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Review

Methods for assessing biochemical oxygen demand (BOD): A review

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ABSTRACT

The Biochemical Oxygen Demand (BOD) is one of the most widely used criteria for water quality assessment. It provides information about the ready biodegradable fraction of the organic load in water. However, this analytical method is time-consuming (generally 5 days, BOD_5), and the results may vary according to the laboratory (20%), primarily due to fluctuations in the microbial diversity of the inoculum used.

Work performed during the two last decades has resulted in several technologies that are less time-consuming and more reliable. This review is devoted to the analysis of the technical features of the principal methods described in the literature in order to compare their performances (measuring window, reliability, robustness) and to identify the pros and the cons of each method.

Contents

1. Introduction 2
2. How to assess BOD? 2
   2.1. Standardised method 2
   2.2. A new generation of assessment methods 5
3. Which alternative for the BOD assessment? 6
   3.1. Methods of BOD measurement 6

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3.1.1. Modified reference method: improvement of the oxygen measurement . . . 6
3.1.2. Photometric methods . . . 6
3.1.3. Manometric methods . . . 6
3.2. Methods of BOD prediction for high-throughput analyses . . . 7
3.2.1. Biosensors based on bioluminescent bacteria . . . 7
3.2.2. Microbial fuel cells . . . 7
3.2.3. BOD biosensors with redox-mediators . . . 8
3.2.4. Biosensors with entrapped bacteria . . . 11
3.2.5. Biosensors based on the bioreactor/chemostat technology . . . 13
3.3. Other approaches . . . 14
4. Technological evaluation . . . 15
4.1. Technical aspect . . . 15
4.2. Biological aspect . . . 15
4.3. Summary . . . 16
5. Conclusion . . . 16
References . . . 16

1. Introduction

The biological measurement "Biochemical Oxygen Demand" (BOD) was selected in 1908 as an indicator of the organic pollution of rivers by the U.K. Royal Commission on River Pollution. The traditional five day period to estimate the BOD_5 parameter was chosen for this test because this is supposedly the longest time that river water takes to travel from its source to its estuary in the U.K. (Great Britain. Royal commission on sewage disposal, 1908). Thereafter, this parameter was adopted by the American Public Health Association Standard Methods Committee in 1936 as a reference indicator to evaluate the biodegradation of chemicals and hazardous substances.

This parameter is defined as the amount of oxygen, divided by the volume of the system, taken up through the respiratory activity of microorganisms growing on the organic compounds present in the sample (e.g. water or sludge) when incubated at a specified temperature (usually 20 °C) for a fixed period (usually 5 days, BOD_5). It is a measure of that organic pollution of water which can be degraded biologically. In practice, it is usually expressed in milligrams O_2 per litre (Nagel et al., 1992).

The BOD_5 has three major applications. First, it is an indicator of the conformity of the wastewater discharge and the waste treatment procedure to the current regulations. Second, in wastewater treatment plants, the ratio between BOD_5 and COD (chemical oxygen demand) indicates the biodegradable fraction of an effluent. Third, the ratio COD/BOD_5 is an indicator of the size of a wastewater treatment plant required for a specific location.

Conventionally, BOD is determined according to the standardised method described in the next paragraph. However, the main disadvantage of this approach is the time required for its achievement (5 days) (Riedel et al., 2002). The standardised method, which allows only a deferred analysis of the wastewater quality, does not appear today to be the most suitable tool for real-time environmental monitoring.

This test is one of the most widespread in the domain of water monitoring; however, the measurement variability in certified laboratories reaches 20% (internal measurements), and this value increases between laboratories (comparative measurements) due to the variability of the microbial populations sampled (Guyard, 2010).

Due to the worldwide use of BOD methods, alternatives have been developed that range from static bioassays to online biosensors. In this review, we are interested in the analysis systems developed during the last twenty years to estimate BOD. Five main development strategies have been used by the research teams to design these new systems. Among these innovative technologies, some were transferred and marketed to industry.

2. How to assess BOD?

Aerobic biodegradation consists of oxidising organic matter biologically. During this process, the organic matter is converted by microorganisms into microbial biomass, eventual transformation products of biodegradation reaction (compounds derived from the initial organic matter), CO_2 and H_2O, according to Equation (1) (Swisher, 1987; Pagga, 1997; Reuschenbach et al., 2003).

$$X_0 + S + O_2 \xrightarrow{N,P,MN} X_f + T_p + CO_2 + H_2O \quad (1)$$

X_0: Initial biomass
S: Organic carbon sources
O_2: Oxygen
N: Nitrogen source
P: Phosphorus source
MN: mineral nutrients
X_f: Final biomass
T_p: Transformation products of biodegradation
CO_2: Carbon dioxide
H_2O: Water

2.1. *Standardised method*

Traditionally, the BOD measurement is performed according to a standardised method, currently named the closed bottle test, described in the International Standards ISO 5815-1:2003 (ISO 5815-1:2003 Water quality – Determination of biochemical oxygen demand after *n* days (BODn) – Part 1: Dilution and seeding method with allylthiourea addition, 2003) (dilution and seeding method with allylthiourea addition) and ISO 5815-2:2003 (ISO 5815-2:2003 Water quality – Determination of biochemical oxygen demand after *n* days (BODn) – Part 2: Method for undiluted samples, 2003) (method for undiluted samples). To mimic the microbial diversity found in the environment, these tests are based on microbial samples generally taken from the environment (unknown microbial diversity, cellular density ≈ 10^5 cells/mL).

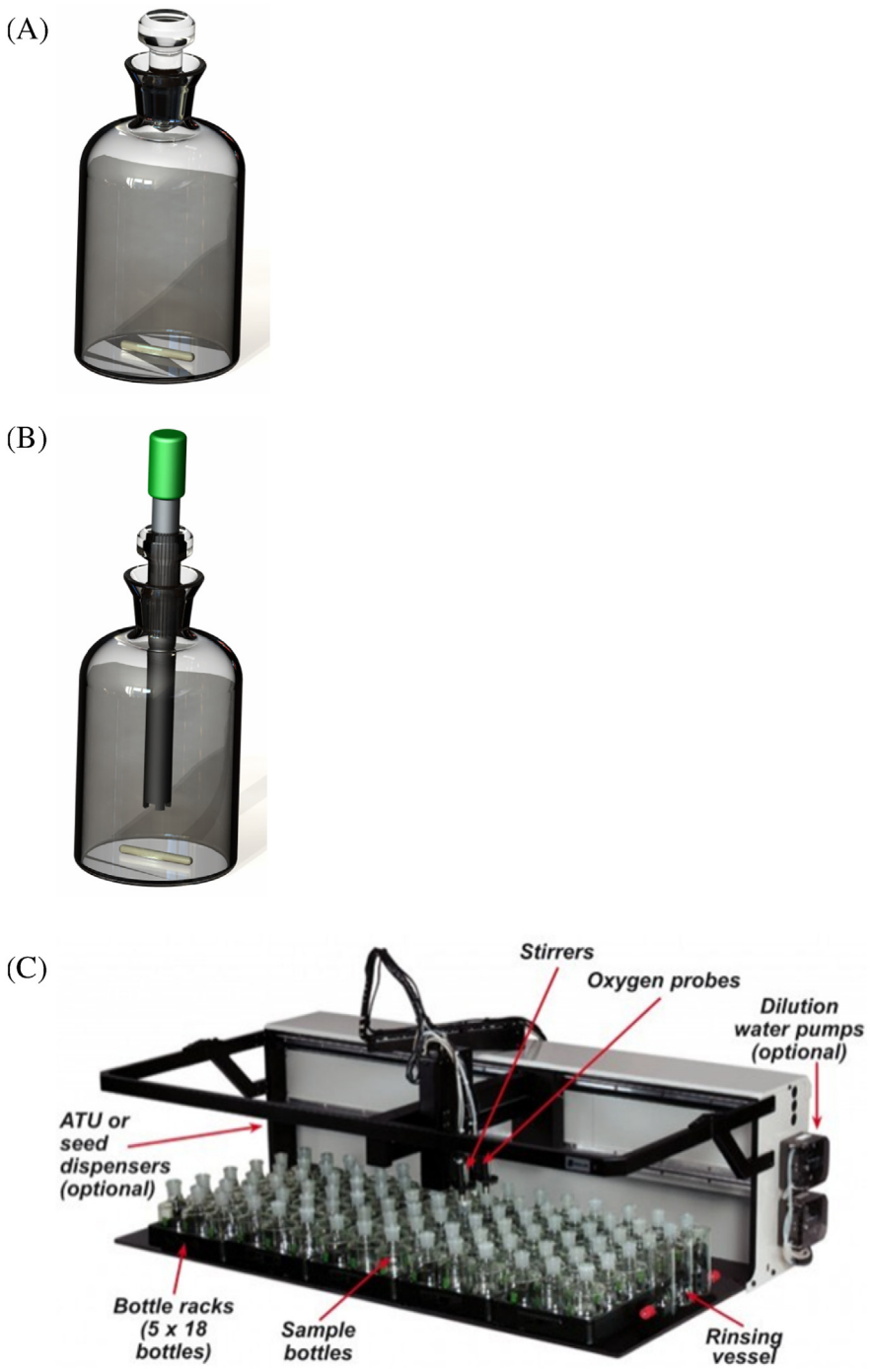

Fig. 1 – Manual (A), semi-automated (B) and automated (C) closed bottle test. With the manual closed bottle, dissolved oxygen is only measured with an electrochemical probe after an incubation period contrary to the semi-automated system and the automated system, which allow measuring continuously the O_2 consumption kinetics of the microorganisms.

Table 1 – Comparison of main strategies of BOD_5 assessment (With a blue background: Method of BOD measurement; without background: Method of BOD prediction).

Technology	Biodegra-dation marker	Transducer	Required time (median)	Benefits	Disadvantages
Reference method	Dissolved O_2	Iodometric dosage Electro-chemical probe	5 days	• Real BOD_5 value • Many reports about field applications • Marketed version	• Long period of analysis • Narrow measurement range (BOD: 0–6 mg/L[a]) • Manual dosage/ Maintenance of probes • Measurement variability (inoculum) • Important working area required
Modified reference method	Dissolved O_2	Optical probe	5 days	• Real BOD_5 value • Non-invasive probes • Many reports about field applications • Marketed version	• Long period of analysis • Narrow measurement range (BOD: 0–6 mg/L[a]) • Measurement variability (inoculum) • Important working area required
Photometric method	Dissolved O_2	Spectro-photometer	5 days	• Real BOD_5 value • Ready to use kit • Small working area required • Many reports about field applications • Marketed version	• Long period of analysis • Narrow measurement range (BOD: 0–6 mg/L[a]) • Measurement variability (inoculum)
Manometric method	Pressure	Manometer	5 days	• Wide measurement range (BOD: 0–700 mg/L) • Many reports about field applications • Marketed version	• Long period of analysis • Indirect measurement • Equivalent BOD_5 • Measurement variability (inoculum) • Important working area required
Biosensor based on biolumines-cent bacteria	Biolumi-nescence activity	Luminometer	72 min	• Short period of analysis • Wide measurement range (BOD: 0–200 mg/L) • Easy to use • Small working area required	• Only one bioluminescent strain = limited range of biodegradable chemicals • Indirect measurement • Predicted BOD_5 • Bioluminescence instability • Very few reports about field applications • No marketed version
Microbial fuel cells	Electrical potential	Amperometer	315 min	• Short period of analysis • Widespread measurement range (BOD: 0–200 mg/L; max 100,000 mg/L[b]) • Low maintenance • Configuration allowing the online monitoring • Marketed version	• Indirect measurement • Measurement variability (inoculum) • Predicted BOD_5 • Few reports about field applications
Redox-mediator	Redox - mediator	Amperometer, luminometer, fluorimeter or Spectro-photometer	15 min	• Short period of analysis • Wide measurement range (BOD: 0–300 mg/L) • Marketed easy-to-use version (fluorescent redox-mediator) which requires a small working area	• Predicted BOD_5 • Indirect measurement • Low accuracy of equivalent BOD_5 assessment • Few reports about field applications
Biosensor with entrapped bacteria	Dissolved O_2	Electro-chemical or optical probe	10 min	• Direct measurement • Short period of analysis • Wide measurement range (BOD: 0–500 mg/L) • Configuration allowing the online monitoring	• Diffusion (oxygen, chemicals) in polymer matrix or membrane • Growth of entrapped bacteria • Few reports about field applications • Predicted BOD_5 • No marketed version

(continued on next page)

Table 1 – (continued)

Technology	Biodegra-dation marker	Transducer	Required time (median)	Benefits	Disadvantages
Bioreactor	Dissolved O_2	Electro-chemical or optical probe	20 min	• Direct measurement • Short period of analysis • Wide measurement range (BOD: 0–500,000 mg/L[b]) • Configuration allowing the online monitoring • Many reports about field applications • Marketed version	• Important required working area • Predicted BOD_5 • Measurement variability (inoculum) • Important calibration phase

[a] Without sample dilutions.
[b] According the manufacturer.

According to these standards, the protocol consists of putting the samples potentially contaminated with organic matter into specific bottles (Fig. 1A), aerating them, and adding a microbial population. The bottles are then hermetically sealed and incubated in a dark room at 20 °C. After an incubation period of *n* days, the dissolved residual oxygen is measured for all analysed samples to estimate the BOD. The standards specify that the oxygen determination must be performed by either the iodometric method (Winkler's method) (*ISO 5813:1983 Water quality – Determination of dissolved oxygen – Iodometric method*, n.d.; Carpenter, 1965) or the electrochemical probe method (*ISO 5814:1990 Water quality – Determination of dissolved oxygen – Electrochemical probe method*, n.d.).

A commercialised semi-automated version is available (Fig. 1B). For this version, an electrochemical probe is inserted into the sealed bottle to measure the dissolved oxygen concentration in the sample in real time. This technical improvement allows the degradation kinetics of the organic matter to be determined in the analysis conditions imposed by the standard. The last version (Fig. 1C) described in this section is an automated version of the standardised test marketed by Skalar (Netherlands). The protocol follows the specifications of the standard method, but the manual steps are replaced by an automated, artificial arm.

This standardised method measures the dissolved oxygen consumption in order to estimate the BOD value (Table 1). However, this test shows some limitations: a significant variability in the results (>20%), due mainly to the microbial population (Guyard, 2010), is observed; a proper working area is required; the duration (usually, 5 days) is not in accordance with industrial demand (quick screening to improve the monitoring of wastewater), and the measurement range of the organic load is limited by the amount of dissolved oxygen. Moreover, this test appears inappropriate for the online monitoring of processes found in wastewater treatment plants. Because of all of these limitations, several research teams are interested in designing new methods to assess the BOD providing alternatives to the standardised tests.

2.2. *A new generation of assessment methods*

The development of methods to assess the BOD is a prolific subject considering the number of published reports describing innovative systems to estimate this parameter. Fig. 2 shows the increases in the numbers of referenced publications since 1977. More than 200 publications relevant to this topic were identified on the "web of science" database, with an annual publication rate of approximately 15 articles per year. In this review, the authors selected the publications presenting industrial or environmental applications or describing a new assessment strategy in this domain.

Among the referenced publications, it is possible to class the assessment methods into 6 main technological categories, including a class dedicated to the modified standard method, as shown in Fig. 3. All these technologies were developed to improve the standard method according to different objectives:

- a decrease in the variability observed with the standard method,
- an increase in the measurement range of the organic load in samples to eliminate the dilution step necessary in the standard method,
- a reduction in the required working area,
- an increase in the analysis frequency to allow the online monitoring of the BOD.

The most frequently represented strategy in the listed literature is based on immobilised bacteria. These methods have been the subject of a second generation of development to improve the measurement by the addition of a pre-treatment step.

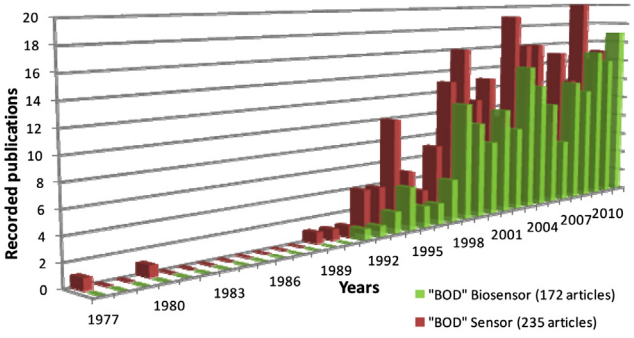

Fig. 2 – Recorded publications in the Web of Science with the following keywords: "BOD"+sensor or "BOD"+biosensor.

Other strategies have been considered to monitor the BOD in the environment based on the technology of microbial fuel cells (respiratory activity), the use of bioluminescent bacteria (cellular activity), the bioreactor or the use of the redox mediators (respiratory activity). The last category, like the method based on immobilised bacteria, has undergone a second generation of development based on the use of an additional complementary mediator.

All technologies quoted in Fig. 3 are described in detail in the following paragraphs.

3. Which alternative for the BOD assessment?

3.1. Methods of BOD measurement

Because of the required time by the tests, the methods presented in this part are essentially designed to perform occasional analyses of BOD or to monitor an effluent with a measurement frequency inferior to one per week. They are primarily methods that present an improvement relative to the simplifying of the standard method (e.g., a decrease in the maintenance, a reduction of the working area or an enlargement of the measurement ranges of the organic load). Nevertheless, these methods are based on the same bacterial population (high variability) and the same required analysis duration (usually 5 days) as the reference method. Consequently, these methods present the same limits for the high throughput monitoring of the BOD in the environment (Table 1).

3.1.1. Modified reference method: improvement of the oxygen measurement

The first level of improvement relates to the oxygen probes. Indeed, according to the standard method, the measurement of the oxygen consumption should be performed with an electrochemical probe (Clark's electrode) or by the iodometric method. The principal advance is the oxygen optode (optical probe) based on a chemical indicator (a dynamic fluorescence quencher) that interacts with oxygen and causes a decrease in the fluorescence emission of the fluorophore proportional to the dissolved oxygen in the sample (Xu et al., 1994; McEvoy et al., 1996; McDonagh et al., 2001; Xiong et al., 2006).

The main advantage of these probes is that they require less care and maintenance than an electrochemical probe. Moreover, with these probes, the analysed sample is not modified during the measurement because the dissolved oxygen is not consumed by the sensor, and the oxygen

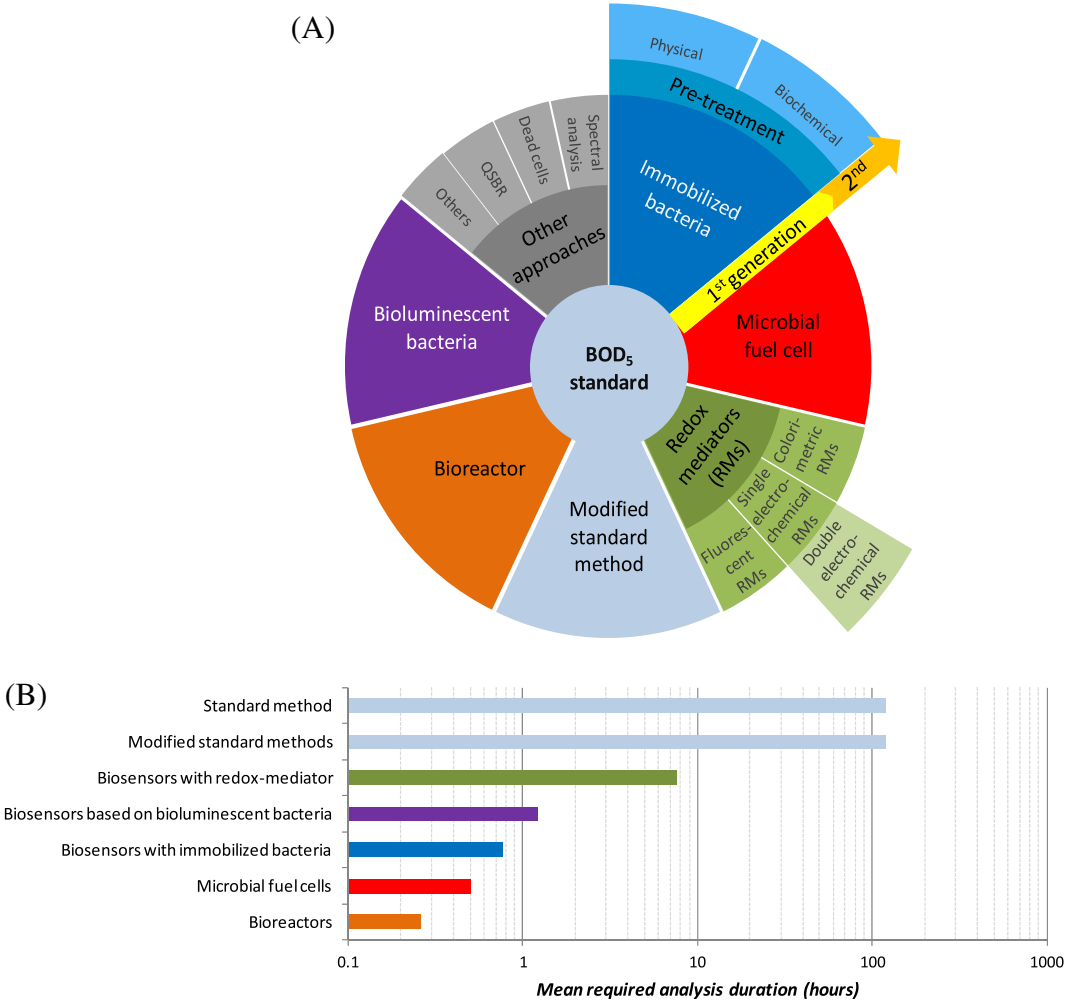

Fig. 3 – (A) The strategies of new approaches to estimate the BOD_5 and (B) their required analysis durations.

measurement is not disturbed by flow velocity or electromagnetic fields (Klimant et al., 1995).

3.1.2. *Photometric methods*

To reduce the working area required to perform a BOD test, Hach Lange (LCK 554 and LCK 555) and Macherey Nagel (BOD5 Nanocolor®) proposed cuvette tests. The tests follow the same protocol as the standard method (dilution of samples with high organic loads, incubation at 20 °C in a dark room, variability of the microbial population, and required analysis time) and are based on the dissolved oxygen consumption by the microorganisms present in the samples analysed during this period.

The dissolved oxygen is analysed before and after the analysis period directly in the cuvettes. In the Hach Lange tests, the dissolved oxygen, after the addition of several reagents to the test cuvettes, forms a red dye proportional to the dissolved oxygen concentration. The measurement of this red dye by spectrophotometry allows the estimation of the oxygen consumption and, therefore, the BOD. The measurement of the dissolved oxygen in the Macherey Nagel test is based on Winkler's method (the iodometric method). These 2 methods provide indirect estimates of the BOD.

3.1.3. *Manometric methods*

The method developed by Caldwell and Langelier (1948) is based on the measurement of pressure decrease due to the oxygen consumption by microorganisms oxidising the organic matter. In practice, the sample bottles are filled with a measured volume of sample. The microorganisms degrade organic substances using the gaseous oxygen trapped in the closed bottle. The carbon dioxide formed by this process is absorbed, generally with sodium hydroxide pellets. The pressure changes are measured by a manometer and converted to oxygen consumption by the device to estimate the BOD value (Fig. 4).

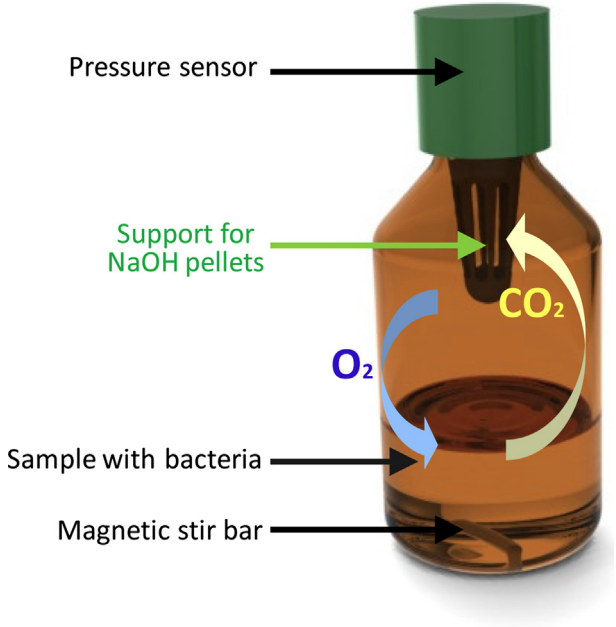

Fig. 4 – Principle of the BOD measurement by the manometric method.

The BMS 6 method proposed by Velp Scientifica is a manual manometric system. The pressure decrease is measured with a mercury barometer and converted to oxygen using a graduated scale. 6 other commercial systems based on pressure sensors without mercury are listed in the literature: the BODTrak™ (Hach Lange, Germany), the Quick Scan BOD Analyzer (Challenge technology, USA), the OxiTop® (WTW), the OxyDirect (Tintometer, Germany), the BOD EVO Sensor (Velp Scientifica, Italy), and the CI-B5 BOD ANALYZER (FanYuan Instrument, China). The measurement heads close the analysis bottles hermetically, and the pressure changes are measured with pressure sensors and converted to BOD.

These alternative methods are very widespread in the industrial sector because they are simple to use. Indeed, these methods allow the measurement of the BOD in samples contaminated by high levels of carbon compounds without making dilutions (0–700 mg/L of carbon versus 0–6 mg/L with the reference method) because of the large pool of oxygen trapped in the bottle.

3.2. *Methods of BOD prediction for high-throughput analyses*

The systems developed in the above paragraph concern technical improvements of the reference method, nevertheless, the required time is stayed the same hence these methods failed to perform high-throughput analyses. The main issue for scientists was to reduce the required analysis duration imposed by the reference test in order to:

- to obtain quickly the analysis results, which would improve environmental monitoring,
- to increase the analysis frequency without increasing the necessary working area.

Several strategies have been considered and are described in details in the following paragraphs (Table 1). However, it is important to realise that the boundaries between these technologies are not always as strict as presented in this review. Indeed, some methods combine several technologies. Consequently, for the sake of clarity, the methods presented below are classified according to the specifications deemed most relevant.

3.2.1. *Biosensors based on bioluminescent bacteria*

To reduce the analysis duration and the variability due to the unknown microbial populations used in the standard method, Sakaguchi et al. (2003, 2007) proposed two BOD assessment methods based on bioluminescent bacterial biosensors. According to the authors, the bioluminescence emission is correlated with the energy produced by the utilisation of a carbon source under aerobic conditions. The BOD is estimated from the bioluminescence emission intensity.

To limit the variability due to the microbial population, the authors used known bacterial strains, recombinant *Escherichia coli* containing the *luxCDABE* genes (from *Aliivibrio fischeri*) under control of the *tac* promoter (plasmid p22luxk described in patent JP,09-056398,A) (Sakaguchi et al., 2003) and *Photobacterium phosphoreum* (a naturally bioluminescent strain) (Sakaguchi et al., 2007).

The first method is based on recombinant *E. coli* (Sakaguchi et al., 2003) and can estimate the BOD_5 in 2 h with limited accuracy. The coefficient of determination (r^2) and the slope (S) of the linear model between the results provided by this device calculated from 7 samples ($n = 7$) are 0.674 and 0.385, respectively. The second system, based on a natural bioluminescent bacterial strain (Sakaguchi et al., 2007), showed better results than its predecessor ($r^2 = 0.995$, $S = 1.018$, $n = 5$) with a reduced response time (25 min).

Nevertheless, the complexity of the bioluminescence reaction can limit the development of these systems because the reaction is regulated by many intracellular and extracellular factors (Watanabe and Hastings, 1986; Meighen, 1993; Sung and Lee, 2004) that can then affect the BOD estimation.

3.2.2. *Microbial fuel cells*

To eliminate the oxygen limitation in the biodegradation reaction, some research teams are working on the development of BOD biosensors based on the microbial fuel cell (MFC) technology. A MFC consists of an anaerobic compartment with an anode (negative electrode) and an aerobic compartment with a cathode (positive electrode) separated by a proton exchange membrane (Fig. 5). In the anaerobic compartment, microorganisms degrade organic matter and generate electrons and protons (Grzebyk and Poźniak, 2005; Rabaey and Verstraete, 2005). The protons migrate from this compartment to the cathode through the membrane, whereas the electrons pass from the anode to the cathode through an external electrical circuit, where oxygen is reduced to form H_2O. The flow of electrons through the electrical circuit generates a measurable current proportional to the microbial biodegradation activity allowing the BOD of a sample to be estimated.

Among the systems described in the available publications (Kim et al., 2003; Chang et al., 2004, 2005; Jang et al., 2004; Kumlanghan et al., 2007; Di Lorenzo et al., 2009) about this subject, few were assessed in real environmental samples. The MFC developed by Kim et al. (2003) shows a good correlation with the BOD_5 obtained with the standardised method ($r^2 = 0.999$, $S = 1.002$); however, the assessment was performed with only a few wastewater samples ($n = 3$).

Only one commercial system based on this technology has been referenced. This BOD sensor, the BOD High Accuracy BOD Sensor 2000 Analyzer (HABS-2000), is available from Korbi Co., Ltd. (Korea). According to the manufacturers, this biosensor is based on "electrochemically-active bacteria attached on the electrode of the MFC" allowing the BOD assessment. No information is available about the nature of these microorganisms (origin, density, diversity). The specifications of the HABS-2000 are: the measurement range is between 0.1 and 200 mg BOD/L (adjustable), the analysis duration is close to 30 min (adjustable), and the measurement errors are lower than 5%. Nevertheless, no environmental application is described in the literature.

Moreover, this biosensor integrates a pre-treatment step to destroy the microflora of the samples to be analysed, specifically, exposure to ultra-violet (UV) radiation using a tangential flow method.

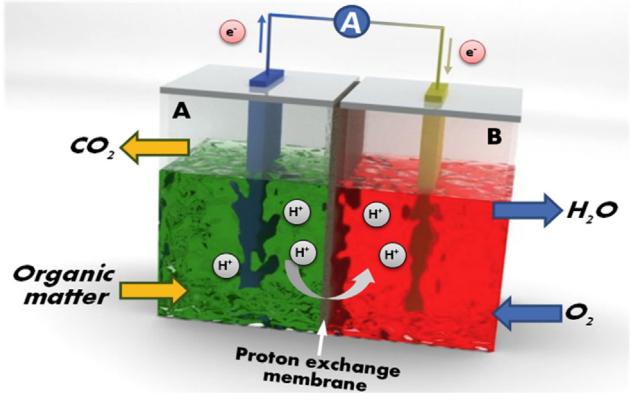

Fig. 5 – Principle of a microbial fuel cell dedicated to the estimation of the BOD. (A) bacterial anaerobic compartment with anode, (B) aerobic compartment with cathode.

3.2.3. *BOD biosensors with redox-mediators*

Usually, microorganisms oxidise organic matter in aerobic conditions. However, when a redox-mediator is present in the medium, it acts as an electron acceptor instead of oxygen (Bennetto et al., 1983; Delaney et al., 1984; Roller et al., 1984; Ramsay and Turner, 1988; Learoyd et al., 1992; Kaláb and Skládal, 1994; Yoshida et al., 2000). Consequently, the quantity of reduced redox-mediator generated by the biodegradation reaction is directly proportional to the metabolic activity (and therefore to the amount of biodegradable organic matter) and allows the assessment of the BOD. The main advantage is that with these redox-mediators, the biodegradation reaction does not depend on of oxygen in the reaction medium (Pasco et al., 2000). Moreover, with a redox-mediator, it is not necessary to dilute the samples to decrease the organic load to be degraded.

3.2.3.1. *Electrochemical redox-mediator biosensors.* The mediator-type biosensors are derived from MFC technology. An electrochemical mediator is added to the compartment with the anode. It is reduced during the metabolic oxidation of the organic matter. The reduced mediator is then re-oxidised at the anode. The difference between the electrical potentials of the anode and the cathode provides a current proportional to the metabolic activity of the microorganisms. The methods detailed below are summarised in Table 2.

3.2.3.1.1. *Single redox-mediator.* The first mediator-type biosensors, described in 2000 by Pasco et al. (2000) and Yoshida et al. (2000), are based on the electrochemical reduction of potassium hexacyanoferrate(III) [$HCF_{(III)}$] to potassium hexacyanoferrate(II) [$HCF_{(II)}$]. The addition of a substrate to medium containing an excess of redox-mediator [$HCF_{(III)}$] results in an increase in the metabolic activity of the bacteria (*E. coli* and *Pseudomonas fluorescens*) and the quantity of reduced mediator [$HCF_{(II)}$]. The reoxidation of $HCF_{(II)}$ generates a current quantifiable by a coulometric (Pasco et al., 2000) or an amperometric (Yoshida et al., 2000) transducer. Following these reports, several studies were performed to improve the performances of these devices (Catterall et al., 2001, 2003; Trosok et al., 2001; Yoshida et al., 2001; Pasco et al., 2004; Oota et al., 2010).

Table 2 – Characteristics of biosensors based on redox mediators.

Type	Microorganisms	Mediator	Transducer	Response time	Correlation with BOD_5			Origins of the validation samples	Reference
					R^2	Slope	*n*		
Electrochemical single redox mediator	*Pseudomonas fluorescens*	$HCF_{(III)}$	Amperometer	15 min	0.66	1.05	7	Sewage treatment plant and food industry	Yoshida et al., 2000
Electrochemical single redox mediator	*Escherichia coli*	$HCF_{(III)}$	Coulometer	60 min	NA	NA	NA	NA	Pasco et al., 2000
Electrochemical single redox mediator	*Candida* sp.	$HCF_{(III)}$	Amperometer	5 min	0.875	1.109	2	Pulp mill effluent	Trosok et al., 2001
Electrochemical single redox mediator	*Proteus vulgaris*	$HCF_{(III)}$	Coulometer	60 min	0.936	0.965	4	Wastewater treatment plant	Pasco et al., 2004
Electrochemical single redox mediator	Artificial consortium (4 strains)	$HCF_{(III)}$	Amperometer	3 h	0.859	0.772	30	Industrial/domestic wastewater	Catterall et al., 2003
Electrochemical single redox mediator	*Candida krusei* sp.	$HCF_{(III)}$	Amperometer	20 min	NA	NA	NA	NA	Oota et al., 2010
Electrochemical single redox mediator	*Pseudomonas fluorescens*	$HCF_{(III)}$	Amperometer	3–20 min	0.616	0.71	59	Sewage treatment plant and food industry	Yoshida et al., 2001
Electrochemical Double redox mediator	*Saccharomyces cerevisiae*	Menadione + $HCF_{(III)}$	Amperometer	15 min	0.712	1.102	6	River and sea samples	Nakamura et al., 2007
Electrochemical Double redox mediator	*Saccharomyces cerevisiae*	Menadione + hydrogen peroxide	Luminometer	8 min	0.925	0.088	3	River samples	Nakamura et al., 2007
Fluorescent redox mediator	*Pseudomonas fluorescens*	Resazurin	Fluorimeter	30 min	NA	NA	NA	NA	Dudal et al., 2006
Fluorescent redox mediator	*Unknown microbial population*	Resazurin	Fluorimeter	15 h	0.842	1.008	49	Domestic wastewater	Rocher et al., 2011
Fluorescent redox mediator	*Pseudomonas putida* mt-2, *Pseudomonas putida* F1, *Burkholderia cepacia* G4 and *Pseudomonas mendocina* KR1	Resazurin	Fluorimeter	10 min	NA	NA	NA	NA	Tizzard et al., 2006
Redox colour mediator	*Saccharomyces cerevisiae*	DCIP	Spectrophoto-meter	10 min	0.726	0.219	3	River samples	Nakamura et al., 2007

R^2, coefficient of determination; slope, slope of the linear model; *n*, number of samples; NA, information not available; $HCF_{(III)}$, potassium hexacyanoferrate(III); DCIP, 2,6-dichlorophenolindophenol.

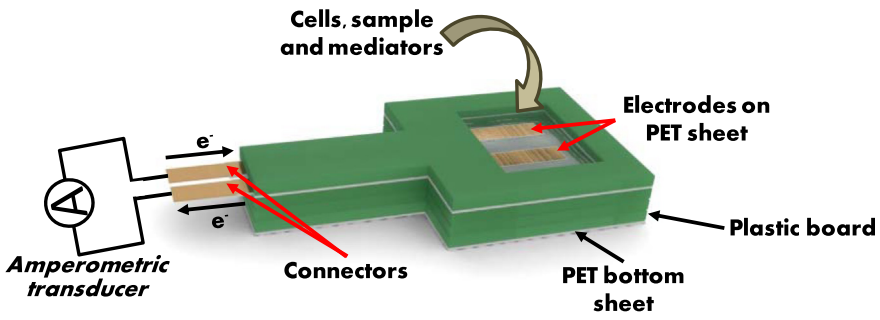

Fig. 6 – 3D modelling of the disposable chip developed by Nakamura et al. (2007a,b,c) to estimate BOD_5.

3.2.3.1.2. *Double redox-mediators*. The biosensors based on a single electrochemical redox-mediator were mainly developed with prokaryotes (Pasco et al., 2000, 2004; Yoshida et al., 2000, 2001; Catterall et al., 2001, 2003; Timur et al., 2007). Baronian et al. (2002) proposed a double-mediator system combining potassium hexacyanoferrate(III) (a hydrophilic mediator) and menadione (a lipophilic mediator) using eukaryotic cells (*Saccharomyces cerevisiae*). Menadione has the ability to cross cell membranes, and it reacts with NAD(P)H to generate menadiol. Menadiol shuttles electrons from the intracellular compartment to the cell wall to reduce the hydrophilic mediator. The reoxidation of potassium hexacyanoferrate(II) generates a current proportional to the cellular activity (Yashiki and Yamashoji, 1996).

This strategy was used by Nakamura et al. (2007a,b,c) in 2006 to design an offline disposable micro-batch-type biosensor (Fig. 6) (Japanese Patent Application Raid-Open Disclosure P2008/96415A) using *S. cerevisiae*. In the presence of biodegradable organic matter, the current generated by the redox reactions in the chip is measured with a two-electrode system and converted into BOD_{DM} ($BOD_{DM} = BOD_5$ estimated by the double-mediator system).

One year later, Nakamura et al. (2007a,b,c) developed an optical system based on previous work. This new BOD biosensor is based on the production of chemiluminescence due to the redox reaction of organic matter by *S. cerevisiae* (Fig. 7). In the presence of biodegradable compounds, menadione is reduced into menadiol. The reoxidation of menadiol generates hydrogen peroxide which reacts with Luminol and hydroxide anions, catalysed by potassium hexacyanoferrate(III), to produce chemiluminescence. The performances of these methods to estimate BOD_5 are summarised in Table 2.

3.2.3.2. *Other redox-mediators*. Dudal et al. (2006) and Tizzard et al. (2006) developed a 96-well microplate method based on resazurin. This blue redox mediator (water-soluble and not fluorescent) penetrates into bacteria and is reduced by cellular activity to resorufin (water-soluble, pink fluorescent compound). The fluorescent intensity emitted in the microplate wells after the incubation period is directly proportional to the quantity of organic matter degraded by the microorganisms in the analysed samples. Therefore, monitoring the fluorescence produced provides an estimate of the BOD. This strategy was used in the commercial test in 96-well microplates (Enverdi) provided by Envolure (a French company founded by Dudal Y. and Pautremat N.), patent WO/2006/079733. With this method, the assessment of the BOD_5 is 8 times faster than with the reference method (15 h compared to the 5 days required by the reference method), and the measurement range is increased from 6 mg BOD/L to 300 mg BOD/L with the reference method without dilution.

This redox mediator can also be used as a redox colour indicator. Indeed, resazurin is a blue dye (optimal absorbance at 605 nm) which turns red after electrochemical reduction to resorufin (optimal absorbance at 573 nm) (Czekanska, 2011). Another redox colour indicator, 2,6-dichlorophenolindophenol, was used by Nakamura et al. (2007a,b,c) to estimate BOD from the cellular activity.

The main characteristics of the methods are summarised in Table 2. According to the data provided by the investigators, it appears that the response times obtained with these BOD sensors are significantly less than with the standard method, 15 h (maximum duration) compared to 5 days. This improvement is most likely due to the redox-mediator, which acts as the electron acceptor instead of oxygen and allows acceleration of the biodegradation reaction.

Others factors can also explain the difference observed with the reference method including the use of pure selected strains (bacteria or yeast) at cellular densities much higher than those employed in the reference test.

3.2.4. *Biosensors with entrapped bacteria*

This strategy, the most frequently represented in the literature, was described for the first time in 1977 by Karube et al. (1977). Since this work, several studies have been published describing systems to estimate the BOD based on immobilised bacteria either with or without pretreatment of the effluent.

3.2.4.1. *Biosensors without pretreatment of the samples*. The principle of this measurement consists of immobilised bacteria that allow direct contact with the measurement electrode. The BOD is estimated from the oxygen consumption measured by the electrode (ratio between the O_2 consumption without and with the sample). According to the authors, this configuration allows, on one hand, a reduction in the analysis time of a sample and, on the other hand, a simplification of the assay.

Different immobilisation modes were used according to the authors; nevertheless, it is possible to class these techniques into three groups. In the first case, bacteria are entrapped inside a polymer network that limits their

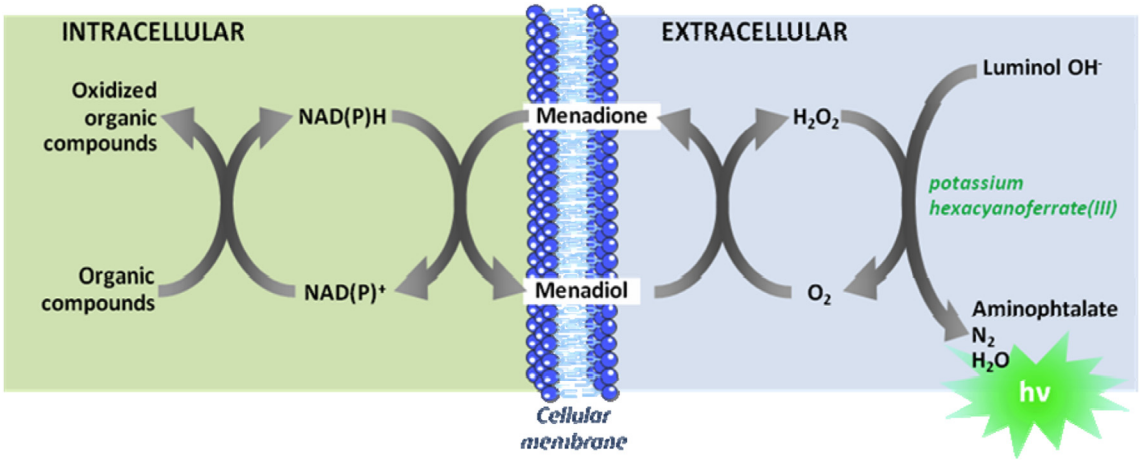

Fig. 7 – Chemiluminescence reaction for estimating BOD (Yashiki and Yamashoji, 1996; Nakamura et al., 2007a,b,c).

movements (Table 3). Several matrices were assessed, including alginate (Kumlanghan et al., 2008), agarose (Raud et al., 2012; Raud and Kikas, 2013), poly(carbamoyl)sulfonate (PCS) (Chan et al., 1999, 2000; Lehmann et al., 1999), silica gel (Oota et al., 2010), sol–gel composite material (silica and poly(vinyl alcohol)-grafted-poly(vinylpyridine)) (Jia et al., 2003; Kwok et al., 2005; Liu et al., 2009, 2011), Al_2O_3 sol–gel (Chen et al., 2002), resin (Yang et al., 1996), polyvinyl alcohol (PVA) (Preininger et al., 1994), polyurethane (Köster et al., 2006), or ormosil-PVA (Dai et al., 2004; Jiang et al., 2006; Lin et al., 2006; Xin et al., 2007). All these polymers are biocompatible and have low toxicity for microorganisms. Nevertheless, the chosen polymer depends on the application. Alginate and agarose hydrogels are particularly easy to use but may not have adequate mechanical stability under some conditions (Chan et al., 1999). PCS and sol–gel polymers are more complex to use but more mechanically resistant. Moreover, sol–gel polymers are described as chemically inert and photochemically and thermally stable. The second immobilisation method consists of sandwiching cells between two polymer layers. Several polymer combinations were used in the studies cited, including dialysis membrane/Teflon® membrane (Liu et al., 2000), cellulose acetate membrane/fluorinated ethylene propylene membrane (FEP) (Li and Chu, 1991), Teflon® membrane/Teflon® membrane (Kim and Park, 2001), Polycarbonate membrane/Teflon® membrane (Suriyawattanakul et al., 2002). The last method of microbial immobilisation consists of adsorbing the cells to solid supports, e.g., porous membranes (cellulose (Chee, Nomura and Karube, 1999), glass-fibre (Arlyapov et al., 2012) or nylon (Rastogi et al., 2003a; Rastogi et al., 2003b)).

In most cases, the oxygen monitoring is performed with an amperometric electrode (Clark electrode). Nevertheless, among the biosensors described above, some systems do not use this type of probe. Indeed, the biosensors developed by Preininger et al. (1994), Lin et al. (2006), Jiang et al. (2006), Kwok et al. (2005), Xin et al. (2007) and Dai et al. (2004) use a fluorescent probe (a ruthenium complex) to monitor the dissolved oxygen consumption resulting from the biodegradation of the organic matter (see 3.1.1.).

Finally, the system developed by Liu et al. (2012) is based on a microbial biofilm formed on the internal surface of glass tube. The principle differs slightly from the methods described above since the microorganisms are not in direct contact with the oxygen probe. The sample to be analysed circulates in a continuous flow inside the glass tube, and the oxygen consumption is measured at the outlet of the tube containing the microbial biofilm.

The specifications of the systems detailed in the literature are described in Table 3.

According to the authors, these biosensors are able to estimate the BOD_5 with an analysis duration that does not exceed 90 min (the minimal reported duration is 70 s). However, in one third of the cases, the validation results are non-existent or irrelevant for environmental monitoring (correlation factor < 0.6).

In most of cases (about 60%), the BOD biosensors are based on only one microbial strain (yeast: 65% or bacteria: 35%) instead of microbial mixture as in the reference method. The recent works of Raud and Kikas (2013) propose an alternative strategy to predict the BOD value based on a bacterial panel composed of seven strains used individually. For this, data provided by each strain are simultaneously analysed with statistical models. The results are hopeful since the predicted BOD values are totally correlated with the measured BOD_7 ($r^2 = 1$ and $S = 1$ and $n = 30$).

3.2.4.2. *Biosensors with a pretreatment of the samples*. To improve the accuracy and the rapidity of the biosensors based on immobilised bacteria, several research teams added a pretreatment step before the measurement phase (Table 4). Some recalcitrant pollutants (i.e., compounds of high molecular weight) can require a lengthy lag phase before becoming biodegradable by microorganisms. Due to the long period of analysis required by the reference method, some of these compounds are counted in the BOD measurement but not in the experimental biosensor assays. The purpose of a supplementary step is to hydrolyse, physically or biochemically, the macromolecules (which are more difficult to degrade) in the samples to compounds of lower molecular weight that can be

Table 3 – Specification of the different immobilised bacterial biosensors.

Type	Immobilisation support	Microorganisms	Response time	Correlation with BOD_5			Origins of the validation samples	Reference
				R^2	Slope	n		
Entrapped cell	Alginate	Activated sludge	10–15 min	0.9995	0.970	31	Different industries	Kumlanghan et al., 2008
Entrapped cell	Agarose	*Aeromonas hydrophyla*	50–90 min	−0.53[a]	0.36[a]	6	Synthetic samples or meat industry	Raud et al., 2012
Entrapped cell	Agarose	*Pseudomonas fluorescens*	50–90 min	0.241[a]	0.375[a]	6	Synthetic samples or meat industry	Raud et al., 2012
Entrapped cell	Agarose	*Pseudomonas fluorescens, Aeromonas hydrophila, Pseudomonas putida, Escherichia coli, Bacillus subtilis, Paenibacillus sp., Microbacterium phyllosphaerae*	NA	1	1	30[c]	Synthetic samples	(Raud and Kikas, 2013)
Entrapped cell	PCS	*Arxula adeninivorans*	100 s	0.88	1.04	16	Domestic wastewater	Chan et al., 2000
Entrapped cell	PCS	*Arxula adeninivorans*	70 s	0.920	0.611	6	Glucose solution	Chan et al., 1999
Entrapped cell	PCS	*Arxula adeninivorans*	70 s	0.898	0.998	16	Wastewater treatment plant	Lehmann et al., 1999
Entrapped cell	Sol-gel composite material	*Trichosporon cutaneum* and *Bacillus subtilis*	>10 min	1	0.9253	5	Synthetic and environmental samples or domestic wastewater	Jia et al., 2003
Entrapped cell	Sol-gel composite material	*Trichosporon cutaneum*	NA	NA	NA	NA	NA	Liu et al., 2009
Entrapped cell	Sol-gel composite material	Microbial population *(BODseed Cole–Parmer)*	10 min	0.971	1.06	11	Wastewater treatment plant	Liu et al., 2011
Entrapped cell	Sol-gel composite material	*Bacillus subtilis*	15–30 min	0.981	0.904	25[d]	Synthetic samples or domestic wastewater	Kwok et al., 2005
Entrapped cell	Sol-gel composite material	Activated sludge	15–30 min	0.984	0.915	28[d]	Synthetic samples or domestic wastewater	Kwok et al., 2005
Entrapped cell	Al_2O_3 sol–gel	Yeast	15 min	0.9996	1.076	6	Food industry	Chen et al., 2002
Entrapped cell	Resin	*Trichosporon cutaneum*	20 min	0.855	1.2	5	Domestic wastewater or food industry	Yang et al., 1996
Entrapped cell	PVA	*Trichosporon cutaneum*	3–10 min	0.927	0.95	12	Wastewater from sewage plant or municipal sewage	Preininger et al., 1994
Entrapped cell	Ormosil-PVA	*Bacillus licheniformis, Dietzia maris and Marinobacter marinus*	3 min[b]	0.814	0.833	6	Seawater	Lin et al., 2006
Entrapped cell	Ormosil-PVA	*Bacillus licheniformis, Dietzia maris and Marinobacter marinus*	4 min[a]	0.902	1.006	22	Seawater	Jiang et al., 2006
Entrapped cell	Ormosil-PVA	*Bacillus licheniformis, Dietzia maris and Marinobacter marinus*	20 min	0.993	0.896	10	Seawater	Xin et al., 2007
Sandwiched cell	Dialysis membrane/ Teflon® membrane	Activated sludge	20 min	0.183	1.05	11	Synthetic samples, domestic wastewater or food industry	Liu et al., 2000
Sandwiched cell	Cellulose acetate membrane/FEP	*Hansenula anomala*	13–20 min	1	0.952	4	River water, food industry and glutamate plant	Li and Chu, 1991

(*continued on next page*)

Table 3 – (*continued*)

Type	Immobilisation support	Microorganisms	Response time	Correlation with BOD_5			Origins of the validation samples	Reference
				R^2	Slope	*n*		
Sandwiched cell	Cellulose acetate membrane/FEP	*Pseudomonas* sp.	13–20 min	1	0.962	3	River water, starch and glutamate plant	Li and Chu, 1991
Sandwiched cell	Teflon® membrane/ Teflon® membrane	*Klebsiella* sp.	NA	0.4023	0.541	8	Carbohydrate solutions	Kim and Park, 2001
Sandwiched cell	Polycarbonate membrane/Teflon® membrane	*Trichosporon cutaneum*, *Bacillus licheniformis*	5–10 min	NA	NA	NA	NA	Suriyawattanakul et al., 2002
Adsorbed cell	Cellulose nitrate membrane	*Pseudomonas putida*	2–15 min	0.839	0.895	14	River water	Chee et al., 1999
Adsorbed cell	Glass-fibre filter	*Candida maltosa*	8–20 min	0.091	0.734	7	Wastewater treatment plant or glucose-molasses plant	Arlyapov et al., 2012
Adsorbed cell	Glass-fibre filter	*Debaryomyces hansenii*	10–17 min	0.97	1.03	7	Wastewater treatment plant or glucose-molasses plant	Arlyapov et al., 2012
Adsorbed cell	Glass-fibre filter	*Candida blankii*	10–22 min	0.588	0.729	7	Wastewater treatment plant or glucose-molasses plant	Arlyapov et al., 2012
Adsorbed cell	Nylon membrane	*Microbes isolated from sewage samples*	10 min	1	0.989	3	Food industry, tannery or pulp and paper industry	Rastogi, Kumar, et al., 2003
Adsorbed cell	Nylon membrane	*Microbes isolated from sewage samples*	5–10 min	0.9997	1.04	5	Industrial wastewater	Rastogi, Rathee, et al., 2003
Biofilm	Glass tube	*Wastewater Microbes*	6–8 min	0.983	0.969	40	Wastewater treatment plant, food industry or environment	Liu et al., 2012

R^2, coefficient of determination; slope, slope of the linear model; *n*, number of samples; NA, information not available; PCS, poly(carbamoyl)sulfonate; PVA, poly(vinyl alcohol); FEP, fluorinated ethylene propylene membrane.

[a] Correlation with the BOD_7.

[b] Response time for 5.0 mg/L BOD.

[c] 5 types of OCDE synthetic wastewater (without addition, with phenol, with milk, with fat or with carboxyl-methyl-cellulose).

[d] 5 types of samples: OECD synthetic wastewater, synthetic wastewater, domestic wastewater, glucose-glutamic acid and a mixture of the OCDE synthetic wastewater and the glucose-glutamic acid.

biodegraded more easily than the parent compounds (Beltrán et al., 1997).

Chee et al. used two physico-chemical pretreatment methods to improve the effectiveness of their immobilised bacterial biosensor (Chee et al., 1999a), ozonation (Chee et al., 1999b) or irradiation with ultra-violet (UV) light (Chee et al., 2001, 2005). From the results obtained with the first pretreatment method, it seems that ozonation does not improve the biosensor performances in contrast to UV irradiation. Indeed, in the two studies based on this technology, the correlation between the biosensor and the reference method increased when the samples were pretreated.

The biochemical pretreatment consists of adding specific enzymes to accelerate the biodegradation of organic macromolecules. Reiss et al. (1998) added an enzymatic pretreatment before the microbial biosensor, which significantly (6%) improved the effectiveness of the short-term BOD biosensor to predict the BOD_5 parameter. However, the system is described for a specific application (samples contaminated with starch). Finally, Kim and Park (2004) proposed a similar approach based on an additional enzymatic pretreatment designed to hydrolyse disaccharides and/or polysaccharides. Nevertheless, the results obtained with the supplementary pretreatment step deviated significantly from the BOD_5 values provided by the reference method. Indeed, some recalcitrant compounds are biodegraded by the enzymatic hydrolysis (pretreatment) during the analysis, whereas they are not degraded with the reference method.

3.2.5. *Biosensors based on the bioreactor/chemostat technology*

The BOD biosensors based on the bioreactor/chemostat technology were mainly developed for online applications. The principle of these biosensors is based on monitoring the oxygen consumption of the microorganisms in contact with the sample, as with the standard method. However, these biosensors are continuously fed with wastewater saturated with dissolved oxygen (Vernimmen et al., 1967). An oxygen probe measures the variations in the respiration rate to deduce the BOD in the sample. Thus, with this technical design, on the one hand, the cellular density is higher than in the environment because of the continuous feeding of organic matter, and, on the other hand, the microbial community within the bioreactor is totally adapted to the organic substrate, significantly reducing the analysis time. The response time is generally less than 45 min, and it allows the effluents to be monitored in real time.

3.2.5.1. *Experimental biosensors*. In this area, some recent studies of new BOD biosensors have been reported over the last decade. In 2004, Liu et al. published a study (2004a, 2004b) of the development of a short-term BOD biosensor for the online monitoring of biological treatment processes (Fig. 8). In this system, free microorganisms (activated sludge collected from a municipal wastewater treatment plant) are maintained in liquid inside a measurement cell with a dialysis membrane to perform the analyses. The response time is nearly 60 s ($r^2 = 0.544$, $S = 0.789$ and $n = 4$).

Two other bioreactor-type biosensors developed by Wang et al. (2010) and Villalobos et al. (2010) are hydride systems featuring bacteria immobilised in PVA/alginate beads in suspension in a traditional bioreactor. The performance of these systems seems interesting. The response time is close to 30 min with a high correlation with the result obtained with the reference method ($r^2 = 0.99$ and $S \approx 1$ (Villalobos et al., 2010; Wang et al., 2010)), but the result needs to be validated because it was determined from a limited number of samples (3 or 5).

In a recent publication, Torrents et al. (2012) proposed a miniaturised reactor-type biosensor based on a microfluidic respirometer. The system consists of a double flow cell in which two microchannels (reaction chamber and electrolyte channel) are separated by an oxygen permeable membrane. The reaction chamber contains the bacteria and the samples to be analysed. The second channel has a constant flow of electrolyte saturated with oxygen. Oxygen diffuses through the membrane from the electrolyte chamber to the reaction chamber to be consumed by aerobic bacteria (unspecified by the authors). The monitoring of the oxygen at the end of the electrolyte chamber provides information on the bacterial activity and allows the estimation of BOD.

3.2.5.2. *Commercial biosensors*. This technology is the most widespread among the commercialised biosensors dedicated to the online monitoring of wastewater. This wide distribution is most likely due to the accumulated experience since the first publication in 1967 (Vernimmen et al., 1967). Fig. 9 is a schematic representation of the architecture of the systems, including Ra-BOD® (Applitek, Belgium), Biox-1010 (Endress + Hauser, Switzerland), MB-DBO (Biosensores, Spain) and RODTOX 2000 (Kelma, Belgium) (Table 5). These biosensors are based on three inoculation strategies.

- For Ra-BOD®, the measurement bioreactor is continuously inoculated with real sludge from a wastewater treatment plant.
- With the Biox-1010 system, the microorganisms colonise small plastic cylinders inside the bioreactor (to protect them against mechanical abrasion caused by turbulent mixing and to increase the contact surface with the sample being analysed) before the measurement is performed.
- In the biosensor MB-DBO, the microorganisms first grow in an independent continuous bioreactor that allows to stabilise the microbial population and to feed the measurement reactor continuously.

The biosensor BioMonitor (LAR, United States) is also a biosensor based on the bioreactor technology; however, this architecture is totally different than the other systems presented above. It consists of four successive reactors that work exactly like an aeration tank (Fig. 10). The oxygen consumption is measured in the gaseous fraction of the last reactor of the measurement channel, and the determination of the BOD parameter is calculated from the difference between the oxygen consumption in the control and the test sample.

According to the manufacturer, this configuration allows more rapid degradation than in the biosensors with only one reactor, and it facilitates the degradation of substances that are difficult to degrade. Due to this improvement, the BioMonitor is able to estimate with reliability and accuracy the BOD_5 in a

Table 4 – Comparison of the performances of the immobilised bacteria based BOD-biosensors with or without pretreatment of the samples.

Type	Immobilisation support	Pre-treatment	Microorganisms	Response time	Correlation with BOD_5			Origins of the validation samples	Reference
					R^2	Slope	*n*		
Adsorbed cell	Cellulose nitrate membrane	None Ozonation	*Pseudomonas putida*	5 min	0.974 0.963	0.919 1.082	16	River water	Chee et al., 1999
Adsorbed cell	Cellulose nitrate membrane	None Ultra-Violet	*Pseudomonas putida*	<10 min	0.896 0.957	0.8704 0.985	10	River water	Chee et al., 2001
Adsorbed cell	Cellulose nitrate membrane	None Ultra-Violet	*Pseudomonas putida*	5–10 min	0.968 0.983	0.849 0.908	21	River water	Chee et al., 2005
NA	NA	None Enzymatic	*Trichosporon cutaneum*	5–6 min	NA[a] NA[a]	NA NA	5	Starch or GGA solution	Reiss et al., 1998
Adsorbed cell	Teflon® membrane	None Enzymatic	*Klebsiella* sp.	15–20 min	NC NC	NC NC	3	Lactose, sucrose or maltose	Kim and Park, 2004

R^2, coefficient of determination; slope, slope of the linear model; *n*, number of sample; NA, information not available; NC, uncalculated value; GGA, glucose-glutamic acid solution.

[a] [%] of the BOD_5 value : 24,3% without pretreatment, 67,9% with enzymatic pretreatment.

sample in only 4 min (Table 5). Moreover, it is important to emphasise the presence of a control channel that allows the estimation of the toxicological impact of the effluent on the microbial community, and limits interpretation errors (underestimation of the BOD value). Nevertheless, no environmental application has been reported in the literature.

The principal advantage of these biosensors is the rapidity of the measurement. However, they are only reliable under specific conditions. On one hand, the parameters of the analysed samples must remain relatively constant during the time of the assay (diversity of the organic load, physico-chemical parameters), and, on the other hand, an important step of calibration is necessary to adapt the system (microbial population or calibration curve) to the samples. Consequently, these devices are efficient for the online monitoring of relatively stable and known effluents but seem to be unsuitable for analysis of diversified samples.

3.3. *Other approaches*

To estimate the BOD, two criteria are usually used: the oxygen consumption (reference method, modified standard methods, immobilised bacteria based biosensors and bioreactor-type biosensors) and the cellular activity (bioluminescent bacteria based biosensors, microbial fuel cells and mediator-type biosensors) due to the biodegradation metabolism. In contrast, the system developed by Chiappini et al. (2010) uses the carbon dioxide produced from the biodegradation process as the BOD indicator. However, the efficiency of this system is relatively low; the error rate was approximately 35% for 5 samples.

Tan and Qian (Tan and Qian, 1997; Qian and Tan, 1998, 1999; Tan and Lim, 2005) proposed to replace the living bacteria by dead cells. Indeed, living cells are generally sensitive to their environment and require careful maintenance of their living conditions to ensure their survival. Consequently, the authors used the enzymatic material extracted from cellular lysates of *Bacillus subtilis* (Tan and Qian, 1997; Qian and Tan, 1998, 1999) or multi-species microbial cells (BODseed, Cole-Parmer E-05466-00) (Tan and Lim, 2005). The enzymes are adsorbed on filters and mounted on an amperometric oxygen probe. According to the authors, the error rate of the BOD_5 estimation is approximately 6% with environmental samples (domestic and industrial wastewaters).

Other systems have been developed, without biological sensors, based on analysing the spectrum of the samples to estimate the BOD (Reynolds and Ahmad, 1997; Hur et al., 2010; Lai et al., 2011; Hur and Cho, 2012). With these approaches, it is possible to monitor water effluents online and in real time, in contrast to all the systems described above. Several spectral markers have been used to estimate the BOD: UV absorbance (Dobbs et al., 1972; Mrkva, 1975, 1983; Bari and Farooq, 1985; Edwards and Cresser, 1987; Reynolds and Ahmad, 1997), fixed-wavelength fluorescence (Reynolds and Ahmad, 1997), synchronous fluorescence spectra (Hur et al., 2010; Lai et al., 2011) and three dimensional fluorescence matrix (Hur and Cho, 2012). Two system architectures are available for online BOD monitoring, the “BOD probe” or the “BOD online analyser”. The three systems, the Stamosens CSM750/CSS70, the carbo:lyser and the STAC (Constant et al., 2009), commercialised by Endress + Hauser (Switzerland), S:CAN (Austria) and Secomam (France), respectively, belong to the first category. They are composed of two parts, a sensor directly dipped in the analysed sample and a transducer. In contrast to the “BOD probe” system, with the “BOD online analyser”, no probe is dipped into the sample. A pump removes the samples, which are analysed in an online UV spectrophotometer. This architecture is used in the BOD Online Analyzer (AWA instruments, Singapore).

In particular cases of the screening of new compounds, there exist predictive models, namely, the quantitative structure–biodegradability relationships (QSBR), able to estimate the biodegradability of compounds from their physico-chemical descriptors (Baker et al., 2004). At first, statistical models are designed by supervised learning from an existing database of molecular descriptors (longest aliphatic chain, molecular linear free energy relation, etc.) of chemical compounds (Geating, 1981; Okey and Stensel, 1996; Raymond et al., 2001; Arora and Shi, 2010; Cheng et al., 2012). Then, these

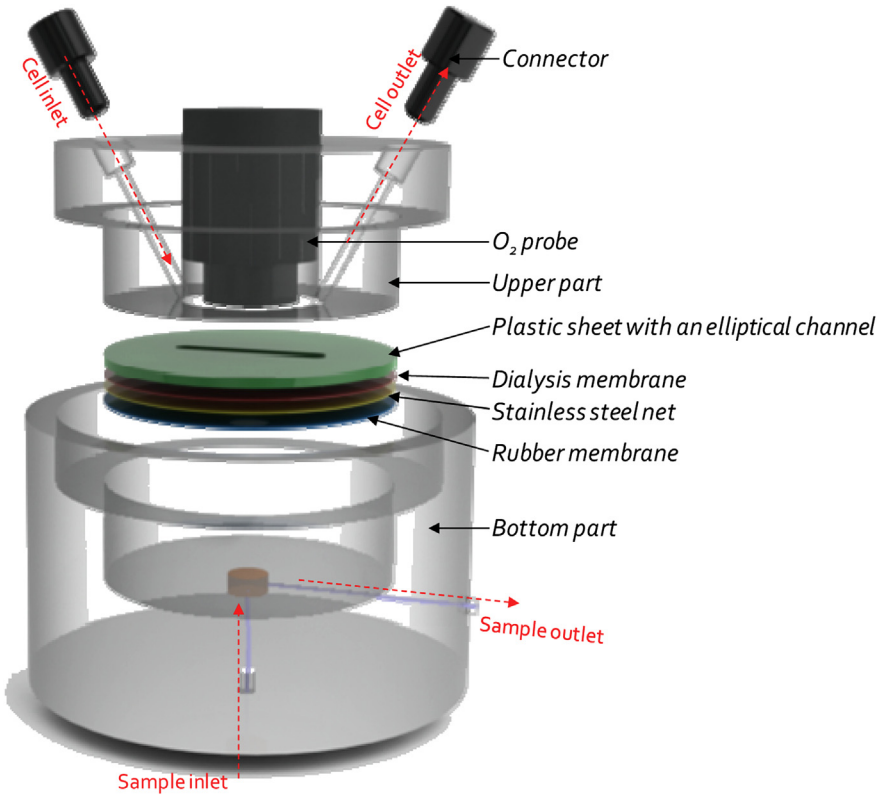

Fig. 8 – Schematic representation of the BOD biosensor based on the bioreactor technology developed by Liu et al. (2004a, 2004b).

models are applied to unknown molecules to predict their biodegradability. In recent work (2012), Cheng et al. (2012) compared the biodegradability prediction obtained from their statistical models with the biodegradability measured with a standardised test (MITI, the equivalent of the BOD test for the longest period, 28 days) on 27 compounds. The results are encouraging as all 27 compounds were correctly predicted. However, with unknown samples without characterisation data (composition, physico-chemical characterisation of present chemicals), as is the case in environmental monitoring, this strategy does not seem applicable.

4. Technological evaluation

In order to compare the main strategies of BOD assessment described above, the main assets and drawbacks of each method are listed in Table 1. These methods are classified in two categories i.e. the real measurement or the mathematical prediction.

4.1. *Technical aspect*

In the first case (real measurement), the BOD measurement is performed on the entire study period (5 days for the BOD_5 assessment) as with the reference method. For this purpose, the oxygen consumption is generally monitored with dissolved oxygen probes (electrochemical or optical) or with pressure sensor. In all cases, many reports of these technologies are available about field applications (environmental monitoring or chemical characterization). However, the delay for analysis constrains significantly the high throughput analyses and notably, the online monitoring of effluents.

The second class described in this manuscript concerns the methods for the BOD prediction. The required time for analysis is significantly reduced by comparison with the measurement methods. Indeed, with these predictive methods, the average time to perform an analysis is about 30 min (min: 70 s, max = 15 h) with a broad measurement ranges (0–500,000 mg BOD/L). Nevertheless, few data about field applications are available and the provided results, predicted from statistical

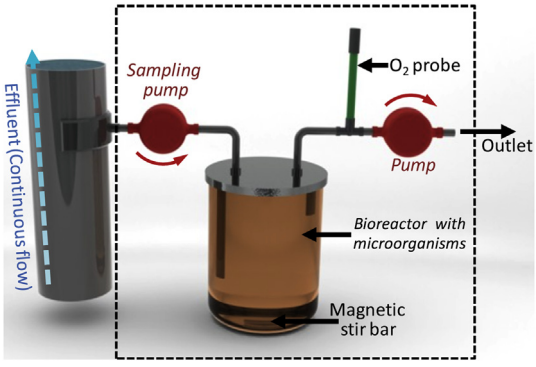

Fig. 9 – Schematic representation of a bioreactor-type biosensor.

Table 5 – Performance of commercial BOD biosensors based on the bioreactor technology.

Name	Manufacturer	Type	Measured parameter	Pre-treatment	Measurement range (mg BOD/L)	Analysis time	Accuracy
Ra-BOD®	AppliTek	Bioreactor	Oxygen consumption	None	20-100,000	30 min	<5%
Biox-1010	Endress + Hauser	Bioreactor	Oxygen consumption	None	5-100,000	3–15 min	3%
MB-DBO	Biosensores	Bioreactor	Oxygen consumption	None	10-1000	30 min	<3%
RODTOX 2000	Kelma	Bioreactor	Oxygen consumption	None	0-500,000	NA	5%
BioMonitor®	LAR	Bioreactor in cascade	Oxygen consumption	None	1-200,000	3–4 min	<5%

NA, information not available.

model of correlation (generally: linear model) are not always reliable ($-0.53 < r^2 < 1$) because, notably, of the calibration methods based on inappropriate reference chemicals or unsuitable microbial inoculums (pure strains).

Among the fastest assessment methods, the biosensors with entrapped bacteria are widespread in literature (more than 50% of publications described in this manuscript), but despite this, no marketed system based on this technology is available (only two technologies are commercially available: the microbial fuel cells and the bioreactors). This absence can be explained by the cellular growth inside the membrane which can modify the characteristics of biosensor (response time, reliability, correlation with the calibration curve) (Jouanneau et al., 2012) and consequently, limit the commercial development of these BOD biosensors.

4.2. *Biological aspect*

Two types of microbial inoculums are usually used, environmental microbial populations (as in the standardised method) or pure strains. The former population can degrade a large panel of organic compounds (Liu et al., 2000). However, the composition of these inocula is not controlled impairing the reproducibility of the BOD measurement (Blok and Booy, 1984; Thouand et al., 1995, 1996). Conversely, in the second case, the use of only one microbial strain (bacteria or yeast) improves the reproducibility of the measurements but limits the biological potential of biodegradation. Some authors addressed the problem by using an artificial microbial mixture of 2–4 strains (Catterall et al., 2003; Jia et al., 2003; Jiang et al., 2006; Lin et al., 2006; Xin et al., 2007) to obtain a sensor combining good reproducibility with a large biodegradation potential. However, these multi-species mixtures are difficult to control in a long-term because of competition for the carbon substrate.

An alternative for the future development would be to use individually several microbial strains in order to preserve important degradation capacities while simplifying the control of cells, as in the publication of Raud and Kikas (2013). The utilization of a microbial panel to estimate the BOD involves the implementation of analytical statistical tools more complex than the usual method based on linear regression used in the majority of the publications. Indeed, this approach requires a multi-parametric analysis of data provided by all strains. Also, with this configuration, it would be interesting to use a QSBR-type model based on biological descriptors instead of molecular descriptors in order to predict accurately the BOD: Qualitative Activity–Biodegradation Relationship (QABR) model.

Finally, it is important to emphasize the absence of toxicity control in the tests presented in this review, except for the BioMonitor described in paragraph 3.2.5.2. The direct consequence of this is the risk of underestimating the BOD value due to an alteration of the microbial population. Consequently, the future development of BOD biosensors would integrate a complementary toxicity sensor.

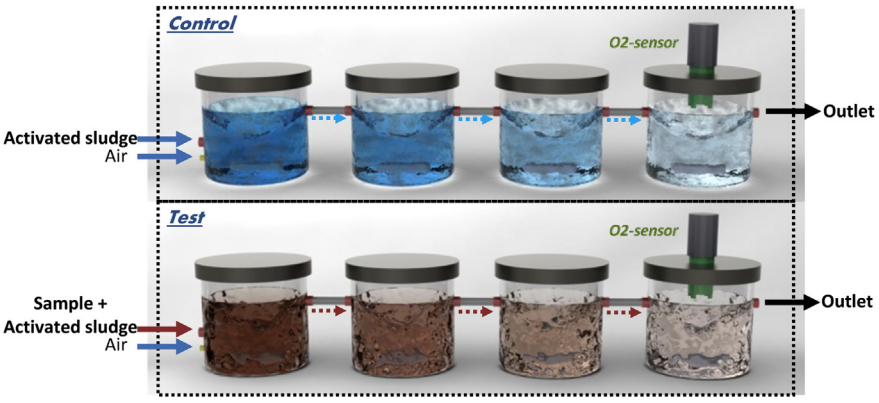

Fig. 10 – BioMonitor (LAR).

4.3. *Summary*

The methods of real measurement are relatively easy to use, technologically reliable (oxygen or pressure probe) and many data about them are available in literature. Nevertheless, they require long analysis periods and, generally, an important workspace. The provided results depend directly on environmental inoculums (20% of variability). Conversely, the predictive methods need a reduced time to perform analyses. The fastest system provides the BOD_5 of a sample in only 70 s instead of 5 days. However, the majority of the described methods requires an average time of 30 min to estimate the BOD_5. To overcome the variability due to the environmental inoculums, recent publications propose to use artificial inoculums or a set of pure bacterial strains. In all cases, the reduced measurement period implies the use of a statistical correlation model to predict a BOD value.

5. Conclusion

This review has identified the main technological strategies designed to measure or to estimate the BOD parameter, used as an indicator of the biodegradability of organic matter. It focuses on the technological aspect of the assessment methods, on the nature of the performed measurement (real measurement or prediction) and on the pros and cons of them.

From a technical point of view, the latest advances show that the "measurement" aspect of biological signals (bioluminescence, cellular activity, oxygen consumption) is reliable and robust. Nevertheless, the research issues should focus on the biological aspect of these systems in order to find a compromise between the standardization of the inoculum and environmental diversity (both of which are opposed on the criterion of the biological variability). These future systems should also integrate toxicity markers in order to limit the risk of BOD underestimating.

The second point concerns the decrease of the workspace which induces a miniaturization of the assessment methods and indirectly of the size of the tested samples. This consequence raises the issue about the representativity of increasingly smaller samples.

The choice of a method for the BOD assessment should take into account the tolerated error rate, the measurement frequency, the nature of the studied matrices (domestic or industrial wastewaters) and the type of considered applications (on-line, in situ, ex situ).

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