

Potential application of biosensor networks for monitoring aquatic systems in support of the water framework directive

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ABSTRACT Improved water quality monitoring techniques based on biosensor, optical, microfluidics and information technologies are leading to radical changes in our ability to perceive, understand and manage the aquatic environment. The EU Water Framework Directive aims to prevent further deterioration and to protect and enhance the status of aquatic ecosystems, to promote sustainable water use based on long-term protection of water resources. This requires real-time environmental monitoring systems to implement and verify compliance with legislation by simultaneously monitoring many different priority chemicals and toxins with simple and cost-effective techniques. Intelligent sensor networks could effectively perform this task, as well as detecting localised sources of pollution and providing an early warning capability. Some recent advances in biosensing techniques using fluorescent labelled antibodies and molecularly imprinted polymers, for the analysis of various contaminants in freshwater and seawater, are reviewed.

1. Introduction

A key challenge of water quality monitoring is the detection of diverse, unanticipated and unpredictable sources of contamination. The solution lies in an interdisciplinary approach, employing hydrological and contaminant transport models, in combination with analysis by means of biosensor technology integrated with advanced data transmission and processing techniques. Contamination of water resources may take place at locations distant from the measurement point. It is therefore necessary to detect a wide range of chemical and bacterial contaminants as quickly and reliably as possible and to predict their migration accurately to provide support to water management. Innovative solutions are currently being developed for real-time monitoring of contamination in aquatic systems, employing miniaturised, low cost biosensor systems that can be deployed in lakes, rivers and aquifers.

This research at the intersection between nanotechnology, biotechnology and information technology is enabling the development of effective solutions to highly complex problems that were previously irresolvable. Monitoring systems based on biosensor and microfluidic technologies could potentially detect and analyse all priority contaminants. Biosensors are able to provide chemically specific, high spatial resolution information and allow completely automated remote operation (Proll *et al.*, 2005). In addition, by networking the biosensor devices via the existing telecommunications infrastructure data can be transmitted between the sampling points and the central control station for continuous monitoring and to provide an early warning and alarm capability. This results in more responsive monitoring systems allowing real-time

monitoring of pollutants and improved assessment of the exposure risk.

The implementation of the EU Water Framework Directive (European Commission, 2000) depends on the availability of reliable and cost-effective measurement techniques. The use of interactive communication infrastructures to link miniaturised analytical devices provides an opportunity for improving the surveillance and protection of aquatic systems. Information on water quality obtained by means of innovative biosensor technologies can assist monitoring programs to verify compliance with legislation (Rodriguez-Mozaz *et al.*, 2004, 2006a) and contribute to the development of improved water management strategies. Real-time analysis methods employed in conjunction with contaminant migration models could form the basis for intelligent decision making to mitigate the effects of pollution.

The integration of biosensor technologies for water quality analysis with informatics systems for rapid, reliable and low-cost detection of contaminants is dependent on optimising sensor response and selectivity. Accurate models will have to be developed to predict contaminant migration and degradation in order for the data to be used to make quantitative predictions, together with appropriate decision making tools to allow real-time interventions. In the case of drinking water it is necessary to consider three distinct sources of contamination:

- (i) at the water source;
- (ii) within the distribution network; and
- (iii) at the point of delivery.

There is increasing interest in the application of innovative technologies for rapid detection of organic pollutants in surface and ground water and water distribution systems. Techniques include the use of fluorescent dye labelled antibodies as biological recognition elements and molecularly imprinted artificial bioreceptors on polymer substrates in immunoassay sensors. To apply biosensor systems for water quality and site monitoring it is necessary to consider factors such as minimum detection levels, the toxicity of the compound, routes of migration and the concentration at which a compound becomes a significant threat to environment and health. Design of biosensor systems capable of detecting the most critical substances, even in the presence of confounding compounds, is an important aspect of this work.

Understanding the migration of contaminants in surface water and groundwater is essential to maintaining water quality and managing contamination of aquifers, lakes and rivers. While water quality monitoring can detect contamination, in order to develop effective remediation strategies and to assess the risk of exposure it is necessary to have predictive models of the migration of pollutants. Contaminant transport takes place generally under non-stationary flow conditions and is governed by interdependent physical processes with different length and time scales and having intrinsically high degrees of uncertainty (Cirpka and Nowak, 2004).

Numerical modelling of reactive transport of pollutants has been used (Watson *et al.*, 2003) in order to identify biodegradation processes and quantify rate parameters for contaminants in groundwater. This model takes into consideration the influence of aqueous geochemical speciation, phase equilibrium, mineral dissolution and precipitation kinetics, abiotic reaction kinetics, and microbial population dynamics and biodegradation kinetics in groundwater flow and contaminant transport. Improved predictive capability was achieved by inputting data acquired by multi-level sampling at actual field sites. These computer simulations can assist in estimating the ability of the aquifer to recover from the effects of

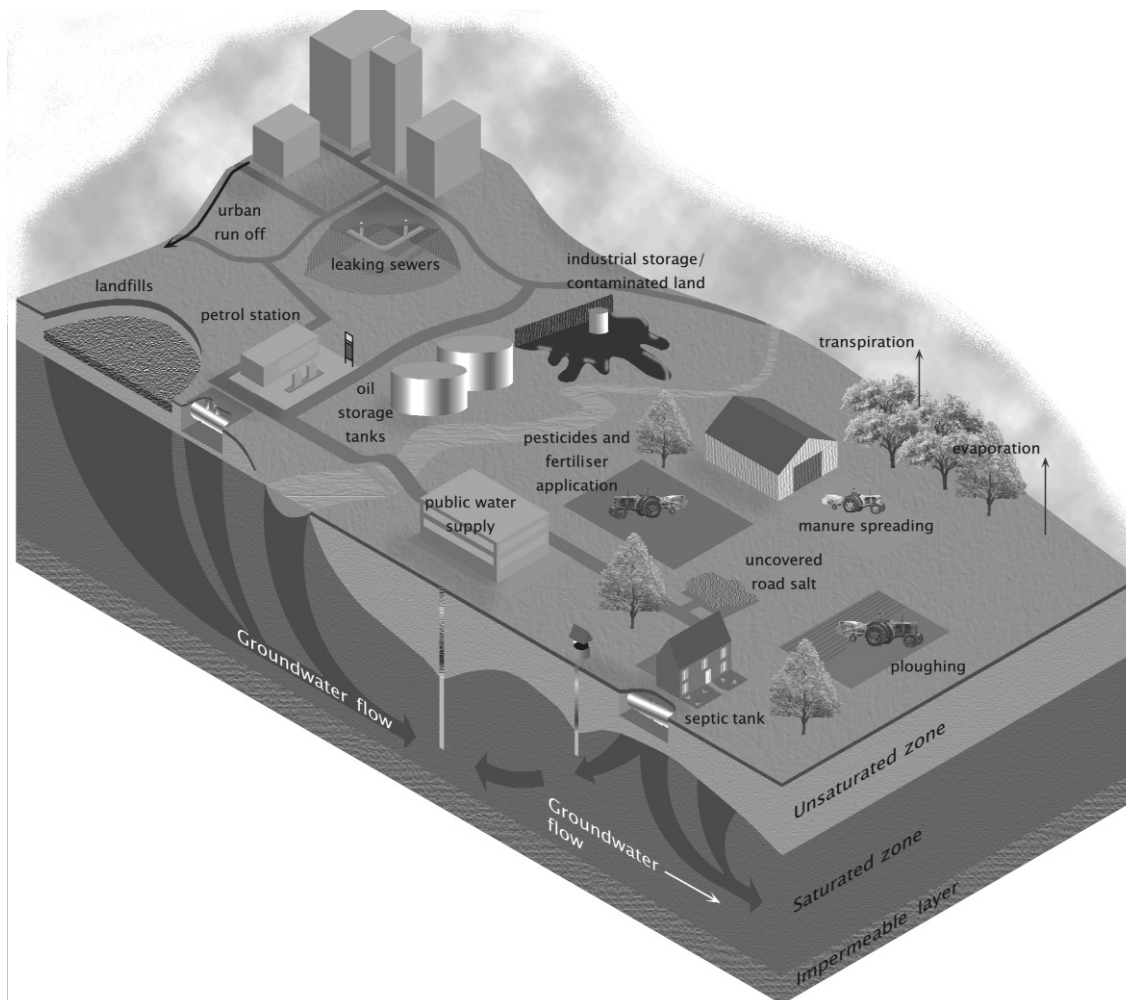


Fig. 1 - Sources of groundwater contamination and their migration paths. Reproduced with the permission of the UK Groundwater Forum ©NERC. All rights reserved.

contamination during a given time interval.

2. Sources of water pollution

Aquatic, terrestrial and atmospheric linkages between nutrient and pollutant cycles contribute to bioaccumulation and ecological risks in aquatic ecosystems. Some of the anthropogenic sources of groundwater contamination and their migration paths are illustrated schematically in Fig. 1 (Downing, 1998). These include the application of fertilisers and pesticides in agriculture, disposal of industrial and domestic waste in landfills, oil storage and refining facilities, sewage discharge and urban run-off. Contaminants migrate from the land surface into the saturated zone of the aquifer through the unsaturated zone due to capillary effects. The speed at which contaminants are transported through the unsaturated zone is determined by the permeability,

porosity, and the depth and degree of saturation of the aquifer, which vary depending on evaporation, transpiration, precipitation, groundwater extraction, and permeability.

An appreciation of the scale and complexity of the problems associated with the analysis of surface water contaminants can be obtained by considering the concentrations of different chemical compounds that may be present in river or lake water. Fig. 2, for example, shows the levels of various pollutants detected in samples of Belgian river waters. The substances present include endocrine disruptors (EDCs), phenols, pesticides, pharmaceuticals, dyes and sulfonates. It is therefore necessary to develop appropriate analytical methods and monitoring tools that are capable of identifying and analysing pollutants even in the presence of great numbers of other chemicals.

Contamination can also occur due to the release of chemicals from materials in contact with drinking water in the supply system or at the point of delivery. Typical materials employed in domestic and industrial water distribution systems include polyvinyl chloride, polyethylene, polypropylene, copper and stainless steel. Other problems that can arise with drinking water quality are related to odour and taste, colour and turbidity, microbial growth and the presence of toxins. Increased emphasis in the future can be expected on the detection and prevention of microbiological and chemical risks in drinking water.

3. Modelling groundwater pollution

In order to understand the migration of groundwater contamination an improved predictive capability of the natural processes occurring in aquifers is required. This can be achieved by acquisition of high quality data, obtained by sampling at horizontal and vertical points within the polluted zone, combined with site simulation using reactive transport models. Microbial and geochemical analysis is performed to determine the change in pollutant concentrations and the underlying processes occurring. Computer simulations of reactive transport are used to quantify the concentration and mass flux within the aquifer in order to assess its ability to achieve the required remediation outcomes within a given time frame. The models combine microbiological ecosystem dynamics with the geochemical reactions and physical transport. At present, the physical and chemical processes are well understood but better knowledge of the microbial processes is required. Obtaining an improved understanding of the processes and interactions taking place in aquifers should make it possible to improve the effectiveness of remediation interventions.

Degradation of contaminants in groundwater to intermediate species takes place as a result of fermentation processes. A numerical model of an aquifer-derived laboratory microcosm has been developed (Watson *et al.*, 2003) that couples microbial growth and substrate utilization kinetics to simulate the dynamic behaviour of fermentation and respiration in groundwater. Biodegrading plumes often have relatively reactive zones around the edges and less reactive zones in the core. In order to model biodegradation and flow, a high degree of refinement is required at the edges in order to improve understanding of plume behaviour. Watson *et al.* (2005a) demonstrated that local refinement of the grid to follow the edges of the moving plume, together with parallel processing, can improve the efficiency of the computation and allow the inclusion of more complex sets of species and reactions to improve understanding of plume degradation processes.

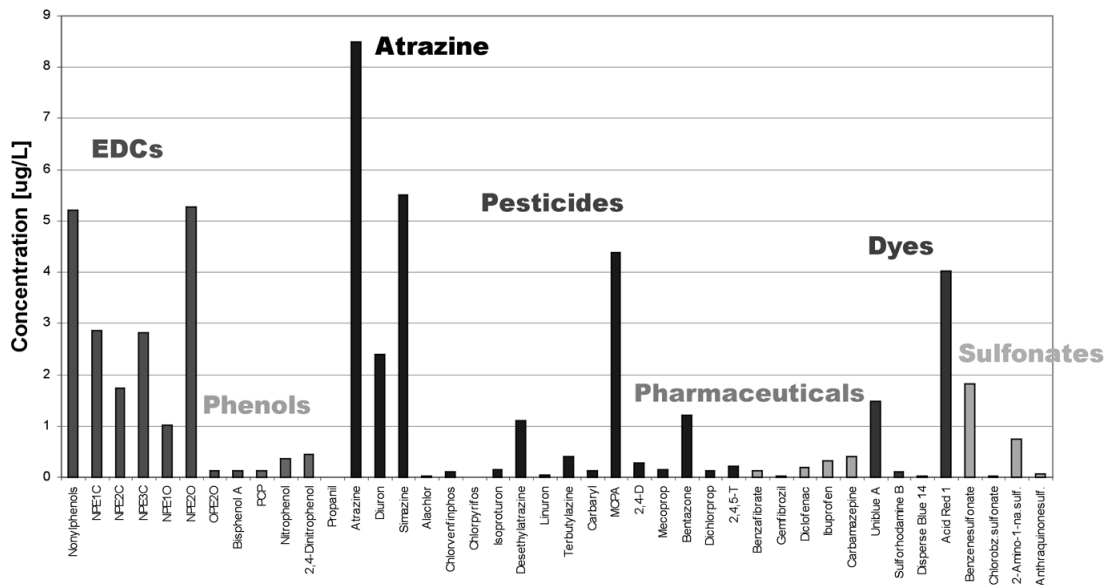


Fig. 2 - Concentrations of various pollutants measured in samples of Belgian river water.

This method was applied to investigate biodegradation of contaminants at a field test site.

Biodegradation effects have been incorporated into a reactive transport model of phenolic contaminants in a groundwater (Watson *et al.*, 2005b). Simulation of biodegradation in the anaerobic plume core allowed the process rates to be estimated from hydrochemical field data obtained from discrete sampling points. Microbial acclimatisation, substrate toxicity toward degradation, bioavailability of mineral oxides, and adsorption of biogenic species in the aquifer were taken into consideration. Processes at the plume core such as fermentation and *Fe(III)*-reduction appear to have potentially greater impact on degradation than those at the plume edge, such as aerobic respiration, denitrification, and SO_4 -reduction.

Mesoscale imaging techniques can be used to determine deposition and transport parameters from non-invasive, non-destructive, high spatial resolution data. Colloid and solute transport in saturated quartz sand were investigated using a fluorescent tracer technique (Bridge *et al.*, 2006). Experiments were carried out at constant flow rates and ionic strengths and relative changes in the mass distribution of the colloid and solute tracers with time were measured. It was observed that increasing ionic strength resulted in a significant reduction in the colloid transport velocity. This change in velocity corresponded to an increasing fraction of colloid mass retained along the flow path.

Rees *et al.* (2007) employed flow reactors containing quartz sand colonised with biofilms as model aquifers to study degrading plumes of acetate or phenol emitted from a point source. The fluorescent tracer technique was used together with chemical and biological sampling to quantify transport and sub-surface chemistry. There was a significant reduction in carbon concentration between the point of injection and the outflow due mainly to dilution but also as a result of biodegradation. Two-dimensional imaging of the aqueous oxygen concentration allowed

Table 1 - European Directives relating to water quality.

2000/60/EC	Water Framework Directive
2006/118/EC	Groundwater Directive
COM(2006) 397 final	Water Quality Standards
76/464/EEC	Dangerous Substances Directive
98/83/EC	Drinking Water Directive
2006/7/EC	New Bathing Water Directive
91/271/EEC	Urban Waste Water Directive
91/676/EEC	Nitrates Directive

quantification of contamination depletion in the plume and provided an indicator of the microbial respiration associated with biodegradation. The microbiological, chemical, and oxygen imaging data showed that biodegradation was greatest at the plume edge. Numerical simulations were compared with the experimental data to verify models of plume degradation processes.

Complex aquifer configurations can be more accurately represented by employing numerical modelling techniques that permit a geometrically flexible choice of discrete points (Matthai *et al.*, 2007). By local refinement of the grid of discrete points, in areas of specific interest, it is possible to predict the flow towards an abstraction well with higher precision (see Fig. 3). Incorporating three-dimensional geological models (Kessler and Matthews, 2004) into the groundwater flow simulations permits an improved understanding of the hydrogeological processes. It would similarly be desirable to increase the number of sampling points for water analysis in field tests, which could be achieved by replacing conventional analytical methods with automated biosensing techniques in order to obtain higher spatial resolution.

4. EU legislation on water quality

The Water Framework Directive (European Commission, 2000) sets out the details for the establishment and implementation of Community-wide legislation for the protection and sustainable use of water resources, including inland surface waters, transitional waters, coastal waters and groundwater. Its main aims are:

- (i) to prevent further deterioration and protect and enhance the status of aquatic ecosystems;
- (ii) to promote sustainable water use based on a long-term protection of available water resources;
- (iii) to enhance protection and improvement of the aquatic environment through the progressive reduction, cessation or phasing-out of discharges, emissions and losses of priority substances;
- (iv) to ensure the progressive reduction of pollution of groundwater and prevent its further pollution;
- (v) to contribute to mitigating the effects of floods and droughts.

Fulfilment of these objectives is expected to contribute to:

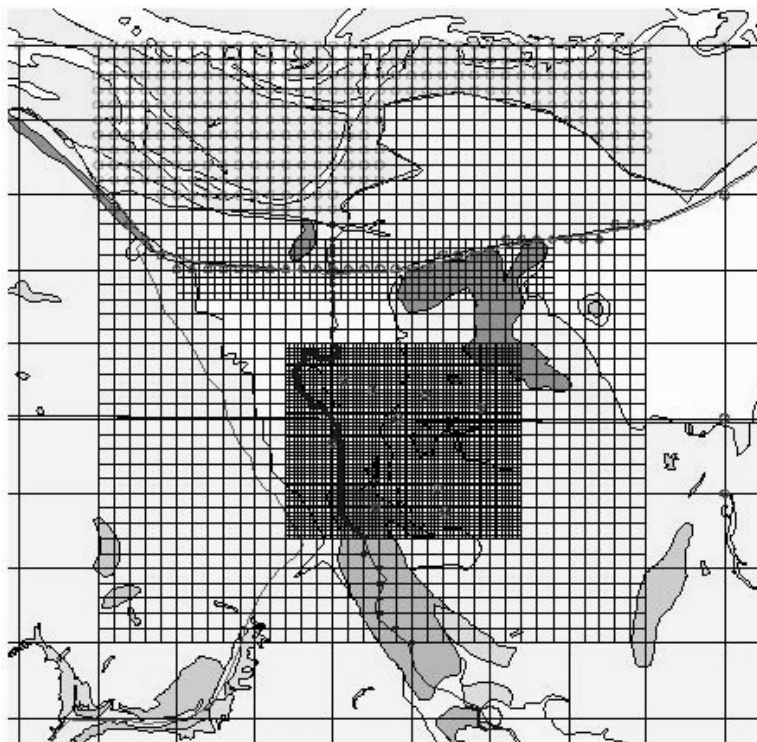


Fig. 3 - Local refinement of the array of discrete points in a ZOOM simulation to simulate small-scale groundwater flow processes more accurately. Reproduced with the permission of the British Geological Survey ©NERC. All rights reserved.

- (i) the provision of the sufficient supply of good quality surface water and groundwater as needed for sustainable, balanced and equitable water use;
- (ii) a significant reduction in pollution of groundwater;
- (iii) the protection of territorial and marine waters; and
- (iv) preventing and eliminating pollution of the marine environment, by the cessation or phasing out of discharges, emissions and losses of priority substances.

Under the provisions of the Water Framework Directive a monitoring programme should be set up within each river basin district to allow collection of physical, chemical and biological data required for assessing surface and groundwater quality. An indicative list of the main pollutants to be monitored is provided. The management plan for each river basin district should contain a summary of the impact of anthropogenic activity on the status of surface and ground waters, including point source pollution and diffuse pollution. One of the aims of the Directive is to achieve a good chemical status for surface water, a standard that would be attained by meeting the environmental quality standards for priority substances and various other pollutants.

A daughter directive regarding the protection of groundwater (European Commission, 2006a) has recently been approved, and a proposal has been made for a second daughter directive (European Commission 2006b) dealing with environmental quality standards for surface water (rivers, lakes, estuaries and coastal waters) and the control of pollutants specified in a list of

Table 2 - Target analytes of the Dangerous Substances Directive and the Water Framework Directive.

DSD	WDF	DSD and WFD
DDT and metabolites	Alachlor	Cadmium and compounds
(DDD, DDE)	Anthracene	1,2-Dichloroethane
Aldrin	Atrazine	Hexachlorobenzene
Dieldrin	Benzene	Hexachlorobutadien
Endrin	Brominated Diphenylethers	Hexachlorocyclohexane
Isodrin	C10-13 Chloroalkanes	Mercury and its compounds
Carbon tetrachloride	Chlorfenvinphos	Pentachlorophenol
Perchloroethylene	Chlorpyrifos	Trichlorobenzene
Trichloroethylene	Dichloromethane	Trichloromethane
	Di(2-ethylhexyl)phthalate	
	Diuron	
	Endosulfan	
	Isoproturon	
	Lead and its compounds	
	Mercury and its compounds	
	Nickel and its compounds	
	Nonylphenols	
	Octylphenols	
	Pentachlorobenzene	
	Pentachlorophenol	
	Polyaromatic Hydrocarbons	
	Simazine	
	Tributyltin compounds	
	Trifluralin	

priority substances. There are currently 33 priority substances listed and these will be reviewed at regular intervals. Some of these substances were already included in the Dangerous Substances Directive (European Commission, 1976). A complete list of all the target substances that are covered by one or both these Directives is given in Table 2.

Maintaining water quality will contribute to ensuring the safety of the drinking water supply. The objective of the Drinking Water Directive (European Commission, 1998) is to protect human health by ensuring that water intended for human consumption is wholesome and clean. Among the various provisions of this Directive are:

- (i) setting of parametric values for the most common substances found in drinking water;
- (ii) establishment of the point of use as the point of compliance with the quality standards;
- (iii) the obligation for Member States to report regularly to the Commission on drinking water quality;
- (iv) the obligation to inform consumers on drinking water quality and measures that they can take to comply with the requirements of the Directive.

The EU has also established limits for the physical, chemical and microbiological parameters

of water used for bathing. The new Bathing Water Directive (European Commission, 2006c) lays down provisions for:

- (i) the monitoring and classification of bathing water quality;
- (ii) the management of bathing water quality;
- (iii) the provision of information to the public on bathing water quality.

The Directive is intended to preserve, protect and improve the quality of the environment and to protect human health. It complements and is integrated into other measures to protect the quality of water (rivers, lakes, coastal waters and groundwater) under the Water Framework Directive. It sets the minimum quality criteria that have to be met by bathing water with regard to:

- (i) the physical, chemical and microbiological parameters;
- (ii) the mandatory limit values and indicative values for such parameters; and
- (iii) the minimum sampling frequency and method of analysis or inspection of such water.

Two other European Directives with relevance to water quality should be mentioned: the Urban Waste Water Directive (European Commission, 1991a) and the Nitrates Directive (European Commission, 1991b). The aim of the Urban Waste Water Directive is to protect the environment from the adverse effects of urban waste water discharges by:

- (i) collection and treatment of waste water;
- (ii) secondary treatment of discharges;
- (iii) pre-authorisation of discharges of urban wastewater, discharges from the food-processing industry and industrial discharges into urban wastewater systems;
- (iv) monitoring of the performance of treatment plants and receiving waters;
- (v) control of sewage sludge disposal and re-use, and treated waste water re-use.

The Nitrates Directive implements measures to reduce the environmental impacts of excess nitrogen, in particular eutrophication, by:

- (i) identification of polluted or threatened waters;
- (ii) protection of living resources and aquatic ecosystems;
- (iii) prevention of eutrophication; and
- (iv) designation of vulnerable zones such as areas of agricultural land making a significant contribution to nitrogen pollution.

5. Biosensing techniques for water quality monitoring

The successful implementation and effectiveness of the Water Framework Directive will depend on the availability of reliable and cost effective monitoring methods. In addition to classical laboratory analytical techniques, sensor and biosensor technologies could play an important role as alternative methods for water quality monitoring. Sensors and biosensors are useful analytical tools for environmental monitoring because they provide rapid and less costly analysis data. Technological advances in this field have led to the development of portable instruments, capable of real time measurements and remote operation. As well as measuring concentration levels, biologically based techniques are able to provide an early warning of potential biological effects due to pollutants in the environment by the use of biomarkers.

Biosensors consist of a biological sensing element, which may be an antibody, receptor,

enzyme or whole cell, interfaced to a transducer, such as an electrode or optical fibre. The two essential properties of a biosensor are its specificity and sensitivity towards the target analyte. The specificity is determined by the biological component while the sensitivity depends on both the biological reaction and the efficiency of the transducer. Two recently published reviews (Farré *et al.*, 2005, Roig *et al.*, 2006) cover advances in sensors, biosensors, bioassays and immunoassay techniques for monitoring water pollution. Biosensors are able to detect and measure concentrations of pollutants and to perform toxicity analysis of water samples. Devices have been developed for the detection of genotoxicity and for monitoring general water toxicity both in the laboratory and at field sites. Biosensors are also available for monitoring PAHs, pesticides and heavy metals, while enzyme assays are being tested to detect phenols.

The Strategic Research Agenda of the Water Supply and Sanitation Technology Platform (2005) has identified as a major priority the development of sensors for monitoring water quality. Innovative biological and electronic sensing techniques and long-life low power systems are needed in order that monitoring and measurement technology can be further improved. This would include the development of in-pipe sensors and could eventually lead to decentralised control of water systems. Water quality monitoring requires measurement and control of chemicals and toxins with simple and cost-effective techniques employing automated real-time analysis with an early warning capability. It is therefore necessary to update current methods that are expensive to operate and have significant delays between sampling and data analysis. The application of detection methods based on biological effects and molecular recognition techniques would result in more efficient monitoring and higher spatial resolution information for improved correlation with health effects. Sensors deployed at raw water sites could monitor spatial and temporal changes in concentrations of pollutants that could be integrated into contaminant migration models to provide real time assessment of water quality.

The use of innovative biomolecular-based monitoring methods would allow the assessment of the ecotoxicological status of water bodies, identification of specific biomarkers and the development and implementation of more sensitive techniques for assessing water quality based on DNA arrays and proteomics. Bioassay and immunoassay techniques for the analysis of toxins and pollutants rely on the use of fluorescent labelled antibodies or receptors that activate a signal when binding occurs. The use of biomarkers is based on the detection of a biological response due to pollutant exposure, for example the activation of a specific gene, before the onset of observable damage such as reproductive disorders, disease or death. Biomarker signals can provide an early indication of environmental and health effects of pollution before its more serious consequences become apparent.

A biomarker is defined as an alteration in a biological response, from molecular through cellular to physiological levels resulting from exposure to, or toxic effects of environmental chemicals (Peakall, 1994). Biomarkers are powerful methods for the detection of pollutants in aquatic systems and assessment of water quality. An extensive review of biomarkers in aquatic organisms has been made by Van der Oost *et al.* (2003). Molecular biomarkers, based on effects at cellular and genetic level are currently under development. These are, potentially, extremely useful indicators because they could allow early detection of ecological damage due to xenobiotics.

Rogers (2006) has reviewed the application of enzyme, antibody- and cell-based biosensors

for environmental monitoring. Enzyme biosensors have advantages that include a stable source of biorenewable material, the ability to modify the specificity by genetic engineering, and the catalytic amplification of the enzyme activity. The disadvantages are the small number of suitable substrates, the limited interaction between pollutants and enzymes, and the poor specificity. Antibody-based biosensors are more versatile because antibodies are available that specifically bind to individual compounds with high affinities. Their disadvantages are the complex assay formats and the limited number of compounds detected by an individual assay in comparison to the numerous compounds in typical samples. Cell-based biosensors include bacteria, yeast, algae and tissue culture cells. Genetically modified microorganisms have been used to construct biosensors that respond to non-specific stressors such as DNA damage, gamma radiation, heat shock, and oxidative stress; toxic metals, organic pollutants and compounds such as nitrate, ammonia and antibiotics.

Rodriguez-Mozaz *et al.* (2004, 2006a) surveyed a range of biosensors for the detection of organic and inorganic pollutants, with particular reference to their application to real-world environmental samples. The toxicity and genotoxicity of substances cannot be adequately assessed on the basis of concentration alone; the use of whole organisms, such as bacterial cells, enables this to be done more effectively. Endocrine disruptors can be monitored by biosensors based on natural estrogen receptors. DNA biosensors are more specific than immunological sensors for rapid identification of contamination by microorganisms, while immunosensors are faster and more robust than the DNA methods and can detect not only contaminating organisms but also the biotoxins they produce. Biosensors and immunosensors have been developed to detect PCBs, and DNA methods to detect PAHs. Immunosensors can be used for the detection of estrone, progesterone and testosterone, and organic pollutants such as Bisphenol A. Enzymatic sensors, based on the inhibition of a selected enzyme are the most widely used type of biosensor for the detection of pesticides. Enzyme biosensors can also be used for detecting the presence of phosphates in surface waters, which is an indicator of eutrophication. Biosensors using bacterial cells and enzymes are effective for detecting toxic metals and bacteria-based biosensors are being developed for the monitoring of nitrates in tap water and urban wastewater.

Dominguez and Alcock (2002) have summarised the conclusions of a workshop on sensor technologies for contaminated sites and groundwater pollution. The workshop dealt with contaminated land and water management and monitoring tools for bioremediation, site characterisation, and groundwater risk assessment. Techniques for environmental monitoring have advanced considerably as a result of the application of the results of fundamental sensor research and the adoption of integrated approaches for the management of water resources. Laboratory and field tests assist in the establishment of quality assurance protocols to enable the exploitation of sensor technologies for monitoring water pollution. Rugged instruments, based on electrochemical or optical transducers, are being developed for field use. The target analytes include chloro-organics, BTEX, PAHs, and heavy metals. Numerical simulations are a useful and inexpensive method for groundwater risk assessment but validation of models to improve their accuracy requires multilevel sampling and multiparameter detection methods. Multiparameter sensing techniques can be applied for general water quality assessment as well as to analysis of priority pollutants. It can be anticipated that novel technologies, such as molecularly imprinted polymers, will increase sensor performance and analytical precision.

Jiang *et al.* (2008) have provided an overview of the application of immunosensors for the detection of pesticide residues. Immunosensors utilise immobilised antibodies or antigens as the biorecognition element and have potential applications for rapid detection and screening. Electrochemical, optical, piezoelectric, or nanomechanical transduction systems can be used. Immunosensors differ from immunoassays in that the transducer forms an integral part of the analytical device, which may be labelled or label-free. There are two types of labelled immunosensors: sandwich assays and competitive assays. In the first type, the antibody immobilised on the transducer surface binds the analyte molecule and a labelled secondary antibody binds to the captured molecule, so that the response is proportional to the analyte concentration. In the second type, the analyte competes for the available antibody binding sites with labelled analyte and the signal varies inversely with analyte concentration. Label-free assays detect the binding of the analyte and the antibody on the transducer surface. The direct type has a response that is proportional to the concentration of analyte. The indirect type has an antigen (pesticide-protein conjugate) immobilised on the transducer surface that reacts with a pesticide-antibody mixture and antibody binding to the immobilised conjugate is inhibited in the presence of the pesticide. Immunosensors have excellent selectivity and sensitivity, can be remotely operated for use in the field, give rapid results and are able to achieve the low limits of detection required by environmental regulations.

Immunoassay techniques allow continuous monitoring of pollutants in a large number of samples. However, systems such as enzyme-linked immunosorbent assay (ELISA) require several hours to complete an analysis. By combining immunoassay methods with sensor systems, pollutants in natural water samples can be analysed rapidly, without the need for intermediate preparation or washing steps. Mauriz *et al.* (2006a, 2006b) have described a portable surface plasmon resonance optical immunosensor system for monitoring organic pollutants in drinking water, river water and groundwater. The sensing mechanism depends on variations of the refractive index close to the sensor surface due to the interaction of the analyte with the immobilised recognition element. Limits of detection (LOD) were 20 ng l⁻¹ for DDT, 50 ng l⁻¹ for chlorpyrifos and 0.9 µg l⁻¹ for carbaryl, which are lower than the maximum concentrations allowed by EU legislation. Covalent immobilization of the analyte derivative by an alkanethiol self-assembled monolayer allowed reuse of the sensor surface for more than 250 regeneration cycles during a month of continuous operation. The analysis time for a complete cycle, including regeneration, was 24 minutes. The small size of the device and the capability for wireless data transmission make it suitable for continuous monitoring applications at remote sites.

Gobi *et al.* (2005) have developed a reusable optical surface plasmon resonance (SPR) immunosensor for direct, label-free detection of 2,4-dichlorophenoxyacetic acid (2,4-D). The 2,4-D analyte was conjugated with bovine serum albumin (BSA) protein and the conjugate was immobilised on the gold film sensor chip by physical adsorption. The antibody 2,4-D-Ab was immobilised by selective immunoreaction on the 2,4-D-BSA physisorbed layer. The sensor showed negligible response to BSA or a non-related antibody, indicating that it was resistant to non-selective adsorption of proteins. Free 2,4-D was detected using a competitive immunoassay format by co-injecting the sample and the antibody over the sensor chip. The system was able to detect concentrations in the range 0.5 ng ml⁻¹ to 1 µg ml⁻¹ with a response time of 20 minutes. The sensor surface was regenerated by flushing it with a solution of 10 ppm pepsin in 0.2M

glycine-HCl buffer (pH 2.0) for 2 minutes. This allowed up to 20 measurement cycles using a single immobilised conjugate layer on the sensor surface without a noticeable reduction in performance.

6. Immunosensors for water analysis

Rapid and cost-effective analytical methods for water quality monitoring are becoming increasingly essential because of the large quantities of natural hormones and other endocrine disruptors discharged into surface waters due to inadequate wastewater treatment. For water quality monitoring applications immunosensors need to have a limit of detection (LOD) and a limit of quantification (LOQ) comparable with those of conventional analysis methods. The application of immunoassay methods for water quality monitoring has however been limited because automated systems were not available for routine analysis. Tschmelak *et al.* (2004a, 2005a) have developed a fully automated RIVER ANALYser (RIANA) immunoassay with an LOD for estrone below 0.20 ng l^{-1} and an LOQ below 1.40 ng l^{-1} . Unlike analytical methods such as GC-MS or HPLC-MS, which typically have a LOD of around 1 ng l^{-1} , this system requires no pre-treatment and pre-concentration of the sample. The high sensitivity is due to the use of an antibody with a high affinity for estrone. The system uses labelled antibodies to detect target analytes and a detection method based on total internal reflection fluorescence (TIRF). Light from a 635 nm wavelength red laser diode, coupled to a glass transducer, is guided by total internal reflection and the evanescent field excites the fluorescent dye at the surface. Sample injection and data acquisition are fully automated and computer controlled. A single analysis cycle, including washing, sample injection and regeneration of the surface takes approximately 12 minutes.

The RIANA system can also be used to detect other organic compounds such as endocrine disruptors, various hormones, pesticides and antibiotics, and is suitable as a rapid and cost-effective water quality surveillance system with an early warning capability. Tschmelak *et al.* (2004b) have described its application for the detection of propanil, a widely-used herbicide that is a common contaminant of water. Optimisation of the immunoassay by using different immobilised derivatives and reducing the amount of the antibody enabled an LOD of 0.6 ng l^{-1} and an LOQ of 4.5 ng l^{-1} to be achieved. The system has also been adapted, by using an appropriate antibody (Tschmelak *et al.*, 2004c), to detect progesterone, a suspected human carcinogen commonly present in surface water. The working range is approximately 1 ng l^{-1} to $10 \text{ } \mu\text{g l}^{-1}$, so the immunosensor is able to determine progesterone levels in water samples over a wide range of concentrations. In the case of progesterone, the LOD was 0.37 ng l^{-1} , which is lower than that for conventional analytical methods.

In order to verify the performance of this optical immunosensor system, the LOD and the LOQ were evaluated statistically from a simultaneous multi-analyte calibration with atrazine, bisphenol A, and estrone (Tschmelak *et al.*, 2004d). It was possible to achieve an LOD of less than $0.020 \text{ } \mu\text{g l}^{-1}$ without pre-treatment and pre-concentration of the sample for all three compounds in a simultaneous analysis. This is comparable with the sensitivity attained with the traditional analytical techniques. Based on this calibration data, real water samples with complex matrices obtained from different water bodies, groundwater sources, and tap water were analysed. The results obtained using the immunoassay technique and conventional analytical methods, like

GC–MS and HPLC–DAD, were comparable for all three analytes. Calibration with isotroturon in the single analyte mode gave an LOD of $0.016 \mu\text{g l}^{-1}$, and an LOQ of $0.091 \mu\text{g l}^{-1}$. The reproducibility, recovery rate and long-term stability of the system were checked by carrying out repeated, independent tests. It was found that over 400 analysis and regeneration cycles could be made without any reduction in performance.

The RIANA immunosensor was applied by Rodriguez-Mozaz *et al.* (2005) to determine the concentration of bisphenol A in various water samples taken at a waterworks, from raw river water to treated drinking water. Human exposure to this compound is of concern because it is a known endocrine disruptor. The LOD for the direct determination of bisphenol A in these water samples was $0.014 \mu\text{g l}^{-1}$. Satisfactory reproducibility was obtained, with a standard deviation between 1.48% and 6.93%. The immunoassay results were validated against those from the traditional method LC–MS. The analyses were in reasonable agreement, after allowance for the characteristic overestimation of immunoassays, and confirmed the removal efficiency of the filtration process employed at the waterworks. Other workers have also employed bioassay techniques to detect bisphenol A. Matsunaga *et al.* (2003) developed an automated immunoassay system using monoclonal antibodies chemically conjugated to bacterial magnetic particles, while Soh *et al.* (2003) used indirect competitive immunoassays based on surface plasmon resonance. Enzyme-linked immunosorbent assays (ELISA) were used by De Meulenaer *et al.* (2002) and Zhao *et al.* (2002) for the analysis of bisphenol A in environmental and biological samples.

Rodriguez-Mozaz *et al.* (2006b) also used the RIANA system for the simultaneous detection of three other environmentally significant pollutants: the pesticides atrazine and isotroturon and the estrogen estrone, in real water samples. Atrazine and the isotroturon are among the priority substances to be monitored under the Water Framework Directive. Estrone is a natural estrogen and has important health and environmental implications owing to its endocrine disruption properties. The immunosensor technique was used with LC–MS to monitor the removal of these three compounds during water treatment, consisting of sand filtration, ozonation, activated carbon filtration and chlorination. The immunoassay results tended consistently to overestimate the concentrations, probably as a result of matrix and cross-reactivity effects.

An improved analytical system AWACSS (Automated Water Analyser Computer Supported System) based on immunsensor technology has been recently developed (Proll *et al.*, 2005; Tschmelak *et al.*, 2005b), that is capable of detecting of up to 32 compounds simultaneously for on-line, unattended, centrally controlled monitoring. The system consists of an optical transducer chip, an autosampler, a personal computer at the sampling site and a remote server. Communication via wireless network between the measurement and control stations allows global management, trend analysis, and early warning. The binding inhibition format is used, with microfluidics technology to control sample injection and fluorescence detection on the surface of an integrated optical chip (see Fig. 4). An optical waveguide distributes light from a semiconductor laser to the individual sensing windows on the chip surface. Each window is sensitive to a specific analyte enabling rapid, simultaneous and high sensitivity detection of up to 32 organic compounds by means of a fibre optic system that monitors the individual fluorescence signals. The performance of the system was evaluated for a number of common pollutants (estrone, propanil, isotroturon, atrazine, bisphenol A, sulphonamides, and progesterone) in samples of tap water and surface water. Detection limits were generally in the nanogram or sub-

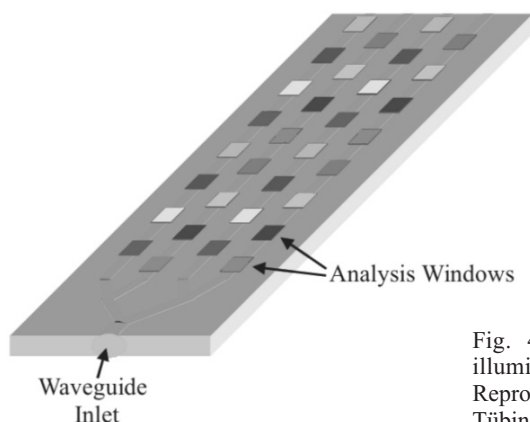


Fig. 4 - Integrated optical chip capable of simultaneously illuminating 32 analysis windows in a miniaturised format. Reproduced with the permission of Dr. Günther Proll, University of Tübingen.

nanogram per litre range; the LOD for estrone was 180 pg l^{-1} .

The performance of the AWACSS system was also compared to that of the conventional analytical techniques SPE-LC-DAD UV, SPE-LC-MS and GC-MS, and the alternative immunoassay methods RIANA and ELISA in inter-laboratory trials (Tschmelak *et al.*, 2005c) using atrazine, bisphenol A and estrone in surface water, groundwater, drinking water and wastewater matrices. Recoveries of atrazine and bisphenol A ranged between 82 and 126% of the actual values, while those for estrone were between 71 and 136%. The AWACSS results were in general less overestimated than those obtained by the ELISA and RIANA techniques. Detection limits of all the analytes considered were in the nanogram per litre range, while selectivity was sufficient to allow trace analysis. A single measurement cycle comprising washing steps, injection of the sample and regeneration of the surface required less than 18 minutes and more than 70 analyses a day were performed in the fully automated mode.

The biosensors discussed so far were developed mainly for the analysis of freshwater but Kröger *et al.* (2002) have also discussed potential applications in studies of marine pollution. While a variety of sensors have been used previously in oceanographic research to determine physical parameters, such as temperature, conductivity, depth and turbidity, there is also a need for real-time chemical and biological information. Continuous monitoring systems are required to detect contaminants, their source, distribution, concentration, persistence and uptake. However, the marine environment presents particular challenges to the integrity of systems deployed remotely and operated unattended for long periods of time. Some of the problems in developing biosensors for the marine environment are the lack of stability of antibodies, enzymes and natural receptors, biofouling of sensors immersed in seawater for long periods and the need for low power consumption electronics and extended lifetime batteries.

7. Molecularly imprinted polymers

The stability of biosensors can be improved by the use of molecular imprinting technology to

create artificial receptors in synthetic polymers by the use of suitable templates (Fig. 5). The target analyte is mixed with a functional monomer and a cross-linking monomer in a solvent and this mixture is copolymerised. The monomers form a complex with the imprint molecule and after polymerization, the functional groups are stabilised by the cross-linked polymeric structure. Subsequent removal of the template by solute extraction leaves binding sites in the polymer containing functional groups complementary to those of the template molecule and able to selectively bind the analyte molecule, in a similar manner, to natural receptors (Wulff, 1995; Mayes and Mosbach, 1997; Kröger *et al.*, 1999).

The molecular imprinting technique can be applied with almost any target molecule, while the affinity and specificity of the artificial receptor depend on the template molecule and the conditions used for polymer synthesis. Imprinting of organic molecules such as pesticides, pharmaceuticals, amino acids, peptides, nucleotide bases, steroids and sugars, is now routine (Haupt, 2001). The complex between the monomers and the target molecule can be formed either by reversible covalent bonding, non-covalent bonding, or a combination of the two. Non-covalent imprinting allows a more flexible choice of functional monomers and target molecules but, due to the greater stability of the bonds, covalent imprinting provides a more homogeneous population of binding sites and higher imprinting efficiency. The specificity and stability of molecular imprinted polymers make them viable alternatives to biological receptors in many applications. Haupt (2003a, 2003b) has reviewed recent advances in this field, with emphasis on immunoassays and biosensors. The artificial recognition sites in the polymers mimic the ability of biological receptor molecules to recognise and bind specific target molecules but they differ in that they are large, rigid, and insoluble, whereas natural receptors are smaller, flexible, and generally soluble.

Biosensors for environmental monitoring are an important application area for molecularly imprinted polymers, including the development of artificial receptors and for algal toxins and pesticides and herbicides. Piletsky *et al.* (2001) have developed a technique for substituting antibodies and receptors in enzyme-linked immunosorbent assays (ELISA) with molecularly imprinted polymers based on aniline and thiophene derivatives. Oxidative polymerization of a mixture containing the template, 3-aminophenylboronic acid, 3-thiopheneboronic acid and aniline was carried out in water and the imprinted polymer was used to coat the polystyrene surface of the microplate. The analytes chosen for investigation were low molecular weight organic compounds, atrazine and epinephrine, and several proteins with different molecular weights, microperoxidase, horseradish peroxidase and lactoperoxidase. Affinities decreased by only 10–20% over a period of two months, indicating good stability of the molecularly imprinted coatings. This was evidently due to partial cross-linking of the polymers during polymerization and fixation of the polymer chains to the surface of the microplate.

Chianella *et al.* (2003) have employed a molecular imprinting technique to synthesise an artificial receptor for microcystin-LR, a toxin produced by freshwater Cyanobacteria that contaminates drinking water supplies. A computational method was used to design a molecularly imprinted polymer with high specificity for microcystin-LR (Chianella *et al.*, 2002). A virtual library of functional monomers was screened against the target toxin and those having the highest binding energy were further investigated by a molecular dynamics simulation. The performance of the artificial receptor was compared to that of monoclonal and polyclonal antibodies. It was

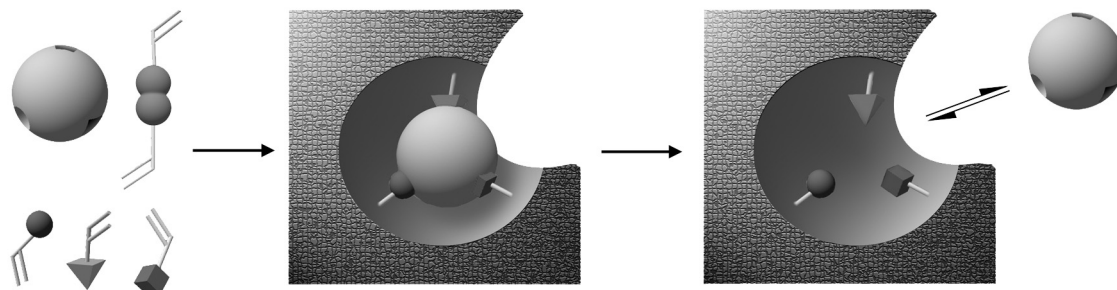


Fig. 5 - Creation of a molecular imprint: copolymerisation of functional monomers with a cross-linker forms an insoluble, cross-linked polymeric network around the template, the removal of which leaves complementary selective binding sites for the molecule. Reproduced with permission from K. Haupt 2003: *Analytical Chemistry*, 75, 376A-385A. ©American Chemical Society.

found that the affinity and sensitivity of the imprinted polymer were comparable to those of polyclonal antibodies, while the chemical and thermal stabilities were better. Cross-reactivity to other toxin analogues was low for the imprinted polymer compared with that observed for the antibodies. The imprinted polymer was used both for solid phase extraction (SPE) and as the sensing element in a piezoelectric sensor. Using SPE allowed preconcentration of the toxin in the injected solutions 100 times greater than that in the original sample, sufficient to achieve a minimum detectable amount of toxin of 0.35 nM, which is lower than the legally recommended limit of 1 nM for microcystin-LR in drinking water.

The covalent method has been applied to produce an imprinted polymer for the detection of organotin compounds (Gallego-Gallegos *et al.*, 2006), which are used in anti-fouling coatings for ship hulls. Tributyltin (TBT) derivatives have been widely applied because of their high toxicity to the target organisms and as a result elevated concentrations of organotins can be found in aquatic environments, both in sea-water and marine organisms such as shellfish. Thermodynamic measurements indicated that the covalent polymers had relatively homogeneous binding sites and improved selectivity compared with polymers synthesised using the non-covalent technique. The influence of electrostatic interactions on the binding behaviour was reduced because the main binding mechanism is reversibly covalent. Evaluation of matrix interference effects by performing tests on complex samples such as mussel tissue (CRM 477) confirmed of the high selectivity of the covalent imprinted polymers.

8. Conclusions

Biosensors integrated with wireless telecommunication systems could revolutionise water quality monitoring:

- nanobiotechnology- based diagnostic techniques can potentially detect all aquatic contaminants and toxins;
- miniaturisation by the application of integrated optics and microfluidic technologies significantly reduces sample volumes;

- multiparameter analysis allows simultaneous measurements for many different contaminants;
- the attainable limits of detection exceed EU requirements (~1 ppb) and are similar to those with conventional analysis techniques;
- remote surveillance and control capability permits continuous real-time monitoring, early warning, and fast response;
- improved spatial resolution and higher sensitivity enables detection of local sources of pollution;
- biosensors have simplified operation and reduced costs compared to the traditional analytical methods.

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