanalytical performance with a low peak-to-peak noise (1 pA), a low and stable background current under the imposed electrophoretic conditions, and a sensitive, reproducible, and stable oxidation response for the chlorinated phenols over many days of use. Diamond outperforms all other bare metal and carbon electrodes in the amperometric detection of chlorinated phenols because of its resistance to deactivation and fouling. In our opinion, this method is very promising and should be tested on a wastewater samples.

To avoid the carbon dioxide formation, Lehnert[70] suggests using thermal desorption (instead of the combustion of loaded activated carbon) and detection by the atmospheric-pressure helium microwave-induced plasma-emission spectrometry after adsorption. The results of microcoulometric detection are available as absolute masses in micrograms. The spectrometric detection results are given as the output voltage and have to be calibrated using standard solutions. The detection limit of 0.2 μg chlorine is reported.

Murphy and McLoughlin[71] tested an optical sensor based on the mid-infrared spectroscopy, utilising a zinc selenide attenuated total reflectance element coated with amorphous fluoropolymer Teflon AF. The highly amorphous low hydroscopic polymeric coating with a large void volume concentrates the chlorinated hydrocarbons within the penetration depth of the waveguide/Fourier transmission infrared spectroscopy and excludes water from the spot. The linear responses are in the mg l^{-1} region and the detection limit 9 mg l^{-1}.

2.4 Sulfur determination

The chemical nature of reduced sulfur compounds makes them difficult to measure. In the pulping industry, reduced sulfur compounds are traditionally determined by purging the sample and analyzing the purged gas by gas chromatography using a sulfur-selective flame photometric detector. In the following sections we summarize the chromatographic and alternative methods.

2.4.1 Chromatographic methods. Jeyakumar[72] suggests a high-performance ion chromatographic method for the determination of sulfite, sulfate and thiosulfate in tannery wastewaters in the presence of common anions. Sulfite, sulfate and thiosulfate are separated isocratically on an anion exchange column using carbonate/bicarbonate solution as the mobile phase and measured by an electrochemical detector. The total sulfate equivalent of these species is then calculated. To reduce the background conductivity of the mobile phase, an anion self-regenerating suppressor is used. In another sample aliquot, all the reduced sulfur species are oxidized by ammonical hydrogen peroxide to sulfate and quantified as sulfate. The difference between the total sulfate equivalent from the first and the second step is attributed to the amount of sulfide. The detection limits for all the ions are 0.5 mg l^{-1} with reproducibility of 10%.

Miura[73] presents an ion-pairing chromatographic method for the determination of sulfide, sulfite and thiosulfate after the conversion of sulfide and sulfite into stable thiocyanate and sulfate, respectively. The sulfide is evolved from an acidified solution as hydrogen sulfide and collected in an alkaline mixture of cyanide and hydrogen peroxide. The sulfite is oxidized to sulfate with hydrogen peroxide in an alkaline medium. Thiocyanate (for sulfide) and thiosulfate are determined chromatographically with a photometric detector (220 nm) and sulfate (for the total of sulfite and sulfate) with a suppressed conductivity detector at 10 μS cm^{-1}. Common anions do not interfere up to 10 mM. The mobile phase of acetonitrile–water (6 : 94, v/v) with 15 mM tetrapropylammonium hydroxide and pH 5.0 was selected as optimal.

Hurse and Abeydeera[74] describe the quantification of sulfur compounds in wastewaters, namely of sulfite, thiosulfate and sulfide, by the combined liquid chromatography method. A sample of wastewater is derived with monobromobimane and separated on a high performance liquid chromatography system in conjunction with a fluorescence detector using the excitation and detection wavelengths of 380 nm and 480 nm, respectively. The recoveries of sulfide, thiosulfate and sulfite were within the range of 98–103%. The in-run relative standard deviations were 1.7% for thiosulfate, 2.1% for sulfite and 3.5% for sulfide.

A high-performance liquid chromatography method for analysis of disulfides and thiols with indirect fluorescence detection without derivatization has been proposed by Pelletier and Lucy.[75] Disulfides and thiols are separated in their native form by ion-pairing chromatography and detected by quenching of the highly fluorescent $Cd(HQS)_2^{2-}$ complex after on-line reduction with of the disulfides with tris(2-carboxyethyl) phosphine. The detection limits were within 2.6 mg l^{-1} (4.3 μM), dynamic working ranges up to 200 μM and the relative standard deviation within 5%. The recoveries were within 87–120%.

Lestremau[76] applied headspace solid-phase microextraction followed by gas chromatography/pulsed flame photometric detection for the analysis of sulfur compounds in industrial wastewaters. Polydimethylsiloxane/carboxen coated fibre was used. A better precision and lower detection limit could be achieved with an extended sampling time (60 min). The quantification of sulfur compounds in wine by the same headspace solid-phase microextraction and gas chromatography-pulsed flame photometric detection technique is reported by Fang and Qian,[77] who used the same Varian 3800 gas

chromatograph, which makes an interesting comparison. The quantification limits for most volatile sulfur compounds in wine are 0.5 ppb/500 ng l^{-1} (15 min extraction time). The sulfur compounds behave differently depending on the wine matrix, but recoveries greater than 80% are achieved for all the sulfur compounds on a polydimethylsiloxane/carboxen fibre.

Gholson[78] developed a methodology using a pulsed flame photometric detector, allowing direct injection of a sample into a gas chromatograph. Recoveries are greater than 80%, precision less than 15%. Calibration is done to the concentration of 20 μg l^{-1}.

2.4.2 Spectrometry. Arowolo and Cresser[79] describe an automated method for the determination of sulfide by gas–liquid separation and a gas-phase atomic absorption spectrometry. Sulfide ions react with 3M HCl and the formed H_2S is purged into a separator. The absorbance is measured at 200 nm with a deuterium hollow cathode lamp. The detection limit is 60 μg l^{-1}, repeatability within 3.3%, and linearity range up to 100 mg l^{-1}. The sample throughput is 20 samples per hour.

2.4.3 Capillary electrophoresis. Font[80] reports a sensitive and selective capillary electrophoresis method for the determination of sulfide in leather industry effluent.

Chen and Naidu[81] report results of sulfur speciation by co-electroosmotic capillary electrophoresis with direct and indirect UV detection at 214 nm. Sulfate, sulfite and HS– can be successfully separated using 20 mM phosphate electrolyte containing 0.75 M tetradecyltrimethylammonium bromide and 15% acetonitrile. The detection limit is 3–8 μM. However, chlorides and nitrites interfere with the determination of thiosulfite.

A rapid capillary electrophoresis (CE) method has been developed for the determination of thiosulfate, sulfide and sulfite species in spent fixing solutions during the electrolytic oxidation by Daunoravicius and Padarauskas.[82] The proposed method is based on the in-capillary derivatization of separated sulfur anions by mixing their zones with the iodine zone during the electrophoretic migration and direct UV detection of iodide formed. Separation time is less than 4 min. The method gives repeatability comparable to that obtained for sulfur anions using the conventional capillary electrophoresis technique.

Sullivan and Douek[83] developed a method for the determination of sulfide, thiosulfate, sulfate, sulfite in kraft liquors by capillary electrophoresis. They found that the quinone-type compounds present in black liquor catalyse the oxidation of sulfide and that the addition of reduced glutathione at concentration of 1 mg ml^{-1} completely stabilizes the sulfide, sulfite and thiosulfate for at least one hour, thus allowing for a quantitative analysis of the analytes.

Kokkonen[84] developed a capillary electrophoresis system allowing ion-specific separation and determination of thiosulfate, sulfate, oxalate, sulfite, hydrogen sulfide, and phosphate in the process water of a paper machine.

Hissner[85] reports the use of capillary electrophoresis to separate the anions, sulfate, sulfite, thiosulfate, thiocyanate and sulfide. The conductivity detection after an electrokinetic sample injection and the improvement of calibration linearity allows the determination of sulfur-containing anions with low limits of detection (8–50 μg l^{-1}).

Liang[86] developed a method of capillary ion electrophoresis with indirect detection for the simultaneous determination of the sulfur-containing anions $S_2O_4^{2-}$, $S_2O_3^{2-}$, SO_4^{2-}, SO_3^{2-}, and S^{2-} and other anions (Cl^-, Br^-, NO^{2-}, NO^{3-}, $(COO)_2^{2-}$, F^-, and PO_4^{3-}) in the corrosion process. The effects of pH, tetradecyltrimethylammonium hydroxide, chromate, 2-(n-cyclohexylamino)ethane sulfonate, calcium gluconate, and acetonitrile on the migration and resolution of the anions and the stability of sulfur-containing anions were systematically investigated. The detection limits, repeatability, and linearity for the anions were comparatively studied at 374, 274, and 254 nm, and the results show that 374 nm is the optimal length. The simultaneous multiwavelength detection at 374, 254, 214, and 195 nm can assist in confirming the identification of UV-absorbing anions.

2.4.4. Optical fibre sensor. The automated flow-through method based on Fischer's coupling reaction of sulfide with *N*,*N*-dimethyl-*p*-phenylenediamine in the acidic medium and Fe^{3+} as the oxidizing agent is proposed by Ferrer[87] (Table 2; Fig. 4 and 5). The methylene blue generated is measured in an

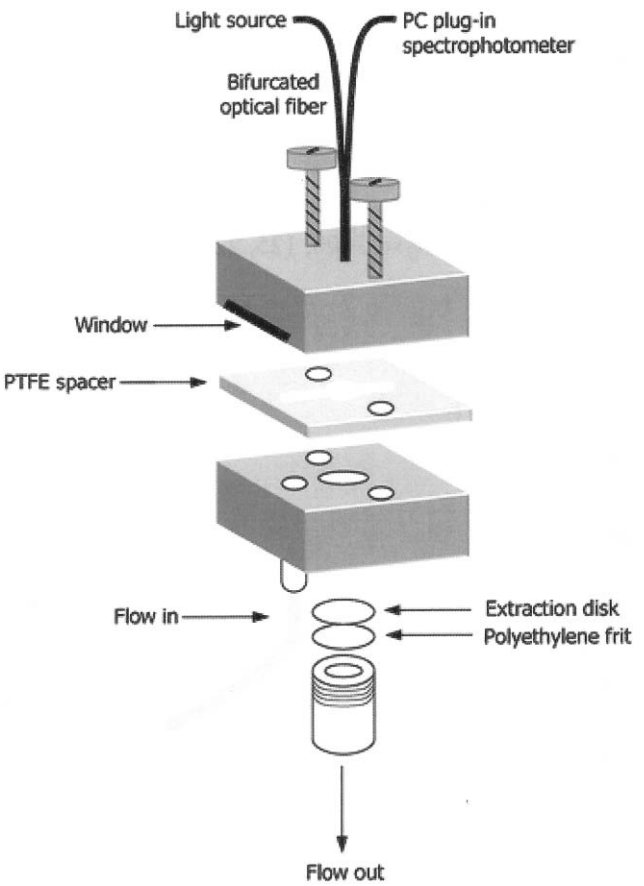

Fig. 5 A schematic view of the flow-through cell for disk-based solid-phase pre-concentration. Octadecyl covalently bonded silica gel disks are used as extraction membranes for sulfide pre-concentration. (Reproduced with permission from ref. 87. Copyright 2005 Royal Society of Chemistry.)

optode cell furnished with an octadecyl-chemically modified disk working as an extraction membrane for the sulfide preconcentration. The throughput is 8 samples per hour.

2.4.5. Total sulfur. In sulfur analysers used in the gasoline analysis, all sulfurous compounds in the sample stream are pyrolysed in an oxygen stream to be converted into SO_2 which is detected by *e.g.* a flame photometric detector based on a gas chromatograph. Ranges as low as 0–10 ppm could be measured.

Iodometric titration can be considered as another method for the determination of total sulfur, as it is realized in *e.g.* some AOX combustion analysers with a sulfur mode. The microcoulometric titration cell comprises a Pt Indicator electrode, a $Pt/I_2/I^-$ reference electrode, a Pt anode, a Pt cathode and a gas inlet. At the Pt anode the following reactions take place:

$$2I^- \rightarrow I_2 + 2e$$

$$I_2 + I^- \rightarrow I_3^-$$

$$SO_2 + I_3^- + 6H_2O \rightarrow SO_4^{2-} + 3I^- + 4H_3O^+$$

In our opinion, such a total sulfur determination method could be tested for applicability with pulp and paper industry effluents. A total sulfur analyser featuring high temperature combustion and a microcoulometric cell for iodometry (also available as a part of AOX analysers) may become an alternative instrument for determination of reduced sulfur compounds in pulping effluents. However, for the present, a gas chromatograph and a pulsed-flame photometric detector remain as the optimum instrumentation for the determination of sulfur compounds in pulping effluents.

2.5 Nitrogen and phosphorus determination

2.5.1 Nitrogen: established methods. The idea of total N determination is an oxidative conversion of ammonia, organic nitrogen and nitrite to nitrate, followed by a quantitation of the resultant and initially present nitrates. The persulfate and persulfate/UV digestion procedures are used.[14]

Nitrate could be quantified by UV-spectrophotometry, as it absorbs light at 210–230 nm.[88] Commercial automatic analysers based on this principle are available. Although computing the second derivative of a sample spectrum could eliminate the background contribution from light-absorbing

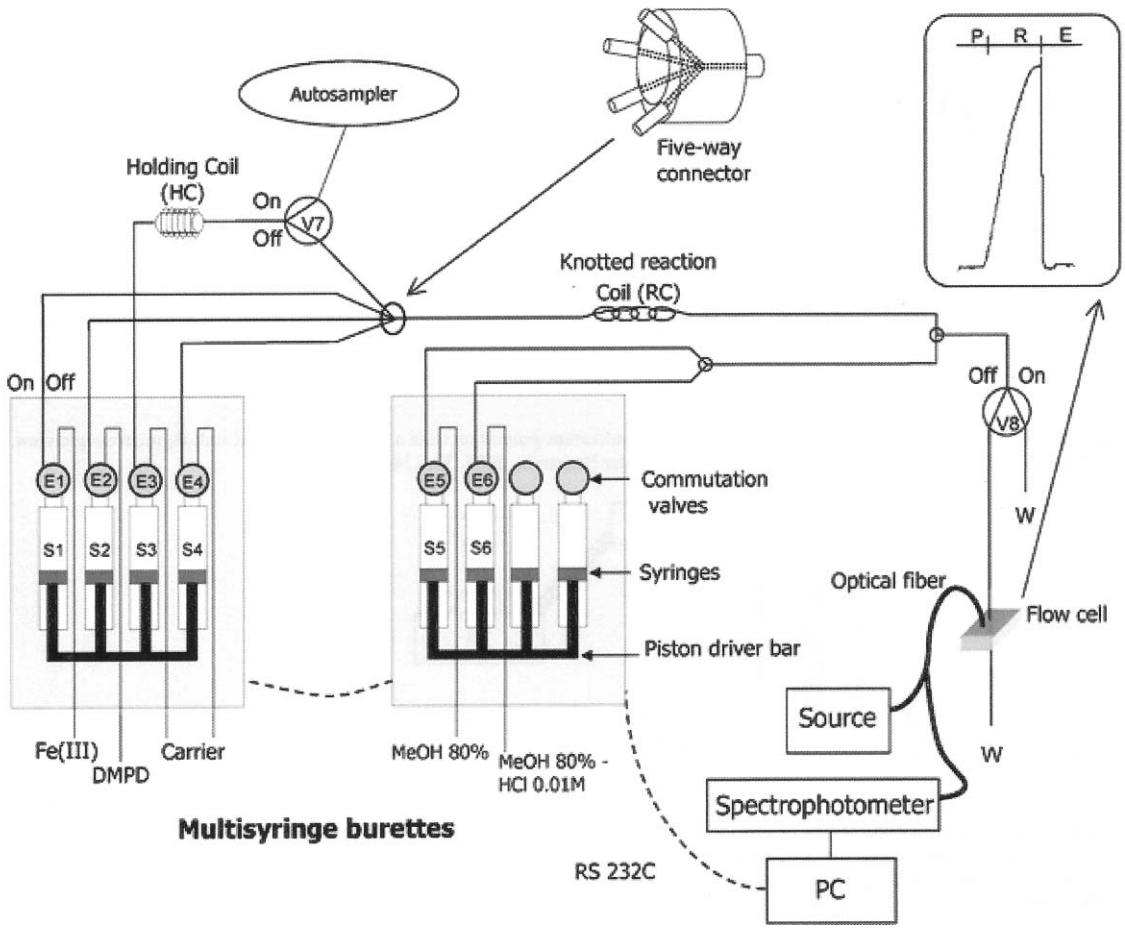

Fig. 6 A schematic representation of the multisyringe flow injection disk-based extraction optosensing manifold designed for the determination of sulfide in environmental waters and wastewaters. The transient analytical signal is also schematically illustrated. (P: pre-conditioning, R: retention, E: elution, DMPD: *N*,*N*-dimethyl-*p*-phenylenediamine monohydrochloride, Carrier: 0.14 M HCl, W: waste). (Reproduced with permission from ref. 87. Copyright 2005 Royal Society of Chemistry.)

natural organic matter,[89] in the case of effluent rich in lignin, the direct UV determination at 220 nm would be held back by lignin compounds which strongly absorb light at 205–280 nm. Hence, despite its tempting simplicity, the direct UV detection of nitrogen should be ruled out for pulp and paper industry wastewaters.

Other possibilities for the nitrate quantitation include ion chromatography, capillary ion electrophoresis, selective nitrate electrode, and reduction to nitrite with hydrazine or cadmium, followed by diazotization and colorimetrical measurements of the resultant azo dye at 520 nm. The automated hydrazine reduction method can determine nitrate over a range of 0.01–10 mg l^{-1} and the cadmium reduction flow injection method works over a range of 0.00025–10 mg nitrite l^{-1}, which is suitable for wastewaters. However, chloride may slow down the cadmium reduction.[14]

A nitrate electrode can measure nitrate over the range 0.14–1400 mg l^{-1}, but it suffers interference from chloride and bicarbonate when their weight ratios to nitrate exceed 10 and 5, respectively.[14]

2.5.2. Phosphorus: established methods. The total P analysis is based on the conversion of condensed, organically bound phosphates into orthophosphate, followed by the quantitation of the resultant and initially present orthophosphate. Organic phosphorus could be converted into orthophosphate by heat and persulfate/UV digestion or by persulfate digestion.[14]

Inorganic condensed phosphates are converted to orthophosphate by sulfuric acid digestion or by nitric acid–sulfuric acid. Persulfate/UV digestion and nitric acid–sulfuric acid method are used.[14]

In principle, orthophosphate could be quantified by ion chromatography and capillary electrophoresis, or by colorimetry. The first two methods are recommended for undigested samples, whereas colorimetry is applied to digested samples in the total P analysis. In the ascorbic acid method, orthophosphate is determined at 880 nm with a molybdate colour reagent over the range of 0.01–6 mg P l^{-1}, which is well applicable to the pulp and paper industry wastewaters.[14]

The ion chromatographic methods for determination of phosphate and total P, as well as other inorganic (reduced and condensed) and organic phosphorus species in water are thoroughly and comprehensively reviewed by Ruiz-Calero and Galceran.[90]

2.5.3 Persulfate method for simultaneous determination of total nitrogen and total phosphorus. The oxidation of nitrogenous compounds for determining total N must occur in an alkaline medium. Conversely, the oxidation of phosphorus compounds for determining total P must occur under acidic conditions. The methods determining total N use a persulfate–sodium hydroxide system to oxidize nitrogenous compounds to nitrate. Accordingly, methods determining total P use persulfate in an acidic medium. During the initial stage of digestion, sample pH is 12. In the final stage of digestion, the NaOH is consumed, causing sample pH to become 2. The digested sample is then analysed for nitrate and orthophosphate, hence yielding the total N and total P results.[14] Total N and total P can be measured simultaneously using a dual-channel analyser that determines nitrate–nitrite by the cadmium reduction method and orthophosphate by the ascorbic acid reduction method. Alternatively, other methods for orthophosphate and nitrate can be used.

An example of the flow injection analysis inline total N manifold could be found in.[14] The manifold includes tubing heater, inline UV-digestion unit equipped with a gas-permeable membrane and a flow cell, and absorbance detector.

In the flow injection analysis manifold for total P, the UV block consists of flexible Teflon (TFE) tubing irradiated by a mercury-discharged UV lamp emitting radiation at 250 nm, and absorbance detector for 880 nm.[14]

Cook and Frum[91] present the results of a single laboratory method evaluation and comparative study of the digestion techniques and the analytical methods for the determination of total N and total P in pulp and paper mill secondary-treated effluents. The sample preservation and storage stability were examined. The investigation of a method utilizing basic to acidic persulfate oxidation for the simultaneous determination of total N and total P is effective at concentrations above 1 mg l^{-1} in pulp mill effluents.

2.5.5 Suggested methods for nitrogen and phosphorus. *Chromatographic methods.* Colina[92] describes a method for the determination of total N, P and sulfur by microwave digestion with hydrogen peroxide followed by ion chromatography. The detection limits based on 0.2 g sediment sample are 0.006% (w/w) N, 0.012% (w/w) P and 0.042% (w/w) S.

Colombini[93] suggests the column-switching ion chromatographic method for the simultaneous determination of total N and total P in wastewater. The samples are oxidized by alkaline–persulfate solution in a microwave oven and analysed by ion chromatography without any sample preparation. The excess of sulfate (4.5 g l^{-1}), generated from the digested persulfate, is eliminated by a system composed of two switching columns. The limits of quantification are 9.0 μequiv. l^{-1} (0.12 mg N l^{-1}) for total N and 3.3 μequiv. l^{-1} (0.10 mg P l^{-1}) for total P.

The detection system showed a reproducibility of 13% for total N and 8.5% for total P. The calibration lines in the range of 5 μequiv. l^{-1}–1000 μequiv. l^{-1} deviated from linearity and were divided into three different weighed linear regression curves. The concentrations tried were within 6 mg l^{-1} for total N and 3 mg l^{-1} for total P.

Spectrophotometry. Roig[94] presents a general scheme for the speciation of nitrogen and phosphorus in wastewater and describes a photo-oxidation/UV system for the measurement of total N (up to 70 mg N l^{-1}) and P (up to 6 mg P l^{-1}). The duration of nitrogen and phosphorus measurements is 20 min. Potassium peroxodisulfate is used as an oxidant and a low pressure mercury lamp as UV irradiation source to convert all forms of nitrogen and phosphorus into nitrate and orthophosphate, respectively. The spectrum deconvolution method is used for the detection. For the nitrate determination, a base of reference spectra is proposed with spectra corresponding to the residual matrix, suspended solids, nitrate and oxidant. The formed nitrite is quantified at 205–335 nm and the orthophosphate calculated from the phosphomolybdate complex

formation. The quantification is carried out at 380–450 nm with recoveries of 85–100%. The results are compared to the theoretically expected and to those obtained by ascorbic acid colorimetry for orthophosphate, and by capillary electrophoresis for nitrate. The agreement is satisfactory.

A linear working range of 0.5–3 mg orthophosphate l^{-1} and a detection limit 0.44 mg l^{-1} is achieved by a spectrophotometric method described by Ghiokas.[95] The reaction of orthophosphates with diphenylamine and molybdenum(VI) in formic acid is utilized. The final product absorbs at 340 nm. Interference with other ions competing for molybdenum ions is absent.

Cerda[96] proposes a flow injection system based on persulfate/UV digestion, converting ammonium, nitrite and organic carbon into nitrate, measuring total N in wastewaters in concentrations of 5–130 mg l^{-1}. The digestion time is 5 s and the recoveries 90–100%. The nitrate is quantified spectrophotometrically at 420–540 nm after reduction to nitrite using hydrazine. The measuring time is 15 min.

Oms[97] developed a manifold for sequential injection analysis for total N in wastewaters based on the oxidation to nitrate with sodium persulfate in basic medium under UV radiation. Samples in the concentration range 1–56 g l^{-1} of nitrogen could be mineralized and measured at 226 nm, although there was interference from organic matter. The linearity range is up to 28 mg nitrogen l^{-1} with COD lower than 1200 mg O_2 l^{-1}, with irradiation period of 5 min and 12 g l^{-1} of persulfate. The detection limit is 0.5 mg l^{-1} and the reproducibility 1.8%. This UV-persulfate instrument has been applied to wastewaters with nitrogen content within the linearity range and even higher (up to 56 mg l^{-1}) with a doubled irradiation time and oxidant concentration. In case of the intensified procedure, good results were obtained when gas bubbles were removed prior to the spectrophotometric measurement.

A linear dynamic range of up to 1.4 mg ammonium l^{-1} for ammonia, which may be not sufficient for wastewaters, is achieved with a method proposed by Meseguer-Lloret.[98] However, an interesting comparison is presented of three methods for the ammonium determination: by an ammonium selective electrode, by the Nessler's reagent, and by the proposed ammonium derivation resulting in the formation of highly fluorescent isoindol measured by a diode array spectrophotometer.

Chemiluminescence. Ammann[99] eliminates the recovery reduction problem encountered in high temperature catalytic combustion when a nitrogen-containing sample is directly injected onto the hot Pt-coated catalyst. The evaporation step was found to be decisive for the quantitative recovery rates. Quantification was done by a chemiluminescence detector, determining NO_x gases in the combustion exhaust.

Colour band formation. Kiso[100,101] suggests a spot colour band formation test for phosphate,[100] and nitrite[101] employing formation of orange azo dye from nitrite and sulfonic acid and 1-naphthol, and phosphoantimonylmolybdenum blue. The length of the colour band is related to the phosphate and nitrite concentration in wastewater. The quantification ranges are 3–18 mg phosphate l^{-1} and 4–20 mg nitrite l^{-1}. The matrix is domestic wastewater with suspended solids content up to 114 mg l^{-1} and COD up to 74 mg O_2 l^{-1}.

Other methods. Okamoto[102] describes a system based on the electrothermal vaporization and inductively coupled plasma atomic emission spectrometry. The phosphate ion is reacted with tungsten to form a stable tungsten phosphate species. Regarding the determination of sulfur, additional chemical modifiers such as copper(II), lead(II) *etc.*, are necessary to retain the analyte on the tungsten boat furnace. The furnace-fusion method or wet-digestion technique on the a tungsten boat furnace is applied to unify the chemical forms of the analytes. Various oxidative and reductive inorganic compounds as well as organic compounds of phosphorus and sulfur show the same sensitivities after the furnace-fusion digestion with hydrogen peroxide. The detection limits are 1.5 ng and 0.12 ng for the phosphorus and sulfur, respectively. The repeatability in terms of the relative standard deviations of 10 replicate measurements of the phosphorus and sulfur are 4.2% and 2.0%, respectively.

2.6 BOD determination

2.6.1 Classic BOD test. The classic BOD_5† test is a complicated and time-consuming procedure that requires considerable experience and skill to obtain reproducible results. The handling of the dilution water, the necessity to run the blank, seed determination, and the glucose–glutamic acid standard every time the BOD analysis is performed, as well as the impossibility of reading results before the incubation time is completed, are often resented in the classic BOD dilution test. The dissolved oxygen is measured initially and after incubation, and BOD is computed from the difference.

The working range of this test is equal to the difference between the maximum initial dissolved oxygen (7–9 mg l^{-1}) and minimum dissolved oxygen residual of 1 mg l^{-1} corrected for seed, and multiplied by the dilution factor. A 300 mg l^{-1} glucose–glutamic acid solution should give BOD_5 198 mg l^{-1} with the standard deviation of 30.5 mg l^{-1}.[14] The dissolved oxygen is measured directly with oxygen electrodes. The Clark oxygen electrode—designed fifty years ago to measure the uptake or production of oxygen by cell suspensions, subcellular particles or enzyme systems—remains the standard for measuring the dissolved oxygen in the BOD test. The Clark cell is also known as the oxygen membrane polarographic detector. A sample is brought into contact with the membrane (usually polypropylene or Teflon) through which oxygen diffuses into the measurement chamber containing a saturated KCl solution and two electrodes: a silver/silver chloride anode and a platinum cathode coated with glass to expose only a very small area of platinum. The electric current between the two polarized electrodes determines the oxygen concentration in the solution. The temperature sensors built into the probe allow compensation for the membrane and the sample temperatures, which affect both diffusion speed and solubility.

† According to a legend, the five-day incubation requirement was set because it took maximum of five days for sewage dumped into a river to reach the estuary in England.

A vast variety of dissolved oxygen probes based on the Clark type polarographic electrodes principle is available on the market. Most often in BOD measurements, the oxygen probe is used and connected to a pH meter. An overflow funnel for use in BOD bottles allows measurements to be made in the same bottle before and after the incubation.

2.6.2 Respirometric method. In the respirometric method, oxygen uptake in the respirometer bottle is measured either by measuring oxygen depletion in the gas phase, or by measuring the mass of oxygen that must be refilled in the headspace to maintain constant conditions. Manometric, volumetric, electrolytic and direct-input respirometers are available.

Convenient automatic equipment for BOD measurements with manometric technique are available. For example, the OxiTop® by WTW GmbH and the BOD sensors developed by Velp Scientifica srl utilize manometric measurements with a pressure sensor. The Velp BOD sensor set has measuring ranges from 0 up to 9999 mg l^{-1} BOD, with results appearing directly on the display of a measuring head. The OxiTop® BOD sensor operates in the pressure range of 500–1350 hPa, with measuring range of 0–4000 mg l^{-1} BOD, accuracy 1 hPa or 0.7% BOD value, and reproducibility of 12% on the glucose–glutamic standard. The measuring head contains a pressure sensor, a timer, a data memory, and an infrared interface communicating with a controller. The results are read directly in mg l^{-1} BOD by the controller. The measuring head stores 180 to 360 data sets (depending on running time) which can be called up at anytime and graphically displayed on the controller.

We have found no research articles reporting the analytic performances of the OxiTop® or the Velp BOD sets in BOD measurements on pulp and paper effluents. However, the respirometric BOD OxiTop® method proves to be a precise and reliable technique for determining the biodegradations of different oils in water.[103,104] Research data are available on the comparison of the analytical performance of the dilution method and the manometric method for BOD results in sanitary landfill leachates with the Velp BOD manometric equipment.[105]

Respirometric tests have many advantages over the dilution BOD method, including the use of undiluted samples and a faster exertion of oxygen demand—as not only the dissolved oxygen, but also the oxygen from air is available and allows continuous monitoring of oxygen uptake. Respirometry can be useful in studies to determine the effects of the nutrification and nitrification inhibition, the pH adjustment of the oxidation rates, the treatability of effluents, the differences in the performances of different microbial innocula, the effects of the known amounts of toxic compounds on the oxygen uptake reaction of test wastewater. A respirogram (respiration rate mg O_2 l^{-1} per hour over time) is used for calculating the concentration of the readily and slowly biodegradable fractions of COD. A peak in a respirogram presents the readily biodegradable COD while the slow decrease of the respiration rate represents the slowly biodegradable COD.[106]

A trial of a respirographic microbial sensor for on-line and off-line BOD measurements has been reported.[107] The system consists of a small conical fluidized bed reactor and the activated sludge is immobilized on a reticulated sinter glass placed in the reactor. For holding the suspended biomass carriers in the reversed conical plenum, a separating grid is placed before the liquid overflow to the oxidation chamber. Before its introduction to the central part of the biosensor, the physically diluted CO_2 in the influent is stripped with air. A data interface device converts the analogue output signals of the pH-controller in the reactor and the CO_2 analyser into digital input signals. The CO_2 analyser is based on infrared spectrometry. Its reproducibility is within 13.8% and the BOD values are 10–170 mg l^{-1}. An overview of the microbiological biosensors on respiratory basis for the measurement of BOD and nitrogenous BOD, as well as for inorganic N-compounds, heavy metals, organic xenobiotics is given by Riedel.[108]

2.6.3 Rapid BOD methods. Both the tedium and duration of the classic test have motivated an intensive search for simpler and faster methods, which has taken a turn mainly to the BOD-biosensor development. A biosensor is defined as an analytical device which converts a biological response into an electrical signal. A biosensor is composed of a biological recognition element (micro-organisms, organelles, cell receptors, enzymes, antibodies *etc.*) producing a biochemical signal and a transducer (such as electrode, piezo electric crystals, optode *etc.*) converting the biochemical signal into an electrical signal. An excellent summary of biosensors development has been authored by Nakamura and Karube.[109]

Since the invention of the microbial electrode BOD sensor,[110] the biofilm-type BOD-sensors remain the main type of BOD sensors. Such biosensors comprise a microbial film sandwiched between membranes and connected to an amperometric oxygen electrode. The microbial respiration is then measured directly and related to the BOD of the sample.[111] A patent by Karube and Nishihara[112] describes the BOD sensor based on *Pseudomonas putida* as an aerobic bacteria combined with an oxygen electrode. The aspiration activity of the bacteria is increased by consuming the organic matter contained in the water sample so as to reduce the dissolved oxygen. As a result, the current from the oxygen electrode is decreased and the change is correlated to the BOD value. The detection limit is about 0.25 mg O_2 l^{-1}, response time within 10 min.

A useful overview of the biofilm-type BOD sensors based on amperometric oxygen probe and respirometric measuring principle listing immobilized microbial innocula, measuring range, response time, repeatability and operational stability can be found in ref. 113. The development is mainly focused on the further enhancement of immobilized film BOD microbial sensors.

Microbial innocula used in BOD biosensors. A culture of the yeast *Trichosporon cutaneum* is used in the BOD biosensor reported by Yang.[114] The lower limit of detection is 0.2 mg l^{-1}, linear dynamic range up to 18 mg l^{-1} and response time 7–20 min. The yeast is directly immobilized on the surface of the miniature oxygen electrodes using an ultraviolet cross-linking resin. Amylase and amyloglucosidase are added to the yeast in a BOD electrode from Prüfgerätewerk-Medingen.[115]

The enzymes (α-amylase and amyloglucosidase) are immobilized by adsorption, cross-linking or covalent coupling and filled into glass columns. Using enzymes as the biological recognition element in a BOD sensor was found to be most suitable for wastewaters containing easily hydrolysable polysacharides. The BOD values obtained with the sensor and with the conventional BOD_5 test are identical.

Thermally killed *Bacillus subtilis* have been utilized by Tan and Qian.[116] The sensor features a biofilm sandwiched between polycarbonate and teflon membranes, a silver anode and a gold cathode. The sensor is immersed in a water-jacketed beaker containing a phosphate buffer. Khan[117] tested BOD Seed, Bi-Chem and Polyseed commercial BOD inocula for BDOC measurements. A biofilm of thermally killed cells of a complex multispecies BODSEED (Cole-Palmer E05466-00) microbial culture has been studied by Tan and Wu.[118]

The following micro-organisms isolated from sewage were used in the research of Rastogi:[119] *Enterobacter cloaca, Citrobacter amalonaticus, Pseudomonas aeruginosa, Yersinia enterocolitica, Klebsiella oxytoca, Enterobacter sakazaki* and *Serratia liquefaciens*. The linearity range was up to 60 mg l^{-1}, the lower detection limit 1.0 mg l^{-1}, reproducibility within $\pm 5\%$.

Ferricyanide-mediated and mediatorless sensors. The ferricyanide-mediated assay has contributed to the development of rapid measurements for BOD.[120–125] The use of ferricyanide results in a significant increase in the rate of biochemical reactions and allows for biodegradative conversion efficiencies similar to the 5-day BOD assay to be achieved in 1 hour. The principle of the amperometric-mediated biosensor is illustrated in Fig. 7 and it is described as follows:[120]

$$CH_2O \text{ (organic substrate)} + O_2 \text{ (electron acceptor)} \rightarrow \text{micro-organisms} \rightarrow H_2O + CO_2$$

$$CH_2O + H_2O \rightarrow CO_2 + 4H^+ + 4e^-$$

$$[Fe(CN_6)]^{3-} + e^- \rightarrow [Fe(CN)_6]^{4-}$$

$$CH_2O + H_2O + 4[Fe(CN)_6]^{3-} \rightarrow CO_2 + 4H^+ + 4[Fe(CN)_6]^{4-}$$

The method is linear between 19 and 150 mg l^{-1} glucose–glutamic acid without dilution and using an immobilized biocomponent, a significant improvement over the standard BOD_5 assay (1–9 mg l^{-1}). The excess of ferricyanide mediator permits a sufficient signal (3 ml of 150 mg l^{-1} glucose–glutamic acid) to measure low BOD values.[120]

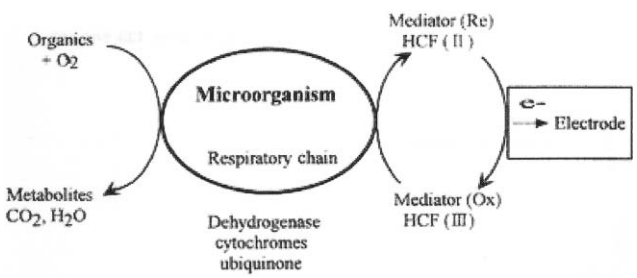

Fig. 7 The principle of the amperometric-mediated biosensor. (Reproduced with permission from ref. 121. Copyright 2000 Royal Society of Chemistry.)

The addition of substrate increases the respiratory activity of the micro-organisms and the accumulation of the reduced mediator; the mediator is subsequently re-oxidized at a working electrode generating a current quantifiable by a coulometric transducer. BOD estimations for the glucose–glutamic acid standard result in an extended linear range of 2–100 mg l^{-1}. The response reproducibility is -10% for a standard containing 10 mg BOD l^{-1}. For the analysis of pulp mill effluents, BOD detection limit is 2 mg l^{-1} with a response time of 5 min.[124]

In the research of Yoshida,[121] a biosensor consists of a three-electrode system with *Pseudomonas fluorescens biovar V* immobilized on the surface of the working electroplated gold electrode and uses hexacyanoferrate as a mediator. The sensor's linear range is 15–200 mg l^{-1}, the response time 15 min, and the repeatability 14%. A schematic diagram of the BOD measuring system is shown in Fig. 8.

The most interesting approach is by Kim,[126] who reports a mediatorless microbial fuel cell. It uses electrochemically active bacteria to transfer electrons to the electrode, thus eliminating the necessity for a ferricyanide mediator. A microbial fuel cell enriched with electrochemically active bacteria is used as a basis of the BOD measurement system.[127,128] The construction of the microbial fuel cell is shown in Fig. 9. When synthetic wastewater is fed to the system, the current generation pattern and its Coulombic yield are dependent on the BOD_5 of the synthetic wastewater. Real wastewater obtained from a sewage treatment plant produced a highly linear correlation between the Coulombic yield and BOD_5 in the system. The measuring time is 45 min, the linear dynamic range up to 150 mg l^{-1}, and the repeatability within $\pm 2\%$.

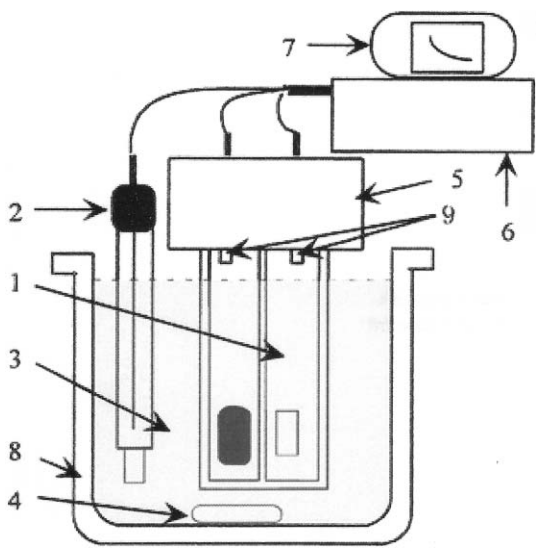

Fig. 8 A schematic diagram of the BOD measuring system and the microbial sensor tip. (1) BOD sensor tip; (2) reference electrode; (3) sample solution containing buffer and mediator; (4) magnetic stirring bar; (5) connector; (6) potentiostat; (7) recorder; (8) thermostated water-jacket vessel; (9) electrical contacts. (Reproduced with permission from ref. 121. Copyright 2000 Royal Society of Chemistry.)

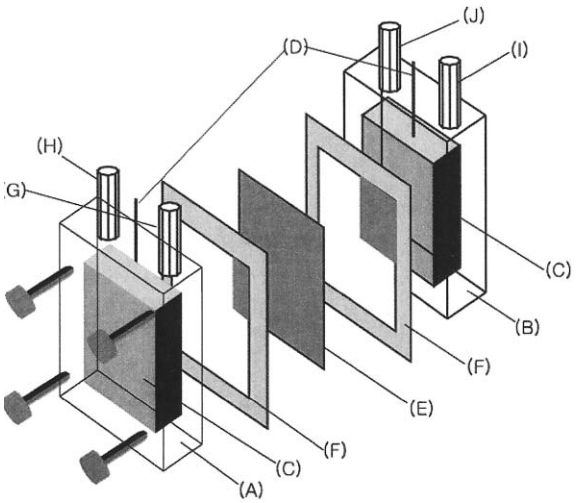

Fig. 9 A schematic diagram of the microbial fuel cell: (A) acrylic plate of the anode compartment; (B) acrylic plate of the cathode compartment; (C) graphite felt electrode; (D) Pt wire; (E) cation exchange membrane; (F) silicon gasket plate; (G) inlet of the anode; (H) outlet of the anode; (I) inlet of the cathode; (J) outlet of the cathode. (Reproduced with permission from ref. 127. Copyright 2000 Royal Society of Chemistry.)

Dai[129] compares the performances of BOD sensing films consisting of layers of an oxygen-sensitive fluorescent material and immobilized organisms (sieved bacteria from sea water and domestic bacilli immobilized in polyvinyl alcohol sol–gel matrix). The linearity range of these optical fiber biosensors is of 0.5–200 mg l^{-1} with the repeatability within 4% for sieved marine bacteria and 0.5–250 mg l^{-1} with repeatability within 2% for a film of domestic bacilli. The response time is within 2–32 min.

Yang[130] compares the performances of the dynamic transient and steady-state measuring methods in a batch-type BOD sensing system consisting of oxygen electrodes functionalized with the yeast *Trichosporon cutaneum*. The dynamic transient method (response time 3 min) and steady-state method (response time within 20 min) were found to be equally reliable for the measurement of wastewater samples. The lower limit of detection is about 1 mg l^{-1} and 0.5 mg l^{-1} for the dynamic transient and steady-state methods, respectively. Reproducibility is within 8% for both methods. The linearity ranges are up to 30 mg l^{-1}.

Chee[131] describes the photocatalytic biosensor using TiO_2 for BOD measurements. The sensor consists of a photoreactor with black-light fluorescent tube, operating according to the following reaction:

$$\text{Organic matter} + O_2 + h\nu\ (TiO_2) \rightarrow CO_2 + H_2O + \text{mineral acids}$$

An oxygen electrode with immobilized biofilm of *Pseudomonas putida* placed on the top of the Teflon gas membrane covers the oxygen electrode, which is linked to both a digital multimeter and an electronic recorder. The sensor's response time is 5–10 min, the detection limit 1 mg l^{-1}, the repeatability 12%, the linearity range up to 8 mg l^{-1}. The degradation of tannic acid with photocatalysis is 52%.

A method for characterization of on-line sensors and quantification of the uncertainty during field operation is considered by Rieger.[132]

2.7 Toxicity

The goal of wastewater toxicity testing is to assess the potential effects of wastewaters on the biota of receiving water body and on the performance of activated sludge in a biological wastewater treatment plant. The developments in assessing municipal wastewater toxicity to activated sludge have been reviewed by Ren.[133] The bioluminescence, respirometric, nitrification/denitrification inhibition assays and molecular-based assays and sensors are discussed.

2.7.1 Identification of toxic pollutants. Klinkow[134] reports toxicity-directed fractionation of organic compounds in tannery wastewater with regard to their molecular weight and polarity. It has been found that the molecular weight and polarity of organic compounds in industrial wastewaters determine their toxicity.[135]

The identification of toxic organic pollutants in wastewaters is mostly done by sequential solid-phase extraction followed by high-performance liquid chromatography fractionation and gas chromatography-mass spectroscopy detection and quantification.[135–138] Castillo and Barcelo[139] used toxicity-based fractionation with liquid chromatography/mass spectrometry for the identification of polar toxicants in industrial wastewaters. Recent studies on the identification of toxic components in pulping wastewaters from totally chlorine-free (TCF) and elemental chlorine-free (ECF) bleaching processes by multivariate statistics of the pyrolysis products of high molecular components have been reviewed by Paasivirta.[140]

2.7.2 Respirometric method. If toxins are present in the wastewater, the biodegradation capability of the activated sludge is reduced, which in turn reduces the respiration rate. Measuring the respiration rate of the sludge gives therefore a direct measure of the wastewater toxicity to the sludge bacteria. The respiration inhibition is reported as EC_{50}, EC_{20}, or EC_{10} values.

Bolton[141] used a twenty-channel, manometric electrolytic respirometer for testing respiration and nitrification inhibition. The sample throughput is about 15 samples per day with the coefficient of variance within 15% depending on the nature of sample.

Gernaey[142] suggests a rapid respirometric procedure using an enrichment nitrifying culture with allyl thiourea as the specific nitrification inhibitor for acute nitrification toxicity screening of wastewaters. The response time is 14 min. The method shows good sensitivity to such toxicants as phenol, 3,5-dichlorphenol, cyanide and metal ions, and acceptable repeatability.

Tzoris[143] tested a portable device, Baroxymeter, based on monitoring respiration of a bacterial culture by pressure measurements and using respiration inhibition as a toxicity alert. 3.5-Dichlorophenol was detected within 5 min from a 1 ml sample.

Commercial activated sludge respirometers for BOD measurements and toxicity test by respiration inhibition and nitrification inhibition are available. For instance, Strathtox™ by Strathkelvin Instruments Ltd provides a real time display of bacterial respiration and calculates respiration inhibition as EC_{50}, EC_{20}, or EC_{10} values. Test times are within 30 min. Marsili-Libelli and Tabani[144] assessed the error sources of an intermittent-flow closed respirometer.

Methanogenic toxicity testing of bleaching effluents with an anaerobic serum bottle technique[145] has been carried out, *e.g.* ref. 146. Methanogenic toxicity batch assays for effluents from elemental chlorine, ECF and TCF bleaching have been reported.[147–149]

2.7.3 Algae and Daphnia. To protect the receiving waters, the principal methods for wastewater toxicity assay over the last decade have been the bioluminescence inhibition methods with marine bacteria *Vibrio fischeri*, the growth inhibition test with algae *Selenastrum capricornutum*,[150–152] the short-term toxicity test and the reproduction of *Ceriodaphnia dubia* in the chronic lethality test.[153]

A toxicity screening assay for wastewaters based on growth inhibition of *Selenastrum capricornutum* as an endpoint is described by Blaise.[150] The exposure time is 72 hours.

An improved algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants is suggested by Lin.[151] Kamaya[152] reports the toxicity of fatty acids to green algae *Selenastrum capricornutum* that is dependent on the number of total carbons and double bonds in the organic molecule, with oleic acid found as the most toxic fatty acid.

The toxicity of TCF bleaching effluent was assessed with short-term and chronic toxicity for *Ceriodaphnia dubia* and short term toxicity for *Escherichia coli*. The reproduction of *Ceriodaphnia dubia* in the chronic toxicity test was inhibited to half of the values observed for the controls with 0.1% concentration of TCF bleaching effluent.[153]

The toxicity of pulping wastewaters was analysed by a modified three-generation *Ceriodaphnia dubia* toxicity test.[154] Galassi and Benfenati[135] investigated the toxicity of entire samples of wastewater and their fractions using *Daphnia magna*.

2.7.4 Bioluminescence inhibition method. This is perhaps the most elegant and convenient of all the toxicity assessment methods reviewed. The luminescent bacteria emit light in the course of their respiration, and thus any inhibition of cellular activity, *i.e.* toxicity, decreases the bioluminescence. A photometer measures the light output from the bacterial reagent upon the addition of different portions of a toxic waste sample and the difference between the blank and the challenged sample is directly related to the toxicity. The exposure time is 5–15 min (sometimes also 30 min) against the 24–48 hours with *Daphnia* or 72 hours with algae. Several commercial protocols based on the measurement of bioluminescent inhibition of *Vibrio fischeri* (Microtox®, ToxAlert®, BioFix® Lumi, ToxTracer®) are available. An important feature is the possibility to use a freeze-dried bacterial reagent which can be stored for a few months.

Since 1992, bioluminescence inhibition protocol Microtox® is established as a standard method in Canada[155] and it is used in the toxicity assay for wastewaters both entering the wastewater treatment plant (WWTP), to protect the activated sludge, and for treated wastewaters to protect the receiving waters. The literature on using this protocol is extensive, *e.g.*ref. 156–160. Later on, the bioluminescence inhibition method has been standardized also elsewhere (see Table 2).

Kostamo and Kukkonen[161] report EC_{50} values to *Vibrio fischeri* for pulp mill effluents before and after the biotreatment. Before the treatment, the EC_{50} values varied between 7% and 46% after 30 min of inhibition. After the treatment, the water became non-toxic.

Apart from *Vibrio fischeri*, other bioluminescent microorganisms are employed in toxicity assays. Methodologies enabling the use of a panel of genetically engineered stress-responsive luminous bacteria for wastewater toxicity assessment are presented by Belkin.[162] A toxicity testing protocol using bioluminescent bacteria Shk1, a genetically modified pseudomonad isolated from the activated sludge, has been reported by Lajoie.[163] The protocol includes a growth of cultures in nutrient broth with tetracycline, storage of cultures at 4 °C, cell activation by re-inoculation into nutrient broth, and toxicity testing by cell injection into the test media. Ren and Frymier[164] determined the toxicity of 98 organic pollutants including phenols and chlorine-substituted aliphatics to bioluminescent bacteria Shk1. An analysis of the quantitative structure–activity relationships allowing predicting the toxicity of different groups of organic chemicals based on the knowledge of their structure was carried out based on the logarithm of the octanol–water partition coefficient.

Ren and Frymier[160] evaluated the toxicity of 7 metals and 25 organic pollutants using the bioluminescent bacteria Shk1 and PM6 and compared these to *Vibrio fischeri* and activated sludge inhibition assays. The PM6 and Shk1 assays responded to toxicants similarly to the activated sludge inhibition assays. A patent by Lajoie *et al.*[165] provides a reporter bacterium—a bacterium that occurs in a biosludge and that contains a nucleic acid that encodes a bioluminescent reporter protein. Gu and Gil[166] present a multi-channel continuous toxicity monitoring system using recombinant bioluminescent bacteria. Cho[167] used groundwater bacteria *Janthinobacterium lividum* YH9-RC luminously modified with *luxAB* for a continuous toxicity test. Luminously modified cells exposed to continuous phenol or wastewater stream showed a rapid decrease in bioluminescence which fell below the detectable range within one minute and was found to be more sensitive than the Microtox® bioassay. The higher sensitivity of the groundwater bacteria as compared to the marine bacteria in Microtox® was ascribed to the limited transport of organic and inorganic pollutants from surface soil to groundwater, thus giving little chance for bacteria in the pristine groundwater to adapt to toxic chemicals. The freeze-drying procedure has been optimized with the assistance of trehalose. A mechanically and electronically developed system featuring 384-multiwell plates was constructed for continuous toxicity monitoring.

The limitations of the bioluminescence method for wastewaters containing surfactants is reported by Sherrard.[168]

Faria[169] suggests a kinetic bioluminescence method with *Vibrio fischeri* for continuous screening of wastewater toxicity. No interference from turbidity or colour was found.

Farre *et al.*[170–172] compare the performances of two bioluminescence inhibition tests for water toxicity: the evaluation of the reproducibility of toxicity assays with Microtox® and ToxAlert®,[170] the comparison of the performances of Microtox® and ToxAlert® 100 to determine the toxicity of industrial wastewaters and of the influents and effluents of a wastewater treatment plant.[171] Both protocols utilize *Vibrio fischeri*. The results show the slightly higher sensitivity of ToxAlert® 100. The reproducibility of different toxicity tests based on the bioluminescent inhibition of *Vibrio fischeri* (Microtox®, ToxAlert®, BioFix® Lumi, ToxTracer®, LUMIStox® LCK 480) has been evaluated.[172] Phenol, 3,5-dichlorophenol and Zn-sulfate solutions were used as toxicants.

2.7.5 Comparative studies on toxicity. The literature comparing performance of the different toxicity tests on wastewaters is extensive. Pedersen and Petersen[173] tested the performances of green algae, *Daphnia magna*, zebra fish, duckweed, and *Vibrio fischeri*. The comparative sensitivity and practicality of the following seven new commercial test assays for the direct assessment of ecotoxicity in industrial effluents was presented by Daniel:[174] *Daphnia magna*, *Selenastrum capricornutum* and *Thamnocephalus platyurus* Toxkits® supplied by Vickers Laboratories Ltd containing dormant, immobilized life stages of the test species; GreenScreen® EM—a yeast based assay for genotoxicity and general acute toxicity supplied by Gentronix Ltd; and CellSense®—a mediated, amperometric whole-cell biosensor based on immobilized activated sludge and *E. coli*.

The toxicity of kraft pulp bleaching effluents to *Vibrio fischeri* and *Daphnia* have been compared by *e.g.* Kennedy[175] and Pintar,[176] who conclude that *Daphnia* is more sensitive to wastewater than *Vibrio fischeri*. The chronic *Ceriodaphnia dubia* toxicity assay was more sensitive than the bioluminescent assay; while nearly all detectable *Vibrio fischeri* toxicity were removed from the wastewaters, residual chronic toxicity towards *Ceriodaphnia dubia* remained regardless of the treatment.[175] In case of *Daphnia magna* the difference was even more remarkable: the wastewaters were treated by catalytic wet-air oxidation. While the toxicity reduction was achieved to *Vibrio fischeri*, the treated waters were all more toxic to *Daphnia magna*.[177] In the studies by Doherty,[178] the results for non-toxic samples are in good agreement for *Ceriodaphnia dubia* and *Vibrio fischeri*. With three samples that were toxic to *Ceriodaphnia*, bioluminescence detected toxicity in two of those samples within 24 hours.

Several reports point out that the respirometric method should be used over the bioluminescence method to protect the activated sludge. In the studies by Gutierrez,[179] assays with a biodegradable reference surfactant show a toxic effect by the bioluminescence method but good biodegradability and no toxicity in respirometry. For the evaluation of the potential toxicity of a compound on a WWTP, the preferred biological material should be the activated sludge itself. The bioluminescence method has proven higher sensitivity to wastewaters than the sludge respirometry method. It is reasonable to assume that a single marine microbial species *Vibrio fischeri* employed in the bioluminescent methods would be less adapted to the testing medium than a heterogeneous population of adapted micro-organisms in the activated sludge. In the studies of Kelly,[180] the discrepancy between bioluminescence of bacterium Shk1 and respirometry toxicity response is ascribed to the fact that the bioluminescent method assesses the soluble toxins concentrations, while the respirometry method assesses the impact caused by the fraction adsorbed on bioflocs. As such, the Shk1 assay is less sensitive than the *Vibrio fischeri* bioluminescence assay and could therefore be more suitable for the influent wastewater toxicity monitoring with bioluminescence.[177] Thus, respirometry is more representative of toxic effects on the activated sludge compared to bioluminescence.

A rapid toxicity pre-screening method is the chemiluminescent peroxidase activity assay based on the peroxidase-catalysed oxidation of luminol by hydrogen peroxide.[181]

Bailey and Young[182] compare toxicity assays using rainbow trout, *Ceriodaphnia dubia*, and algae *Selenastrum capricornutum* for pulp and paper mill effluents. The sensitivity of the standard algal growth inhibition test to pulp and paper mill wastewaters is shown.

Tarkpea[183] assessed the toxicity of the conventional elemental chlorine, ECF and TCF bleaching effluents with Microtox®, and the lethal and the reproduction *Nitocra spinepes* test. TCF bleaching effluents were the least toxic, and the conventional bleaching effluent and ECF the most toxic. The macroalga *Ceramium strictum* was found to be most sensitive species to the bleaching effluents.

Dalzell[184] compares the toxicity assessments by nitrification inhibition, respirometry, adenosine triphosphate luminescence and enzyme inhibition of activated sludge, and the *Vibrio fischeri* toxicity test. The bioassay with *Vibrio fischeri* is more sensitive than the tests utilising activated sludge, and it is recommended that the *Vibrio fischeri* test should not be used to determine the potential effect to activated sludge by an unknown pollutant. With activated sludge, nitrification inhibition was the most sensitive test. It was shown that tests used in combination are more adequate than using only one test and at least two toxicological tests using sludge matrix should be used to protect the activated sludge.

LeBlond and Duffy[185] show that the *Selenastrum capricornutum* growth assay is unsuitable for gold and zinc mining effluents, whereas the Microtox® assay is a useful screening tool. The bioluminescent bacterium Shk1 is less sensitive than the Microtox® assay and therefore more suitable for influent wastewater toxicity monitoring.[177]

Ren and Frymier[186] examined the data of four bioassays obtained from the literature: Polytox®, activated sludge respiration inhibition, the *Nitrosomonas* and *Tetrahymena* assays and their own data on the continuous Shk1 using multidimensional scaling. The results of the two-dimensional mapping using similarity coefficients suggest that Polytox®, activated sludge respiration inhibition, *Nitrosomonas* and *Tetrahymena* assays and Shk1 bioluminescence assay would make an effective test set to assess the toxicity of wastewater to activated sludge.

Hernando[187] evaluated the acute toxicity tests for the monitoring of wastewater treatment using the *Vibrio fischeri* bioluminescent test Biotox, the *Selenastrum capricornutum* growth inhibition test and the *Daphnia magna* acute immobilisation test. The reproducibility calculated as relative standard deviation was between 5% and 22.3%. The *Daphnia magna* test was the most sensitive permitting the detection of toxicants in effluents from wastewater treatment plants for which the toxicity results of *Vibrio fischeri* were not toxic. No correlation between toxicity and TOC results was found. It is pointed out that the use of single organisms to evaluate wastewater toxicity cannot provide an adequate assessment of the risk. The appropriate way would be to use a set of toxicity tests with representative organisms of different biological organizations.[186,187]

Stauber[188] compares the toxicities of ECF and TCF bleaching effluents by Microtox®, the phytoplankton growth inhibition test, the macroalgal fertilization test, the sea urchin fertilization test, the scallop larval abnormality test, and the fish larval survival test. The sea urchin fertilization and scallop larval abnormality tests are the most sensitive to the effluents. Toxicity assays for discharge from a paper mill show that the order of sensitivity of the analysed toxicity tests is from the most sensitive to the least sensitive: Microtox®, chronic *Ceriodaphnia* = acute *Ceriodaphnia*, chronic sublethal biossay with *Selenastrum capricornutum*, acute rainbow trout.[189]

O'Connor[190] assessed the toxicity of TCF bleaching effluents by the acute lethal toxicity tests to *Ceriodaphnia, Daphnia magna* and *Vibrio fischeri* (with Microtox®), and the chronic sublethal toxicity test to *Ceriodaphnia* reproduction. Peroxide-based bleaching effluents exhibit relatively high chronic toxicity to *Ceriodaphnia*.

We conclude that the sensitivity towards wastewaters decreases in the following order: consortium in activated sludge, modified bioluminescent bacteria Shk1, bioluminescent *Vibrio fischeri*, and *Daphnia*. This means that using *Daphnia* would always give the highest toxicity results than other methods. Hence, for protecting the receiving waters, the bioluminescence protocol with *Vibrio fischeri* could be optimal, whereas for protecting a biotreatment plant, toxicity should be assessed with the activated sludge respirometry. If equipment for respirometric BOD is available, toxicity tests for sludge could be done with the same equipment.

2.7.6 Toxicity biosensors. A patent by Upton and Pickin,[191] describes a detector where various bacteria (nitrifying, denitrifying or carbon-degrading) are immobilized by encapsulation in a polyvinyl alcohol medium and exposed to a fluid. The encapsulation allows a high number of specific bacteria to be quickly captured and retained within the medium providing a good response from the detector.

Gu and Gil[166] suggest a multi-channel continuous toxicity monitoring system composed of a parallel combination of several two-stage mini-bioreactor systems connected by a fibre optic probe to a luminometer. Different bioluminescent recombinant strains of *E. coli* are cultivated separately in different channels, which allow a specific bioluminescent response from each channel for the classification of toxicity.

In a whole-cell bacterial biosensor, electric current is obtained from bacterial electron transport chain by using electron mediators which aid the current flow between the bacterial cell and the transducer. The produced current is proportional to the metabolic activity.

Farré *et al.*[171] used an amperometric biosensor, Cellsence, with *E. coli* as the immobilized biological component. The biosensor is constructed of a screen-printed working electrode on which bacteria are immobilized, and an Ag/AgCl reference electrode on a polymer substrate. The biosensor is immersed in a constantly stirred substrate and redox mediator solution and provides a continuous signal. The response to the toxic challenge is a suppression of metabolic activity related to the concentration of the toxicant. A mathematical model assuming a hyperbolic relationship between the exposure time of the biosensor biocatalyst and the inhibition was used to fit the measured data and to produce time-dependent inhibition values. Farré and Barcelo[136] employed the Cellsence biosensor based on the inhibition of *Pseudomonas putida* to assess the acute toxicity of wastewater and compared its performance to the bioluminescence inhibition test ToxAlert® 100 using *Vibrio fischeri*. Although *Vibrio fischeri* demonstrated better sensitivity and repeatability, the advantage of Cellsence was that it can function in highly turbid and coloured waters.

Lucarelli[192] tested an electrochemical DNA biosensor constructed of an immobilized double-stranded calf thymus DNA on the surface of a disposable carbon screen-printed electrode. The analytic signal was the oxidation signal of the guanine base obtained by a square wave voltammetric scan. The results were comparable to those of the bioluminescence test.

3 Laboratory set up

In principle, all the non-biological key summation parameters of principal interest (total organic carbon, total nitrogen, total phosphorus, total organochlorine, and even total sulfur) could be determined by combustion and the separation of combustion products by the chromatographic methods. Already in 1969, Dugan and Aluise[193] had described an analyser for dynamic microdetermination of carbon, hydrogen, nitrogen, sulfur and oxygen. The procedure is based on the uncatalysed dynamic flash-combustion of the sample under an oxygen–helium atmosphere of 1070 °C in a quartz tube. The retention and separation of the combustion gases (NO_2, SO_2, CO_2 and H_2O) is done by chromatographic columns in liquid nitrogen. Thus, an 'all-purpose' analyser based on combustion and elemental analysis should be considered possible.

The modern combustion-based multi-purpose analysers are available allowing measurement of *e.g.* TOC and AOX, total N and total P, TOC and total N, total sulfur with one apparatus, simultaneously, or by switching modes. In some cases, the attempt to analyse all the parameters with one instrument is not optimal due to matrix interferences, so a meticulous selection of the instrument is recommended. We suggest the following set of instruments for this purpose: a TOC analyser, an analyser for chlorine (AOX), and a total N and total P analyser.

Measuring the respiration rate of the activated sludge could answer for the biological parameters such as BOD and toxicity (at least to activated sludge). Hence, the biological parameters (BOD and toxicity) may be determined by the same respirometric equipment.

3.1 Recommended instrumentation

A wastewater laboratory focusing on pulp and paper industry effluents must be capable of detecting and quantifying the following parameters: total suspended solids, pH, electrical conductivity, COD, AOX, total N and total P, reduced sulfur compounds, BOD, and toxicity to aquatic biota or activated sludge. Moreover, the TOC analysis could be used for rapid measurements of organic load. Analyses requiring determination sensitive to individual compounds are beyond the scope of the daily routine, but should be provided when such characterisation is needed.

We recommend the following instrumentation for an analytical laboratory for wastewaters with low throughput of samples:

– pH meter.

– Conductivity sensor.

– Drying oven for operation at 105 °C for suspended solids determination; analytical balance capable of weighing to 0.1 mg, desiccator, aluminium weighing dishes, filtration apparatus with reservoir and 40–60 μm fritted disc as filter support, or membrane filter funnel.

– TOC analyser with high temperature combustion.

– AOX analyser featuring high temperature combustion of loaded carbon sample in oxygen flow, a microcoulometric cell for argentometry, and a mode switch allowing POX and EOX measurements.

– Gas chromatograph and a pulsed flame photometric detector for determination of sulfur compounds.

– Total N analyser featuring flow injection analysis manifold for UV-persulfate digestion, hydrazine or cadmium reduction, and a unit for azo-dye detection at 550 nm.

– Total P analyser featuring UV-persulfate digestion and acidolysis with addition of molybdate reagent and colorimeter for 880 nm wavelength.

– 150 °C heater with a set of commercially available closed tubes containing the exact doses of dichromate reagent and direct-reading spectrophotometer for Cr^{3+} and Cr^{6+} measurements at 600 nm and 420 nm for standard COD test.

– Incubation cabinet keeping temperature at 20 °C, BOD manometric equipment set featuring magnetic stirring, brown-glass incubation bottles closed with manometric sensor-measuring heads allowing detection in negative pressure changes and equipped with a light-emitting diode display or an infra-red interface which is connected to a controller, and an oxygen electrode for classic dilution BOD test.

– Bioluminescence system for toxicity assessment.

– Gas chromatograph coupled with mass spectroscopy for the determination of fatty and resin acids and chlorinated organic species.

It should be noted that many new COD methods that have been suggested lately in the research literature appear worth testing on pulp and paper industry effluents and including in a laboratory toolkit. These include, for example, the amperometric methods based on PbO_2-modified electrodes; the mixed-acid digestion and single sweep polarography; the flow injection analysis with permanganate cation-exchange and the chemiluminescence system.

Conclusions

Being a multifarious blend of suspended, colloidal and dissolved matter—both organic and inorganic, neutral, acidic and basic, oxidisable and non-oxidisable, stable and unstable, toxic and non-toxic—wastewaters present up till now a considerable challenge for qualitative and quantitative analysis, not to mention the tedium of many standard methods. The choice of methodologies and analytical equipment that can provide reliable, selective, sensitive, rapid and cost-efficient determination and quantitation of aqueous pollutants is an extensive task requiring assessments based on an adequate understanding of the underlying analytic principles as well as on the properties of wastewater samples, the comparison of the analytic performance on certain analytes, and the cost analysis of the equipment provided by various manufacturers.

The key parameters to consider when planning a modern laboratory for measuring pulp and paper industry wastewaters are total suspended solids, pH, electrical conductivity, COD, AOX, total N and total P, reduced sulfur compounds, BOD, and toxicity to aquatic biota and to activated sludge. The BOD and COD parameters yield information on the organic pollution causing oxygen depletion in aquatic ecosystem. AOX accounts for the chlorinated organic pollution which can have mutagenic effect on aquatic biota along with the toxicity. Total N and P are checked to avoid the eutrophication of the receiving waters. The pH of the discharged waters should be neutral, or nearly neutral.

High-temperature combustion methods remain as the principal procedures used for the TOC analysis of wastewaters when a quick assessment of organic load is needed. A very attractive approach towards TOC is ICP-AES which besides lower cost seems to offer a possibility to develop a single system capable of giving information about TOC, DOC, IC and heavy metal contents in waters. The adsorption–pyrolysis–argentometric titration method continues to be the optimal choice when determining the total chlorinated organic in wastewaters.

Automated flow-through methods based on the Fischer's coupling reaction are suggested for sulfur analysis with the subsequent detection of methylene blue, capillary electrophoresis, and chromatographic methods such as high performance liquid chromatography with fluorescence detection and gas–liquid separation with gas-phase atomic absorption spectrometry. For the present, the gas chromatograph and the pulsed-flame photometric detector remain as the optimum instruments for the determination of sulfur compounds in pulping effluents.

Ion chromatography methods, spectrophotometry and chemiluminescence have been suggested for nitrogen and phosphorus determination. A simultaneous determination of total N and total P could be realized with the persulfate/UV

digestion and a dual-channel analyser that determines nitrate–nitrite by the cadmium reduction method and orthophosphate by the ascorbic acid reduction method.

An intensive research activity is targeted to the COD and BOD analyses. With COD, researchers are pursuing improvements in digestion to shorten the processing time and to eliminate the need for hazardous and expensive additives. A noteworthy approach in the COD test development is the microwave-assisted and ultrasound-assisted dichromate digestion featuring significantly reduced digestion times compared to the standard methods. In addition, using a mixed acid as oxidant has shown good results in this area. Fenton's reagent employed in the oxidation of wastewaters could be suggested as the direction for further development of the digestion methods used in the COD analysis. The introduction of thin semiconductor films oxidising organic matter and producing photocurrent directly proportional to the COD content is so far the most interesting development in COD optimization. Recently suggested amperometric methods based on PbO_2-modified electrodes, mixed-acid digestion and single sweep polarography, flow injection analysis with permanganate cation-exchange, and chemiluminescence system appear worth testing on pulp and paper industry effluents and including in a laboratory toolkit.

Although the classic BOD dilution test remains in use, the respirometric method is becoming more and more recognized and preferred because of its clear advantages: the possibility of including undiluted samples, the faster exertion of oxygen demand, and the continuous monitoring of the oxygen uptake. The measurement of bacterial respiration activity is used in both BOD and toxicity assessments. While in BOD sensors the aspiration activity of the aerobic bacteria is increased by consuming organic matter from wastewater so as to reduce dissolved oxygen and thus by measuring its variation to obtain a BOD value, in toxicity sensors the respiration activity of bacteria is inhibited by the toxicants in wastewaters and by the variation in dissolved oxygen. In principle, BOD and toxicity may be determined by the same respirometric equipment.

Currently, the hottest area is the development of biosensors for rapid assessment of BOD. One of the most interesting applications is the microbial fuel cell enriched with electrochemically active bacteria as the basis for a BOD measurement system. Biosensors are still applicable to wastewaters containing quickly assimilable organics, but they do not account for the full range of biodegradable organic matter and hence cannot replace the BOD_5 method as of today.

In laboratory assessments of wastewater toxicity to aquatic biota in receiving waters, the growth inhibition of *Selenastrum capricornutum*, the acute and the chronic lethality of *Ceriodaphnia dubia*, and the bioluminescence inhibition of *Vibrio fischeri* still prevail, with the bioluminescence protocols becoming increasingly preferred. Although the bioluminescence inhibition of *Vibrio fischeri* is a frequently used toxicity assay both for waters discharged to the receiving water body and for waters entering the biological wastewater treatment system, the toxicity assessment by the bioluminescence protocols using *Vibrio fischeri* should be restricted to the former. The respirometry of the activated sludge and the bioluminescence inhibition with genetically modified bacteria isolated from a consortium of Shk1 are shown to be better options to protect the activated sludge.

The introduction of advanced analytical techniques allowing rapid, selective, sensitive, and reliable determination of aqueous pollutants is a hot area of interest for analysts dealing with environmental monitoring—not only due to their supreme environmental importance alone, but also because of the diversity of the fundamental phenomena and the elegance of the interdisciplinary applications involved in the development of wastewater analytical techniques.

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