

Biochemical oxygen demand sensor arrays

K. Pitman, M. Raud and T. Kikas*

Estonian University of Life Sciences, Institute of Technology, Kreutzwaldi 56, EE51014 Tartu, Estonia. *Correspondence: timo.kikas@emu.ee

Abstract. Biochemical oxygen demand (BOD) is one of the most widely utilized parameters in water quality evaluation. BOD as a parameter illustrates the amount of organic compounds susceptible to biochemical degradation in the water. The BOD test lasts for at least 5–7 days or even up to 21 days. An incubation time this long is not acceptable for monitoring purposes or system control. In order to shorten the BOD measurement time, a multitude of biosensors have been proposed. Unfortunately, BOD biosensors have several limitations, such as short lifetime, limited substrate range, precision etc. Some of those limitations can be overcome by using microbial sensor-arrays. Such bioelectronic tongues can achieve the much wider substrate range usually attributed to multiculture sensors and still maintain the long lifetime of a single culture sensor. This is achieved by separating different cultures from each other in the array and using the signals of separate sensors to produce summarised information via statistical analysis. The purpose of this review is to give a short overview of BOD measurements and discuss the potential of using sensor-arrays for BOD measurements.

Key words: sensor-array, BOD sensor-array, electronic tongue, biosensor, biochemical oxygen demand.

INTRODUCTION

Water quality monitoring is an important aspect of water management with regard to pollution control. One of the most important water quality parameters is biochemical oxygen demand (BOD). This parameter was first introduced in 1917 and published in *Standard Methods* (Bourgeois, 2001). BOD is determined by means of an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewater, effluents and polluted waters (APHA, 1985; Tan & Wu, 1999; Bourgeois, 2001). The standardized test measures the oxygen required for the biochemical degradation of organic material and the oxygen used to oxidize inorganic material, such as sulphides, ferrous iron and reduced forms of nitrogen, unless their oxidation is prevented by an inhibitor (APHA, 1985). The results of a BOD test characterize the total content of biochemically oxidizable organic substances in the water as well as the ability of the water to self-cleanse (Ponomareva, 2011).

In a standardized BOD test, a sample is placed in a full, airtight bottle and incubated under the specified conditions (20 ± 1 °C, in the dark) for a specific time (APHA, 1985). The incubation period is 5 or 7 days according to the American (APHA, 1985) or Swedish standard (Liu & Mattiasson, 2002), respectively. The BOD value is calculated based on the difference between the initial and final dissolved oxygen concentrations (APHA, 1985; Liu & Mattiasson, 2002). The BOD value is measured in milligrams of

oxygen per litre or cubic decimetre ($\text{mgO}_2 \text{ l}^{-1}$ or $\text{mgO}_2 \text{ dm}^{-3}$). The BOD_5 values in surface water layers usually fall into the range of 0.5–4 mg l^{-1} (Ponomareva, 2011), while in industrial wastewaters BOD_5 may be as high as 30,000 mg l^{-1} . The precision of the method is around 15–20% (Namour & Jaffrezic-Renault, 2010).

The BOD test has been the most widely used method to measure organic pollution in water samples because of its wide applicability to different type of samples as well as its simplicity (Liu, 2014), since it requires no expensive equipment. However, due to the prolonged incubation time, it is not suitable for the monitoring or control of wastewater treatment systems where fast feedback is necessary (Raud, 2012a).

One way to overcome the long delay between the measurements and the results is to use biosensors. Depending on the measurement method, BOD biosensors can give results within 5 to 30 minutes (Kim, 2006; Kibena, 2012). Many papers on BOD biosensors have been published and these biosensors have been developed and marketed by various manufacturers in both biofilm and bioreactor-type configurations (Rodriguez-Mozaz, 2006). The purpose of this paper is to give an overview of the biosensors used in BOD measurements and to direct more attention to the possibility of using sensor-arrays for BOD measurements.

BIOSENSORS FOR BOD

A biosensor is defined as a self-contained integrated device capable of providing specific quantitative analytical information. A biosensor consists of a biological recognition element (Luong, 2008; Lagarde & Jaffrezic-Renault, 2011; Su, 2011), which is in direct spatial contact with a transduction element (Thévenot, 2001; Xu & Ying, 2011). A variety of transducers have been used in biosensors, such as electrochemical, colorimetric, optical, acoustic, luminescence, and fluorescence transducers. Furthermore, different biological sensing materials have also been used, such as microorganisms, tissues, organelles, receptors, enzymes, antibodies, nucleic acids, aptamers, cofactors, etc. The most frequently used ones are enzymes and microorganisms (D'Souza, 2001; Kissinger, 2005; Su, 2011).

The first BOD biosensor was reported by Karube in 1977 (Karube, 1977). It consisted of a dissolved oxygen electrode and a membrane impregnated with the yeast *T. cutaneum*. Since then, many BOD biosensors based on various measurement principles and biological sensing elements have been reported. Various microorganisms, including yeasts and viable cells of bacteria such as *Arxula adenivorans*, *Bacillus polymyxa*, *Bacillus subtilis*, *Candida*, *Escherichia coli*, *Hansenula anomala*, *Issatchenkia*, *Klebsiella*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Saccharomyces cerevisiae*, *Serratia marcescens*, *Torulopsis candida*, *Trichosporon* etc., have been used for the construction of BOD biosensors (Liu & Mattiasson, 2002; Raud, 2010b; Lagarde & Jaffrezic-Renault, 2011; Ponomareva, 2011). Microorganisms have been used in the form of a single pure culture, mixtures of several pure cultures, or mixed cultures, such as activated sludge or the BODSEED culture (Tan & Wu, 1999; Rastogi, 2003). BOD sensors based on a single strain have relatively good stability and a long service life (Kim, 2006), but the sensor-BOD value will be limited due to the narrow substrate spectrum of one microbial strain (Liu & Mattiasson, 2002; Raud, 2012), which may lead to an underestimation of BOD. In order to construct a BOD biosensor with a wider substrate spectrum, mixtures of several microbial strains or mixed cultures have

been used (Suriyawattanakul, 2002). However, compared to single strain biosensors, mixed culture biosensors have decreased stability and a shorter service life due to the different life-spans and growth rates of various microorganisms used in consortia (Liu & Mattiasson, 2002). Thermally killed cells have been used to overcome the instability of microbial consortia and to achieve a longer service life for biosensors (Ponomareva, 2011). Thermally killed cells do not need a periodic nutrients supply. On the other hand, living cells need careful maintenance and a supply of nutrients and minerals during storage (Liu & Mattiasson, 2002).

Most of the reported BOD biosensors fall into one of two types – biofilm and respirometric (also called bioreactor-type) biosensors. Biofilm-type BOD biosensors are based on measuring the change in the dissolved oxygen concentration due to the respiration of microorganisms in the proximity of the transducer (Ponomareva, 2011). Microorganisms may be immobilized directly onto the transducer or immobilized and placed as a separate film or membrane in close proximity to the transducer. The transducer is most commonly a dissolved oxygen sensor. Respirometric or bioreactor-type biosensors, on the other hand, are biosensors where the microorganisms are not attached to the transducer but float freely in the measurement solution and the dissolved oxygen concentration is measured directly from the solution. These systems provide a constant measurement of the respiratory activity of a microbial suspension (Ponomareva, 2011).

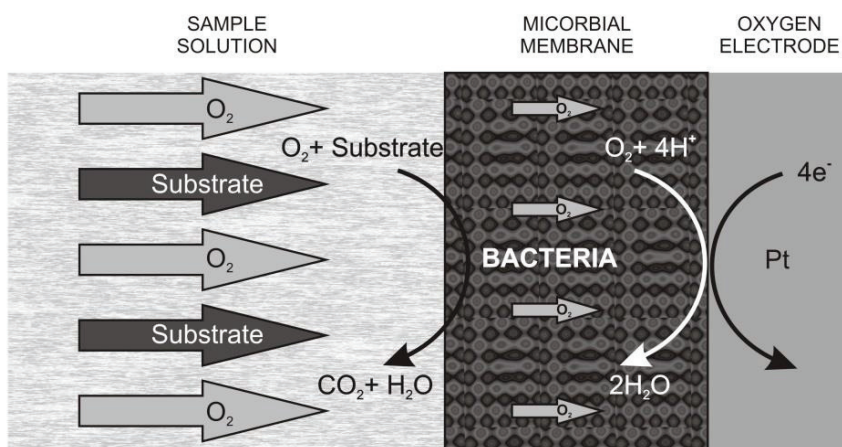


Figure 1. The basic principle of a microbial biosensor based on an amperometric transducer.

The working principle of a typical biofilm-type BOD biosensor is illustrated in Fig. 1. The microorganisms are immobilized or placed onto the oxygen electrode and the biosensor is immersed in the measurement solution. In a clean measuring solution the biosensor achieves a steady state current slightly lower than in the measurement medium, as most of the dissolved oxygen diffuses through the membrane but some is used up by microorganisms. When a sample containing biodegradable substrates is added to the measuring solution, the microorganisms start using oxygen for the assimilation of substrates at a certain rate and the measured oxygen concentration

decreases to a new and lower steady state. The decrease in the concentration of dissolved oxygen is proportional to the concentration of added biodegradable substrates. Based on the decrease in measured oxygen concentration, BOD can be calculated (Liu & Mattiasson, 2002).

With both biosensor types, the signal can be analysed using either the steady-state method or the kinetic method. The first one derives BOD from the current difference between two steady states, before and after adding the sample. It is often also called the end-point method. The kinetic method, on the other hand, uses the time derivative of the current right after the addition of the sample, and is also known as the initial rate, quasi-kinetic or dynamic transient method (Pasco, 2011).

The duration of measurement is 5–25 min in the stationary mode and 15–30 s with the initial rate method (Ponomareva, 2011). Recovery time is 15–60 min in the stationary mode and more than 10 min when using the initial rate method (Liu & Mattiasson, 2002). Hence, the initial rate method is preferable where a constant BOD monitoring is necessary, for example, when controlling a wastewater treatment plant or analysing a large number of samples (Ponomareva, 2011). The sensitivity of the initial-rate method, however, is twice as high as that of the stationary mode (Liu & Mattiasson, 2002).

The BOD values gained from BOD sensors do not always match the conventional BOD results due to differences in the measuring principles. The conventional BOD test has an incubation time of 5 or 7 days. In the course of this time the microorganisms can assimilate easily degradable compounds but also they have time to induce the necessary enzymes for the degradation of refractory compounds. However, during the short measurement time of a biosensor, the immobilized microorganisms are able to assimilate and thereby detect only easily degradable compounds, which may result in an underestimation of BOD values.

The problem with the underestimation of BOD could be overcome by choosing a suitable calibration solution. The most common calibration solutions are: a solution of equal parts of glucose and glutamic acid (GGA) (Ponomareva, 2011) and a synthetic wastewater according to the recipe established by the Organisation for Economic Cooperation and Development (OECD). Due to its simple composition, the GGA solution is unsuitable for studying samples of a more complicated composition (Liu & Mattiasson, 2002). Better results have been obtained with the OECD synthetic wastewater, as its composition closely resembles that of municipal wastewater (Liu & Mattiasson, 2002). Other artificial wastewaters have also been used for the calibration of BOD sensors (Chee, 2005; Chee, 2007). The ideal calibration solution would be as close to the composition of the wastewater to be analysed as possible (Liu, 2000; Liu & Mattiasson, 2002). Therefore, there is no universal calibration solution; rather, it must be chosen based on the composition of the sample to be later analysed.

Other ways to achieve a better match between the BOD values measured by different methods consist in preselecting microorganisms that have wide substrate spectra and are able to assimilate specific refractory compounds found in wastewater, or pre-incubating the living cells in a solution whose composition is similar to the sample to be analysed (Liu & Mattiasson, 2002). The pre-incubation helps living cells to start producing the enzymes that otherwise would not be present in the cells, thus widening their substrate spectrum.

BIOSENSOR ARRAYS

The principle of sensor-arrays is based on an analogy to the biological organization of the olfactory and taste systems of mammals, where millions of nonspecific receptors in nose and taste systems respond to different substances. The idea of artificially reproducing the natural response of a human to environmental stimuli was first published in 1943 (Vlasov, 2005); however, the first attempts to design an artificial olfactory system for smell were made in the 1960s (Vlasov, 2008), while non-specific sensor-arrays became commercially available in the mid-1990s (Bourgeois, 2003). According to the IUPAC (The International Union of Pure and Applied Chemistry) definition, ‘an electronic tongue is a multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate data analysis’ (Vlasov, 2005; del Valle, 2010). The basic principle of a sensor-array is shown in Fig. 2. Each sensor in an array produces an individual signal, which may not always correlate with the samples’ composition. The summarised signal of the sensor-array is analysed using statistical multivariate analysis methods, which enable extracting qualitative and quantitative information about the samples. Arrays of gas sensors are termed ‘electronic noses’ while arrays of liquid sensors are referred to as ‘electronic tongues’ (Escuder-Gilabert & Peris, 2010).

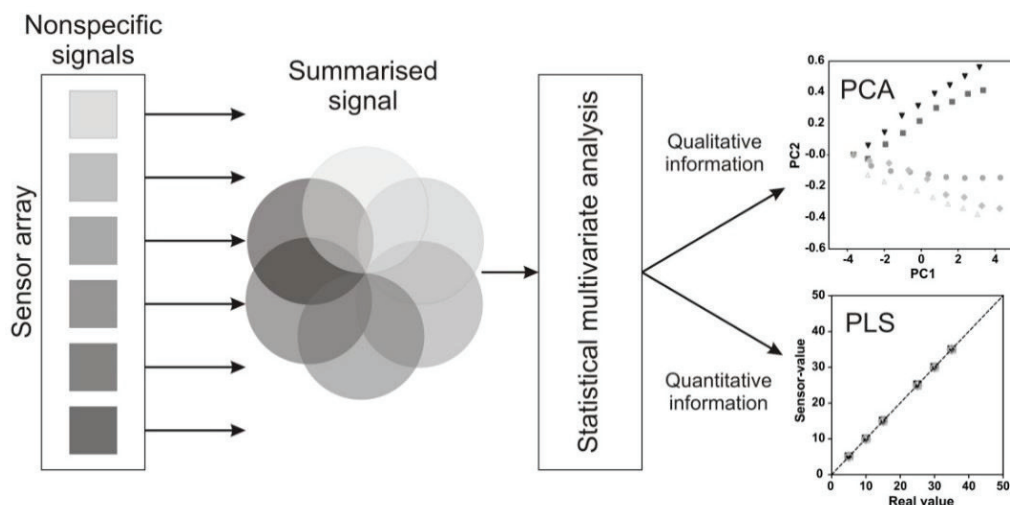


Figure 2. The working principle of a sensor array.

The most typical feature shared by electronic nose and electronic tongue systems is that an array of low selective and cross-sensitive sensors is conjugated with data processing and pattern recognition methods (Vlasov, 2008). Cross-sensitivity in this context is the ability of a sensor to respond to a number of different compounds in a solution and produce a stable response in the sample (Vlasov, 2005). Thereby, when the sensors are responding to several different substrates, the sensor-array creates a chemical image of the sample (Hruškar, 2010) or a signal pattern, which can be related to certain features or qualities of the sample (Krantz-Rülcker, 2001). In this way, the limited selectivity of each individual sensor will be compensated by the data processing, which

allows the determination of a species in the presence of its interference (del Valle, 2010). Electronic tongues are a powerful tool in the rapid assessment of information of complex solutions (Riul Jr, 2010).

Various sensing principles can be employed in sensor-arrays. The most widespread are electrochemical (del Valle, 2010) and optical sensors (Krantz-Rülcker, 2001; Vlasov, 2005; Witkowska, 2010); however, other techniques, such as surface acoustic waves (Krantz-Rülcker, 2001), piezoelectric mass sensors (del Valle, 2010) etc. have been reported. Usually, a single sensor-array consists of sensors of the same type; however, sensors based on different principles of signal transduction may be used in the same sensor-array. The number of sensors in the array may vary from 4 to 40 (Vlasov, 2005), depending on the analytical task and the number of different sensing materials available. Usually, a sensor-array consists of an excessive number of sensors compared to the analytes to be detected and is thereby applicable to different analytical tasks (Vlasov, 2005).

Various biosensor-arrays have been developed for different purposes. Several biorecognition elements can be used for biosensor-arrays, for example, microorganisms, cofactors or enzymes. The most widely used biorecognition elements are enzymes belonging to the classes of oxidoreductases and hydrolases (Solna, 2005). Enzymatic biosensor-arrays are promising pre-screening methods for rapid and simple measurements and an express analysis of many pollutants, which can function either directly as substrates or as inhibitors of the enzymes selected for the sensing array (Solna, 2005).

Multisensor electronic tongue systems are suitable for a diversity of analytical tasks, both conventional and nonconventional for chemical sensors. In recent years, much attention has been given to electronic tongue applications such as industrial and environmental monitoring, and quality control (Vlasov, 2008) (e.g. fermentation processes), as electronic tongues are capable of fast, inexpensive, automated and on-line control (Witkowska, 2010).

SENSOR-ARRAY DATA ANALYSIS

In case of a single biosensor, the linear regression model (LRM) is often used for data analysis (Badertscher & Pretsch, 2006). However, since sensors used in the array may respond to all analytes, a vast amount of multidimensional information is generated (del Valle, 2010). This complex data can be processed using multivariate analysis methods (del Valle, 2010; Vlasov, 2008). Multivariate treatment makes it possible to transform the complex responses of a sensor-array into a format that is easier to interpret. It has been shown that the use of biosensor-arrays with multivariate analysis can be a promising approach for simple, fast, reproducible, selective and sensitive detection of different compounds in various samples and provides both a qualitative and quantitative overview of sample compositions (Solna, 2005).

Qualitative information from sensor-array data is used for the classification and identification of samples. The most commonly used method for this purpose is the principal component analysis (PCA), which is widely used in statistical analysis to present the data (Riul Jr, 2010). PCA makes it possible to explore multivariate data and reduce its noise without loss of information; in addition, the significance of individual components can be assessed (Riul Jr, 2010). PCA is a linear multivariate analysis method

(Hines, 1999; Solna, 2005) whose mathematics is based on matrix algebra (Massart, 2004). PCA decomposes the initial data matrix into latent variables in such a way as to preserve as much variance as possible in the first principal components (PCs). Through this method, loading and score plots can be produced which show the relationship between the variables and the samples, respectively, as well as their influence on the system. The groupings in the score plot can be used for classification, since the more similar samples are grouped together (Krantz-Rülcker, 2001). PCA requires no prior knowledge of the samples and the data is presented in as few variables as possible.

In order to extract quantitative information from biosensor-array signals, a multivariate calibration method must be applied to connect the observed signals with the identity of an analyte and its concentration. However, for the calibration of sensor-arrays, a large amount of various samples are needed in order to divide the samples into two sets: training and test samples. There are several multivariate regression models for calibration available – these can be either linear or non-linear methods (Nascu, 1999). The most widely used methods are partial least squares (PLS) and principal component regression (PCR), which are both factor-based (Correia, 2005) linear calibration methods. PCR is conducted in a similar manner to PCA; however, when used in calibration, PCR performs a linear least squares regression of the dependent variable against the scores of the significant PCs (Hibbert, 1998). On the other hand, in PLS, a principal component analysis is performed on both the dataset and the corresponding actual values (Krantz-Rülcker, 2001). The difference between PCR and PLS is that PLS includes information about the function vector in the model while PCR does not (Hines, 1999). PLS is specially devised for quantification purposes and mainly used in multi-determination applications (del Valle, 2010). The partial least square (PLS) regression method is very useful in predicting a set of dependent variables from a large set of independent variables (Hruškar, 2010).

In case of non-linear data, other methods for data treatment are required. For non-linear data, artificial neural networks (ANNs) methods are widely used. ANN is a massively parallel computing technique, especially suited to non-linear sensor responses and very similar to human pattern recognition (del Valle, 2010). ANNs are distributed computing systems composed of processing units connected by weighted links that can be assembled in one or more layers, resembling the structure and functioning of the human brain (Hruškar, 2010; Riul Jr, 2010). Thereby, ANN creates models that are non-linear (Hibbert, 1998; Krantz-Rülcker, 2001; Hruškar, 2010) and non-parametric (Hines, 1999).

BOD SENSOR-ARRAYS

Various types of BOD sensor-arrays have been reported. Not all of them are based on biosensors but also chemical sensors have been applied. Some BOD sensor-arrays are outlined in Table 1.

Table 1. Overview of BOD sensor-arrays

Transducer	Sensor modification method	Immobilization method	Calibration solution	Application	Data analysis
Clark-type oxygen electrodes (Yang, 1997)	<i>Trichosporon cutaneum</i>	Photo-crosslinkable resin ENT-3400	GGA	BOD	Calibration graph
Polypyrrole conducting polymer sensors (Stuetz, 1999) (Bourgeois & Stuetz, 2002)	N/A	N/A	Wastewater samples	BOD	ANN, PCA
The sensor chip with four platinum containment electrodes (König, 2000)	<i>Sphingomonas yanoikuyae</i> B1, <i>Candida parapsilosis</i> ,	Poly(vinyl alcohol)-PVA	Synthetic wastewater	BOD, PAH*	Calibration graph
Polypyrrole conducting polymer sensors (Onkal-Engin, 2005)	N/A	N/A	Wastewater samples	BOD	Multiple discriminant analysis, canonical correlation analysis, ANN
8 screen printed Pt and Pt-graphite electrodes (Tønning, 2005)	Enzymes: tyrosinase, horseradish peroxidase, acetyl cholinesterase and butyryl cholinesterase	cross-linking with glutaraldehyde	N/A	N/A	Drift correction, PCA
CCD camera (Sakaguchi, 2007)	<i>Photobacterium phosphoreum</i> IFO 13896	Sodium alginate gel	GGA	BOD	Linear calibration graphs
8 metal electrodes (Au, Pt, Rh, N/A Ir, Ag, Ni, Co, Cu) (Campos, 2012)	N/A	N/A	Wastewater samples	BOD, COD*, NH ₄ -N, PO ₄ -P, SO ₄ -S, acetic acid, alkalinity	PLS
Clark-type oxygen electrodes (Raud & Kikas, 2013)	7 different microorganisms	Agarose	OECD synthetic wastewater	BOD	Sheffe test, PCA, PLS

*PAH – polycyclic aromatic hydrocarbons, COD – chemical oxygen demand.

Stuetz and colleagues used a non-specific electronic nose, which consisted of 12 electrodes coated with a polypyrrole-based conducting polymer doped with different dopants to monitor wastewater samples. The concentration of biodegradable organic matter as determined by BOD was measured in samples collected from different parts of the wastewater treatment facility. The BOD values were derived from the odour profiles of different samples and ANN was applied for data analysis. The results were compared to the corresponding conventional 5-day BOD values and a good correlation was obtained. However, a linear correlation between the sensor responses and BOD was only evident for up to 4 weeks (Stuetz, 1999). A similar approach of using a polypyrrole-based conducting polymer sensor-array for odour analysis was also applied later to a BOD analysis (Bourgeois & Stuetz, 2002; Onkal-Engin, 2005). In that study, a good correlation between odour and the corresponding BOD values as well as good classification accuracy were achieved. However, classification was difficult due to the large variability of wastewater, especially in facilities where domestic and industrial loads frequently alternated (Onkal-Engin, 2005).

Campos and colleagues applied a voltammetric electronic tongue, which consisted of 8 electrodes made of different metals, and PLS was used for data analysis to monitor various parameters in the influent and effluent of the wastewater treatment plant. The sensor-array showed relatively good predictive power for the determination of some parameters; therefore it might be possible to use this technology for semi-quantitative analysis (Campos, 2012).

A biosensor-array utilizing different enzymes was used to extract qualitative information, i.e. to study the quality of the wastewater treatment. However, the sensor performance was not easily characterized due to its decreasing sensitivity over time and the effect of inhibiting compounds. These problems were mitigated by using drift correction algorithms (Tønning, 2005).

One of the first biosensor-arrays with immobilized microorganisms was reported by Yang and colleagues, who used thin film technology to prepare miniaturized Clark-type oxygen electrodes. This dual-type BOD sensor consisted of two oxygen electrodes – one cathode functionalized with yeast and the other without it – and two anodes. The yeast *Trichosporon cutaneum* was immobilized onto the cathode with photo-crosslinkable resin and the GGA solution was used for sensor calibration, while the difference between the outputs of the two oxygen electrodes was used to estimate the BOD. The sensor was also used for an analysis of real samples and the results obtained were in good correlation with the conventional 5-day BOD values (Yang, 1997).

A different approach was employed by Sakaguchi, who used a biosensor-array based on immobilized luminous bacteria in arrayed holes on a microchip. Several different samples were analysed at the same time, since only one strain was used in all the micro-holes. The system used a digital CCD camera to detect the luminescence as well as a mobile PC, making on-site measurements available (Sakaguchi, 2007).

Konig and colleagues immobilized two different microbial strains, one of which was a PAH-degrading bacterium, into separate platinum electrode cavities. The biosensor chip was integrated into a flow-through system to measure the oxygen consumption of the immobilized microorganisms. Good correlations of BOD₅ and sensor-BOD results were achieved. In addition, while both strains responded to glucose, only the PAH-degrading strain gave signals with a naphthalene solution; as a result, the naphthalene concentration was successfully estimated with the sensor-array. Although

high concentrations of toxic substances were supposed to be present, none of the microbial sensors showed any decrease in sensitivity after measurements with these real samples (König, 2000).

Seven microbial cultures were used to construct a biosensor-array to measure BOD in different synthetic wastewater samples containing refractory compounds. The Scheffe test, PCA and multivariate calibration methods were applied to extract qualitative and quantitative information about the different biosensors and wastewater samples. A good correlation between sensor-array measured BOD values and BOD₇ values was obtained. In addition, PCA enabled the separation of samples according to their type and BOD₇ value, making it possible to extract qualitative information about the samples (Raud & Kikas, 2013).

DISCUSSION

Despite the fact that the first BOD biosensor was developed 38 years ago, investigation and development of new devices is still active. The majority of BOD biosensors use microorganisms or a combination of microorganisms as a biological recognition element. Therefore, one main field of study is finding the most suitable microbial cultures for any particular analytical purpose. However, a single culture does not have sufficiently wide substrate spectrum to analyse diverse samples despite the fact that a single culture is more stable than a consortium of several bacterial cultures. To overcome this problem, several sensor-arrays have been proposed for BOD measurements. The first proposed sensor-arrays did not make use of statistical analysis and were thus cast aside. The second wave of sensor-arrays did use statistical analysis, but also utilized chemical sensors with no specificity of the biorecognition element. This led to a summarised signal with no qualitative information. Only in recent years have sensor-arrays been proposed that utilize both statistical multivariate analysis and specific biorecognition elements. Biosensor-arrays utilizing a variety of microorganisms make it possible to conduct measurements with several cultures at the same time, which helps to save time, since information from several biosensors is received simultaneously and a more complex signal is obtained. Applying a multivariate statistical analysis to this kind of signal will yield both more accurate quantitative information and qualitative information.

There is a need for the development of new on-line monitoring techniques, since the standard BOD test is too time-consuming for process control in water treatment systems. On-line measurements are available when automated biosensor-arrays are used. Automated measurements, fully controlled by the computers, are noticeably less labour-intensive and thus measurement precision increases since human error is minimized.

Many new technologies, such as screen-printing and microfabrication, are available that enable the construction of miniaturized biosensor-arrays. Smaller, miniaturized biosensor-arrays lead to less chemical usage and consequently cheaper measuring technology. In addition, using smaller sensors makes it easier to develop portable devices, which enable conducting field measurements. Small but automated on-line biosensor-arrays like these can give real-time information about wastewater parameters and make it possible to operate the treatment plants over the network.

New data analysis methods provide other ways to interpret biosensor-array results. It has been shown that various multidimensional data analysis methods are making it

possible to extract more information from data than ever before. Various classification, calibration and information extraction methods are available, some of which do not require linear models and are even self-learning, such as ANN. Data analysis has become more complex, using sensor-arrays instead of a single biosensor. However, biosensor-arrays with complex data analysis provide more precise results.

Still, there are more problems to be solved. Sensor drift is a big problem with sensor-arrays. It may be caused by the ageing of a sensor, temperature or pressure changes, or the ageing of the biological recognition element (Bourgeois, 2003). Achieving a longer and more stable service life for biological recognition elements, guaranteeing easy and effective maintenance of the measurement system, and overcoming the toxic effect of samples to microorganisms are just a few of those challenges. Another problem, a political one, lies in the fact that it takes time before new devices and methods are accepted by governments and proper legislation is issued to encourage the use of biosensor-array systems.

CONCLUSIONS

Biosensors have been investigated for more than thirty years. Over that time a number of biosensors for the determination of a variety of analytes have been developed, out of which BOD biosensors are probably the most widely reported microbial biosensors. In the past decade various sensor-arrays comprising a set of different sensors and multivariate analysis methods for signal analysis have been developed. Sensor-arrays have been used for BOD measurements; however, there is still room for development. By combining several technologies, such as the application of several specific microorganisms, the miniaturization of sensors and sensor-arrays, the flow-through technology, and the complex multivariate technology for data analysis, superior results could be achieved. A biosensor-array of that kind would be small and fully automated, and precise, multifaceted information could be obtained about the samples.

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