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Microbial BOD sensors for wastewater analysis

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Abstract

The field of biosensors for measuring biochemical oxygen demand (BOD) is reviewed. Particularly, BOD sensors constructed on the biofilm configuration are discussed regarding performance characteristics like linearity, response time, precision, agreement between BOD values obtained from the biosensors and the conventional 5-days test, as well as toxic resistance to various compounds and operational stability. The techniques for improving the agreement between the sensor BOD and BOD_5 are described. Information provided also includes BOD biosensors based on respirometers and other measuring principles, the commercial BOD instruments, as well as the current limitations of BOD biosensor development.

Keywords: BOD sensors; Biosensors; Biochemical oxygen demand (BOD); Short-term BOD; Biofilm; Wastewater

1. Introduction

The determination of biochemical oxygen demand (BOD) is an empirical test in which standardised laboratory procedures are used to determine the relative oxygen requirements of wastewater, effluents and polluted waters. The BOD values indicate the amount of biodegradable organic material (carbonaceous demand) and the oxygen used to oxidise inorganic material such as sulphides and ferrous iron. It also may measure the oxygen used to oxidise reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The BOD test has its widest application in measuring waste loading to treatment plants and in evaluating the BOD removal efficiency of such treatment systems [1].

BOD has been determined conventionally by taking a sample of water, aerating it well, placing it in a sealed bottle, incubating for a standard period of time at $20\pm1^{\circ}C$ in the dark, and determining the oxygen consumption in the water at the end of incubation [2]. According to the American standard, the incubation time is 5 days and the BOD values based on this standard are called BOD_5 for short [1], whereas the incubation time is 7 days in the Swedish standard and the abbreviation is BOD_7 [3].

The conventional BOD test has certain benefits such as being a universal method of measuring most wastewater samples, and furthermore, no expensive equipment is needed. It has, however, the limitation of being time consuming, and consequently it is not suitable for on-line process monitoring. Thus, it is necessary to develop an alternative method that could circumvent the weakness of the conventional BOD test described above.

Fast determination of BOD could be achieved by the biosensor-based methods. A biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with a transduction element [4]. The BOD biosensors can be generally classified as biofilm- and respirometer-type. Both types of sensor are based on respirometric principle. However, BOD biosensor developments based on other principles are also available. It should be noticed that BOD biosensors make it possible to determine a short-term BOD (BOD_{st}) that is not identical with the conventional BOD value in all cases.

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Therefore, studies on the correlation between the BOD_{st} and BOD_5 are needed. Nevertheless, BOD_{st} is a more valuable parameter for control purposes because it represents the oxygen demand within the time constraints of an aerobic treatment and is intimately related to the specific microorganisms of the treatment process. The BOD_5, on the other hand, merely characterises the impact on the receiving water [5,6].

The biofilm-type BOD sensors have generated a great interest among scientists due to various advantages including rapid analysis, simplicity, compact design, low cost and the possibility of instrumentation for on-line applications. Work on development of this type of BOD biosensors has been going on for more than two decades. The first report was published in 1977 [7] Following this, different kinds of biosensor design and various modifications have been studied.

This paper summarises the present development of biofilm-type BOD sensors based on literature review and experiences from earlier work. The measuring principles and sensor fabrication will be explained first. The next section will discuss the performance characteristics of the BOD biosensors. The techniques for improving the agreement between the sensor BOD and BOD_5 are also covered. In order to give a complete picture of BOD biosensors, respirometers specifically designed for BOD_{st} measurement and BOD biosensors based on other principles are also briefly introduced in a separate section. Finally, some commercial BOD instruments are described, and their performance is discussed.

2. The biofilm-type BOD biosensors

2.1. The measuring principles

Most of previously reported BOD sensors are biofilm-type whole-cell-based microbial sensors, which rely on measuring the bacterial respiration rate in close proximity to a suitable transducer. A common feature of these sensors is that they consist of a microbial film sandwiched between a porous cellulose membrane and a gas-permeable membrane as the biological recognition element. This microbial film is an immobilised microbial population that can biooxidise the organic substrate to be quantified. The response is usually a change in concentration of dissolved oxygen (DO) or other phenomena such as light emission. A physical transducer is used to monitor this process. The result is a change in an electrical or optical signal. The signal is amplified and correlated to the content of biodegradable material measured.

A common design and a typical response curve of a biofilm-type BOD sensor are shown in Figs. 1 and 2, respectively. DO diffuses from the aerated phosphate buffer through the dialysis membrane into the immobilised cell layer, where part of the oxygen is consumed by the immobilised microorganisms. The remaining oxygen diffuses through the gas-permeable Teflon membrane and is detected by the oxygen electrode. A steady-state current can be observed at the beginning representing equilibrium between the oxygen diffusion and the endogenous respiration rate of the immobilised bacteria. When a wastewater sample is injected into the sensor system, assimilable organic substrates diffuse through the dialysis membrane and are assimilated by the immobilised bacteria, resulting in an increase in the bacterial respiration rate and oxygen consumption for biooxidation processes. Therefore, less oxygen can diffuse through the Teflon membrane and be detected by the oxygen electrode. The current will then decrease until a new equilibrium value for oxygen is achieved. When the buffer is re-injected into the system, the remaining wastewater sample will be diluted and washed out. As soon as the respiration rate of the microorganisms decreases, the endogenous respiration rate is progressively restored. Since the process is controlled by substrate diffusion, the sensor signal should to a certain extent be proportional to the concentration of easily biodegradable organic substrates in the sample.

There are two measuring techniques available for biofilm-type BOD sensors, *viz.* the steady-state (also entitled as end-point, dynamic) method and the initial-rate (also entitled as quasi-kinetic, kinetic, dynamic transient) method. In the steady-state method, the current difference (ΔI) between the two steady states is used for the BOD_{st} estimation. The measuring time is normally 15–20 min followed by 15–60 min recovery time. In the initial-rate method, the initial current change ($\Delta I/\Delta t$) after sample addition is used instead as the sensor response. This parameter reflects acceleration of the bacterial respiration rate and, to a certain extent, is proportional to the substrate concentration. In this case, the sensor response is normally recorded for 15–30 s followed by a recovery time of <10 min.

The correlation between values obtained using these two methods has been studied. High correlation ($r^2 = -0.998$) in terms of reliability was reported for both standard GGA solution (containing 150 mg/l glucose and 150 mg/l glutamic acid with a measured BOD_5 value of 200 ± 10 mg/l) and wastewater samples from a food factory. No significant difference of sensor repeatability is observed, but the sensitivity is twice as high in the initial-rate method [8]. Liu et al. [9] also demonstrated the good agreement observed from the ratios between the measurement results obtained in the initial-rate and steady-state modes, i.e. 0.98:1, 1.08:1 and 0.88:1 for the samples of municipal wastewater, wastewater from a food processing factory and the GGA solution, respectively. In comparison to the steady-state mode, the sensor BOD measurement in the initial-rate mode has the advantage of being faster, thereby

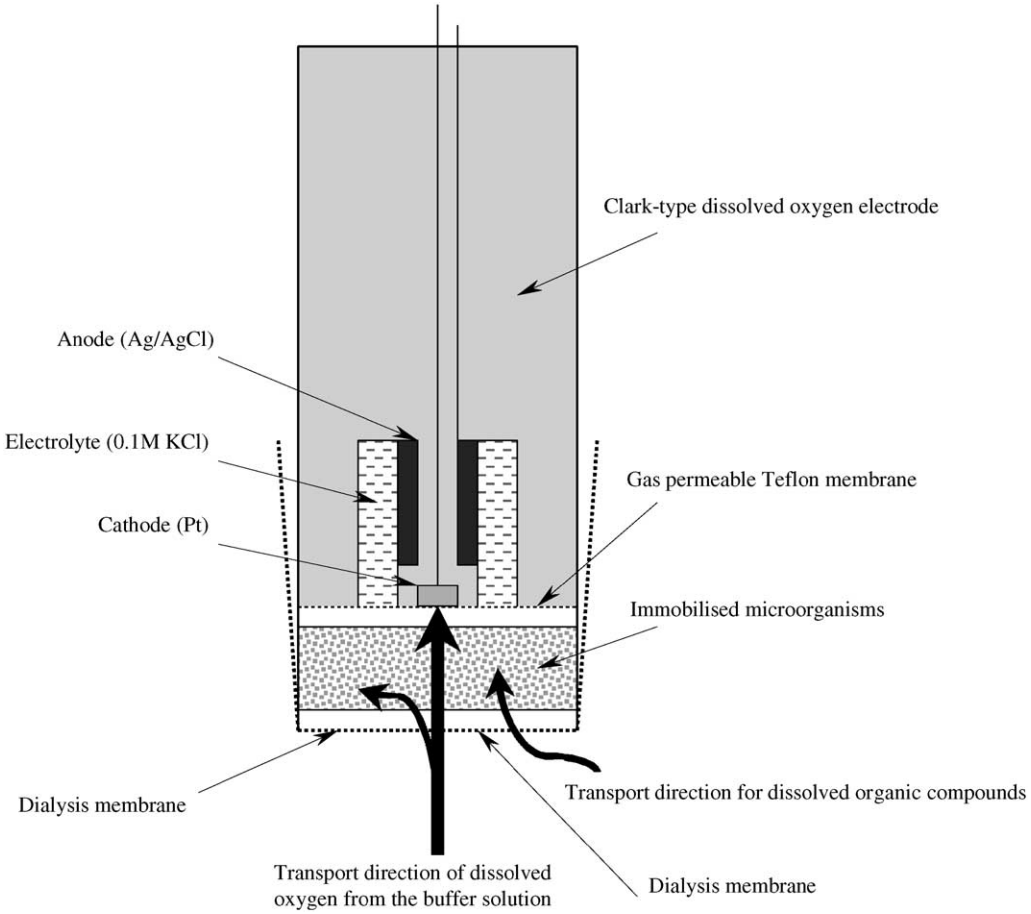

Fig. 1. Schematic presentation of a BOD biosensor. An immobilised microbial population in combination with a Clark-type oxygen electrode.

allowing more frequent measurements and being more suitable for on-line monitoring when the purpose is process control.

2.2. Biological recognition element

Different microbial strains have been used as the biological recognition element for fabricating BOD sensors. These include pure cultures (e.g. *Arxula adeninivorans*, *Bacillus polymyxa* D-21, *Bacillus subtilis*, *Hansenula anomala*, *Klebsiella oxytoca* AS1, *Pseudomonas putida*, *Serratia marcescens* LSY 4, *Torulopsis candida*, *Trichosporon cutaneum*), a mixture of two identified microbial strains (e.g. *Bacillus subtilis* and *Bacillus licheniformis* 7B, *Rhodococcus erythropolis* and *Issatchenkia orientalis*), induced microbial consortium, activated sludge, and even thermally killed bacteria.

In general, single-culture-BOD-sensors have an advantage in a stable sensor performance over a desired period, but the detection will be limited by the narrow substrate spectrum of the single strain. In contrast, BOD sensors based on a complex bacterial population, such as that found in activated sludge and microbial consortia [7,9–13] have a good detection capacity for a wide substrate spectrum but an unstable sensor performance due to instability in the composition of the consortia over time. As most of the previously reported BOD sensors were designed with the aim of good precision and operational stability, a mixture of two identified microbial strains has been used to overcome the problem of narrow substrate spectrum detection. Furthermore, this leads to a stable sensor performance over a reasonable period [13–15].

BOD sensors based on heat-killed cells have also been reported. It was found that *Bacillus subtilis*, killed by exposing vacuum-dried cells to 280°C for 2.5 min, still retained sufficient multiple enzyme activities to catalyse the oxidation of organic substrates and could act as the

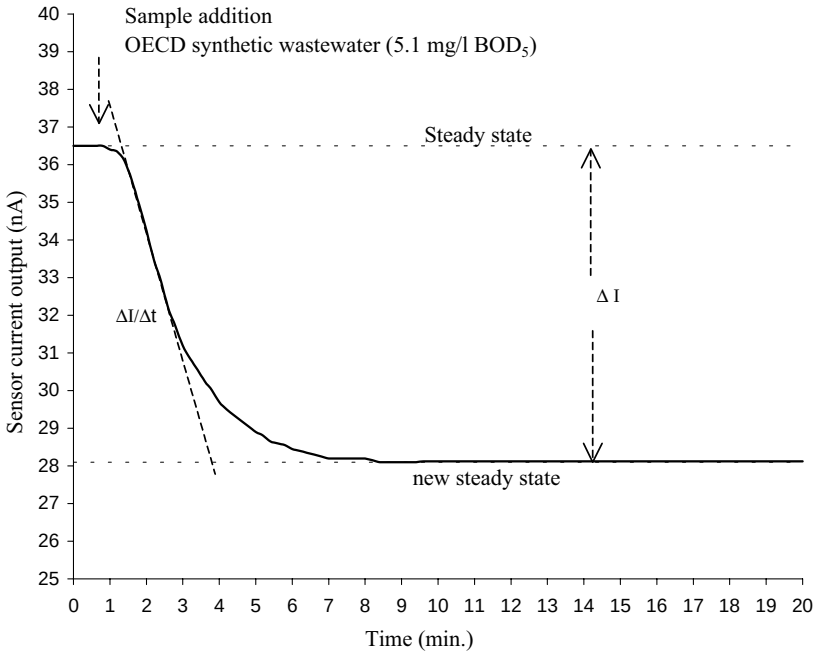

Fig. 2. A typical response curve from the BOD biosensor for a sample of the OECD synthetic wastewater with BOD_5 value 5.1 mg/l. The initial current change ($\Delta I/\Delta t$) and current difference (ΔI) between two steady states were the sensor responses for sensor BOD measurements in the kinetic and dynamic modes, respectively [9].

biological recognition element. Sensors based on heat-killed cells can be stored in a phosphate buffer at room temperature over long idling period without periodic service and nutrients supply [13,16–18]. Biosensors based on living cells, on the other hand, require careful maintenance and supply of nutrients and minerals during prolonged storage.

2.3. Transducers

The most commonly used transducer for fabricating BOD biosensors is the Clark-type DO probe, which is an amperometric sensor developed by Clark in 1956. This DO probe is a two-electrode system consisting of a silver anode and a gold or platinum cathode covered with an oxygen permeable membrane. A Clark-type DO probe has a limited lifetime because of the consumption of the electrolyte and oxidation of the anode metal [19]. As a consequence, drift in calibration values occurs over a period of time. Therefore, it is necessary to change the electrolyte and clean the anode surface regularly.

There is an increasing demand for miniaturised and disposable BOD sensors. Consequently, the fabrication and utilisation of such miniaturised and disposable oxygen probes based on conventional semi-conductor technology have been reported. Yang et al. [20,21] used a commercial miniature Clark-type oxygen probe (15 mm × 2 mm × 0.4 mm) that was fabricated on a silicon substrate using micromachining techniques. They also produced miniature Clark-type DO probe arrays (26 mm × 76 mm × 0.9 mm) using the thin film technology. Chan et al. [22] used another commercial miniature oxygen probe (59 mm × 7.5 mm) that was manufactured using thick film technology. The probe is in a three-electrode configuration, i.e. a Pt-working electrode, an Ag/AgCl reference electrode and a Pt auxiliary (counter) electrode.

Optical sensors have been developed for measurement of DO and also been used for construction of BOD sensors. Preininger et al. [23] reported an optical fibre for transmission of fluorescence signals emitted from oxygen sensitive material, Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) perchlorate, in close proximity to the immobilised yeast cells. A similar technique was tested by Li et al. [25] for respirometric monitoring during the incubation of the conventional BOD_5 measurement. Chee et al. [26] described a BOD biosensor using a commercial DO optical fibre sensor measuring fluorescence quenching by oxygen. The major advantages by using optical oxygen sensors over amperometric oxygen probes are that no oxygen is consumed and long-term stability of the optical sensors is achieved which results in that no calibration drift

occurs and inertness for sample flow rates and stirring. The optical methods offer as option of designing disposable sensors at a low cost.

2.4. Immobilisation

The microbial cells are immobilised most commonly by simple adsorption, e.g. the cells are placed directly on a porous cellulose membrane by suction, or hydrogel entrapment, i.e. the cells are entrapped on the surface of a porous matrix membrane by using an aqueous solution of polyvinylalcohol (PVA) [15,27–30] or poly(-carbamoyl)sulphonate (PCS) [31,32]. For the purpose of developing a disposable BOD sensor, an ultraviolet cross-linking resin (ENT-3400) was used to immobilise the cells directly on the surface of a miniature oxygen electrode [20]. As an alternative to using disposable-type BOD sensors, the sensor can be designed for easy replacement of the biofilm. A BOD sensor using magnetic activated sludge was prepared by mixing magnetite powder with activated sludge. The magnetic sludge was then simply layered onto the Teflon membrane of a magnetic cathode by magnetic attraction [11].

3. Performance characteristics of biofilm-type BOD biosensors

Following the first biofilm-type BOD sensor reported by Karube et al. [7,33], various sensor designs and modifications have been presented. As summarised in Table 1, the sensor performance characteristics, such as linearity of the calibration curve, response time and operational stability, varied depending on the species of microorganism used, the calibration solution, the measuring techniques and the operational conditions. However, optimisation of a biosensor system is a complex task since often improvement of one property leads to impairing of another one. Some of the most important characteristics of a BOD biosensor are described here.

Table 1
Biofilm-type BOD sensors based on amperometric oxygen probe and respirometric measuring principle

Immobilised microbes	Measuring range (mg/l BOD)[a]	Response time (min)	Repeatability (± %)	Operational stability (days)	References
Trichosporon cutaneum	<60[d1]	<18[b]	<6	17[g(30,i,1,n)]	[34]
	4–100[d1]	5[b], 0.5[c]	3.3	48[g(30,h,i,n)]	[27,28,35]
	0–110[d1]	3–10[b]	4	7–30[g(i,4,i,y)]	[23]
	3–150[b]				[36]
	0.2–18[d1]	7–20[b]	8	d.s.[f1]	[20]
	10–70[d6]	5[b]	<3	40[g(30,i,i,n)]	[37]
Hansenula anomala	0.01–0.2 mM[d4]	15–20[b]			[38]
	1–45[d1]	13–20[b]	6		[53]
Pseudomonas sp./	1–30[d1]	<15[b]	4.1		[39]
Pseudomonas putida	1–40[d1]	13–20[b]	6		[53]
	0.5–10[d2]	2–15[b]	10	10[g(30,4,i,n)]	[41]
	1–10[d2,j]	15[b,j]	20[j]		[26]
Klebsiella oxytoca AS1	<44[d1]	2.5[b]			[42]
Bacillus subtilis	2–22[d1]	0.25[c]	5	30[g(30,h,i,n)]	[27]
Bacillus subtilis (heat killed)	<80[d1]	25[b]	2.4–3.4	140[g(25,h,i,y)]	[17,18]
Arxula adeninivorans LS3	8–550[d5]	1.2[c]	5	30[g(37,i,i,n)]	[29]
	2-550[d1]	1.2[c]	10	40[g(37,i,1,i)]	[31,32]
	1.24–4.55[d5]	1.7[c]		62[g(i,i,i,y)]	[22]
Mycelia of *A. adeninivorans* LS3	2.61–524[d5]	1.2[c]		110[g(37,i,i,i)]	[43]
Torulopsis candida	7–75 ppm[e1], <500 ppm[e2]	1[b,e1], 10[b,e2]			[45]
Serratia marcescens LSY 4	44 ppm[d1]	15[b]			[46]
B. subtilis + *B. licheniformis*	<70[d1]	4–15[b]	5	22[g(30,4,1,y)]	[15]
	<80[d1]	0.25–0.5[c]		60[g(30,30,i,i)]	[30]
Rhodococcus erythropolis + *Issatchenkia orientalis*	17–1275[d5]	0.25–0.5[c]	<5		[47]
	6–600[d1]	0.25–0.5[c]	<5	40	[48]

Table 1 (*continued*)

Immobilised microbes	Measuring range (mg/l BOD)[a]	Response time (min)	Repeatability (± %)	Operational stability (days)	References
Citrobacter sp. + *Enterobacter* sp.	6–18[d1]	8[b]	<11		[49]
Bacteria isolated from soil	<22[d1]	10–15[b]	7.5	10[g(30,i,1,n)]	[7]
	0–350 ppm[d1]	30[c]	6	10[g(30,i,i,n)]	[50]
Bacteria isolated from	2–22[d1]	<10[b]	9–12	20[g(30,i,i,i)]	[12]
activated sludge	3.3–32.8[d1]	6[b]	1.3	48[g(30,4,4,y)]	[10]
Thermophilic bacteria	<10[d1]	7[b]		40[g(50,50,i,y)]	[51]
Induced microbial consortium	<40 [d3]	0.5[c]	4.7–5.6	<7[g(h,h,1,n)]	[9]
Multi-species culture (BODSEED)	0–45[d1]	45–60[b]	2.9–4.8	20[g(25,i,i,y)]	[13]
Multi-species culture (BODSEED) (heat killed)	0–45[d1]	35–50[b]	8.5–12.4	20[g(25,i,i,y)]	[13]
Activated sludge	<60[d1]	30[b]	5	e.r.b[f2]	[11]

[a]Measuring range for calibration solutions.
[b]Measuring in steady-state mode.
[c]Measuring in initial-rate mode.
[d1]Glucose and glutamic acid (GGA) solution.
[d2]Artificial wastewater (AWW).
[d3]OECD synthetic wastewater.
[d4]L-lactate solution.
[d5]Gluose solution.
[d6]Alanine and glutamic acid solution.
[e1]Flow though assay.
[e2]Flow injection assay.
[f1]d.s., Disposable sensor.
[f2]e.r.b., Easy replacement of the biomembrane for short time use.
[g(α,β,γ,δ)]Operational stability under certain condition. α, Operating temp. (°C); β, storage temp. during the measurement interval (°C); γ, time interval (day) between measurements; δ, reactivation of microbial cells before new measurement (*y*, with; *n*, without).
[h]Room temperature.
[i]Not specified.
[j]Optical transducer.

3.1. Linearity of calibration curve

Linearity in response from a BOD biosensor over a certain concentration range is a measure of the detection capacity to analyse wastewaters with varying substrate concentration. A broad linear range is desirable for reliable and accurate measurement over the concentration range. As demonstrated in Table 1, the linearity of BOD biosensor in steady-state measuring mode was reported up to maximum BOD value varying from 10 to about 150 mg/l, whereas sensor output varies in a linear mode in a range from 4.55 up to 1275 mg/l when using the initial-rate mode. The linearity is related to the sensor fabrication, type and density of the cell preparation. BOD sensors with a high cell density biofilm are generally more sensitive in sample analysis but have a more narrow linear range. Furthermore, linearity is also influenced by the sensor sensitivity to particular organic substrates. A BOD sensor will give a different linear characteristic when comparing the sensor signals from calibration solutions and samples with varying organic substrate composition. Comparison of the linearity could only be done in cases where the same calibration solution is applied.

3.2. Response time and recovery time

BOD sensor response-time varies depending primarily on the applied measuring techniques. As shown in Table 1, the sensor signal has normally been recorded for 5–25 min in the steady-state mode, whereas it takes only 15–30 s to complete a measurement in the initial-rate mode. In the steady-state mode, the time taken to reach a new steady state also depends on the substrate concentration in the sample, that is, more time will be spent for analysing a sample with a higher concentration of substrate [15,17].

In order to achieve a good repeatability, the sensor has to return to the baseline condition corresponding to its original response in a well-aerated buffer solution.

Generally, the time required for baseline recovery is more than that for signal recording, i.e. 15–60 min and 5–10 min for steady-state mode and initial-rate mode, respectively. Recovery time also increases with the time taken for the measurement and may be as high as several hours [30].

3.3. Precision

The purpose of BOD sensor development was a fast alternative analytical method that had at least an equally good performance (precision and bias) as the existing conventional test. According to ISO 5725-6 that has been adapted to the intralaboratory situation, errors may be of random or systematic character. Precision covers random errors while trueness (bias) covers systematic errors. Precision is divided into repeatability and reproducibility. Repeatability is the standard deviation of a tested single sensor by one operator under the same conditions, while reproducibility is the standard deviation of a series of tested sensors by more than one operator under different conditions.

According to the APHA standard for 5-day BOD test, analysis of a GGA solution check with an average BOD_5 value of 198 mg/l and a standard deviation of 30.5 mg/l is intended to be the reference of single-laboratory test [1]. This is about $\pm 15.4\%$ of variability in precision. As indicated in Table 1, the repeatability of previously reported biofilm-type BOD sensors varies from $\pm 2.4\%$ to $\pm 10\%$ for the single-strain sensors, $\pm 5\%$ to $\pm 11\%$ for the sensors based on the mixture of two identified strains, and $\pm 1.3\%$ to $\pm 12.4\%$ for those multi-strains based sensors. However, only few data are available regarding the reproducibility of the BOD sensors. Sakai et al. [11] reported the sensors based on magnetic activated sludge with reproducibility within $\pm 5\%$. Liu et al. [9] reported that a sensor based on an induced microbial consortium had reproducibility varying from $\pm 9.3\%$ to $\pm 15.0\%$ depending on the applied wastewater samples. More investigation is needed in order to make a conclusion in this characteristic.

3.4. Agreement between the BOD_{st} and conventional BOD

There is no measurement for establishing bias of the conventional BOD procedure. The GGA solution check is the only reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique [1]. Comparative tests are therefore carried out between the results of the sensor BOD measurement and the conventional BOD analysis for the related sensor performance check.

The previously reported BOD sensors have been applied to analyse different kinds of wastewater. It has been noted that in many cases BOD_{st} of these real samples estimated by BOD biosensors are not identical with the results obtained by the conventional test. This is due to the different measuring principles and variable composition of the wastewater samples, therefore BOD_{st} is only analogous to the conventional one. The conventional BOD procedure covers a period of incubation time over 5 or 7 days, which reflects the various metabolic reactions of a mixed microbial population. The incubation includes a microbial adaptation period for microbial growth and induction of the necessary enzymes for assimilation of desired compounds, and a period for enzymatic hydrolysis of polymers (e.g. starch and proteins). Microbial oxygen consumption measured by the conventional method is therefore the sum of the oxygen used to oxidise both easily assimilable compounds and biodegradable polymers. The conventional test has been recognised as an industrial standard and universal method for analysing different kinds of wastewater. In contrast, the BOD estimation with biofilm-type sensors is the test with selected microbial strains. The bacteria are pre-immobilised without requirement of further multiplication of the cells. After immobilisation and fabrication of sensor, only a period of a few hours is essential for sensor stabilisation. Owing to the very short recording time during which degradation of polymers will not take place, BOD biosensors normally can only give responses to those fast and easily assimilable compounds in wastewater samples. The analysis gives insight into the current metabolic process of organic compounds in the wastewater, and therefore is a fast test of biological activity, providing a "snapshot" [48]. Furthermore, the procedure of sensor BOD analysis does not cover a microbial adaptation period to induce the necessary enzymes for assimilation of the desired compounds. As a result, the estimated BOD will also depend on the variety of organic compounds in wastewater samples. However, it is possible to minimise the problems by selecting suitable microbial strains as biological recognition elements and introducing a sensor pre-incubation step and a sample pre-treatment step. These will be discussed later in the paper.

As illustrated in Table 2, wastewaters with a high content of fast and easily assimilable compounds, such as the wastewaters from food and fermentation industry, generally give a less variable and more accurate BOD_{st} estimation than other types of wastewater, such as municipal wastewater and wastewaters from chemical and pharmaceutical industry. This is due to the high concentration of polymers and/or recalcitrant compounds and low concentration of fast and easily assimilable compounds in these wastewaters. Therefore, the BOD biosensors are more applicable to specific wastewaters with high concentration of fast and easily assimilable compounds.

Table 2
BOD values estimated by biofilm-type BOD sensors and conventional 5-day test for various wastewater samples

Wastewater source	Immobilised microbes	BOD_5 (mg/l)	Sensor BOD (mg/l)	(Biosensor BOD/BOD_5) ratio	References
Municipal wastewater	*Trichosporon cutaneum*	252	165	0.65	[20]
		486	825	1.70	
		832	973	1.17	
	Arxula adeninivorans LS3	131	98	0.75	[29]
		123	86	0.70	
		180	123	0.68	
		112	89	0.79	
		53	37	0.70	
		108	86	0.80	
		153	77	0.50	
		114	46	0.40	
		166	175	1.05	
		169	195	1.15	
	Mycelia of *A. adeninivorans* LS3	82.6	121.2	1.47	[43]
		59.0	40.7	0.69	
		116.0	124.0	1.09	
		52.3	54.2	1.04	
		49.5	42.8	0.86	
		19.6	22.5	1.15	
		28.2	26.3	0.93	
	Bacillus subtilis (heat killed)	168	182	1.08	[18]
	B. subtilis + *B. licheniformis*	170	154	0.91	[30]
	Induced microbial consortia	68	191	2.81	[9]
	Bacteria isolated from activated sludge	61.1	67.6	1.11	[10]
	Multi-species culture (BODSEED)	51.6	38.6	0.75	[13]
		75.7	62.1	0.82	
	Multi-species culture (BODSEED) (heat killed)	51.6	41.3	0.80	[13]
		75.7	54.2	0.72	
Food industry	*Trichosporon cutaneum*	152	155	1.02	[34]
		4000	4250	1.06	
		8000	8764	0.91	[28]
		425	357	0.84	[20]
	Hansenula anomala	479	484	1.01	[53]
		52250	49750	0.95	
	Pseudomonas sp./ *Pseudomonas putida*	39800	38300	0.95	[53]
		1954	1740	0.89	
	Bacillus subtilis (heat killed)	348	319	0.92	[17]
		585	567	0.97	[18]
		332	305	0.92	
		1473	1388	0.94	
	B. subtilis + *B. licheniformis*	151	147	0.97	[15]
	Bacteria isolated from activated sludge	350	378	1.08	[10]
		198	297	1.50	
		53.7	55	1.02	
	Induced microbial consortium	411	401	0.98	[9]

Table 2 (*continued*)

Wastewater source	Immobilised microbes	BOD_5 (mg/l)	Sensor BOD (mg/l)	(Biosensor BOD/ BOD_5) ratio	References
Fermentation industry	*Trichosporon cutaneum*	2700	2275	0.84	[27]
	Bacillus subtilis	2700	2245	0.83	[27]
	B. subtilis + *B. licheniformis*	15040	15640	1.04	[15]
	Bacteria isolated from activated sludge	10700	11600	1.08	[10]
Chemical industry	*Trichosporon cutaneum*	726	853	1.17	[28]
	Torulopsis candida	1326	1657	1.25	[45]
	Multi-species culture (BODSEED)	400	395.8	0.99	[13]
	Multi-species culture (BODSEED) (heat killed)	400	341.4	0.85	[13]
					[30]
Pharmaceutical industry	*B. subtilis* + *B. licheniformis*	2076	1897	0.91	
		1032	1042	1.01	
		699	689	0.99	
		659	701	1.06	
	Bacteria isolated from activated sludge	250	185	0.74	[10]
	Multi-species culture (BODSEED)	638	772	1.21	[13]
	Multi-species culture (BODSEED) (heat killed)	638	724	1.13	[13]

Under defined conditions it is possible to deduce the BOD_5 values from the BOD_{st} with the help of specific conversion coefficient. It is essential that the qualitative composition remains relatively constant and that only the concentration is altered. The coefficient of conversion is applicable only to particular stages of an individual sewage treatment plant [48,52].

3.5. Against toxic compounds and microbial contamination

One problem emerging is the poisoning of immobilised microbial cells by toxic compounds in the wastewater. The toxic effect of heavy metal ions and other substances (e.g. CN^- and phenol) on the BOD sensor response has been investigated. Owing to difference in resistance to toxic chemicals among microbial strains, BOD biosensors with different microbes may not show identical resistance patterns to such toxic substances.

Riedel et al. [28] reported that 50 mg/l of Cu^{2+}, Zn^{2+} and Pb^{2+} had only little effect on a *Trichosporon cutaneum* BOD sensor, while drastic inhibition is observed by Hg^{2+} and Cd^{2+}. Li and Chu [53] reported that 50 mg/l of Fe^{2+}, Zn^{2+}, Mn^{2+} and Cu^{2+} had virtually no effect on *Hansennula anomala* or *Pseudomonas* sp. based sensor, whereas the same concentration of Hg^{2+}, Ag^{2+} and 100 mg/l of Cu^{2+} had apparent effects on *Pseudomonas* sp. sensor. Li and Tan [54] demonstrated that 4 mM Fe^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} and Cr^{3+} had a negligible effect for a mixed *Bacilli* BOD sensor. The presence of Co^{2+}, Zn^{2+} and Ni^{2+} even promoted the assimilation rate of the organic compounds, whereas Mn^{2+} suppressed the microbial respiration rate. Ag^+ and Cu^{2+} were strong deactivators. For the BOD sensor based on a heat-killed *B. subtilis*, the analysis was unaffected by 5 mM of Al^{3+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Ni^{2+} and Pb^{2+}. Over-estimation of BOD was reported for samples containing Zn^{2+}, Mn^{2+} and Sn^{2+} in the same concentration, whereas under-estimation was observed in case of Cu^{2+}. The sensor completely and irreversibly lost its BOD sensing ability in presence of 0.5 mM of Ag^+ or Hg^{2+} [55].

The use of resistant strains for sensor preparation is therefore of great interest. A heavy metal resistant BOD sensor using *Alcaligenes eutrophus* was reported to function for estimation of BOD in presence of 4 mM Ni^{2+}, Cu^{2+}, Zn^{2+} [56]. An arsenic resistant BOD sensor based on *Pseudomonas putida* can even determine BOD in presence of 1000 mg/l of As^{5+} [57]. Ohki and his co-workers also indicated that the BOD sensor based on *Klebsiella oxytoca* AS1 showed a higher resistance to phenol and CN^- than the *Trichosporon cutaneum* based sensor [42].

It is possible to eliminate the toxic effects of heavy metal ions by using a chelating agent to reduce the concentration of free heavy metal ions by complexation.

Two chelators, ethylene diamine tetra-acetate (EDTA) and sodium diethyl dithiocarbamate (DDTC), were reported to effectively suppress the interference of some heavy metal ions like Ag^{+}, Cu^{2+}, Mn^{2+}, Sn^{4+} and Zn^{2+} [54,55]. Another interesting approach is by membrane modification. Li and Tan [58] described a modification by covering a BOD biosensor with a poly(4-vinylpyridine)-coated polycarbonate membrane. The membrane prevents the passage of heavy metal ions without loss of selectivity or sensitivity for measurable substrates. Kim et al. [46] reported another membrane modification by graft polymerisation of sodium styrene sulphonate on the surface of the porous teflon membrane to reduce inhibitory effects of Zn^{2+} and Cd^{2+}. Furthermore, phenotypic modification of the microbes by immersing the sensor in a solution containing heavy metal ions can also eliminate their toxic effects [46,54].

Prevention of contamination by other microbes is also important for a reliable biofilm-type BOD sensor. Contamination can influence the analytical results by degradation of target substrates and consumption of DO. Moreover, erosion of the covering cellulose dialysis membrane may also occur, which is due to enzymes secreted by the bacteria from external sources. However, antibacterial reagents can be used to rinse and immerse the measuring system in order to reduce contamination by microorganisms under anaerobic conditions. The sensor microorganisms are stable under aerobic conditions. Such antibacterial reagent can be solution of 0.1% of a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one, 2-methyl-4-isothiazolin-3-one and Mg salts or a mixture of 5-chloro-2-methyl-4-isothiazoline-3-one, 2-methyl-4-isothiazolin-3-one, benzylalcohol and Mg salts [48].

3.6. Operational stability

A stable sensor performance over a desired operational period is essential for a reliable biosensor system. The operational stability of a BOD biosensor may vary considerably depending on the sensor configuration, the microbes used, as well as the immobilisation method. Furthermore, it is also strongly dependent on the external limiting substrates and inner diffusion. Finally, it varies considerably depending on the operational condition.

The operational stability of biofilm-type BOD sensors is primarily related to the stability of the immobilised microorganisms. If a mixture of different types of microorganisms is selected, complex patterns of the mixed culture behaviour must be taken into consideration. The possible patterns of behaviour of two species in a homogeneous culture might include: (1) the influence of the same/different growth-limiting substrate concentration(s) on the specific growth rate (μ) of each species, which represents the rate of growth per unit amount of biomass, (2) product of one species is substrate for the others, (3) inhibitory product of one species is the limiting substrate of the other species, (4) the predator-prey interactions [59]. In a heterogeneous microbial culture, more complex effects can occur. Consequently, the constituent of microbial mixed cultures may vary with time, which results in unstable sensor performance of the multi-strains based sensors after a certain period of operation. The pure microbial cultures, on the other hand, have a much better stability under the equal cultivation and storage condition and therefore tend to be the biological recognition elements of BOD biosensors for long-term operation.

Operational conditions cover a series of factors, which can also considerably influence the stability of a BOD biosensor. Among them, the frequency of measurement deeply affects stability of the sensor. Other factors also include sample composition, sample concentration, buffer composition, the average measuring time, presence of external nutrient supply during analysis interval, as well as the operation and storage temperatures. In general, a frequent analysis of samples results in high stability, whereas an infrequent sample measurement and long pauses give low stability probably due to increased microbial death rate [48]. Furthermore, sensor storage in the buffer at 4°C and reactivation of the immobilised cells by immersing the sensor into a solution with the desired nutrients for a few hours before the new measurements can partly recover deterioration of sensor sensitivity. This is probably due to reactivated metabolic activity of microbes by stimulus of existing cells and growth of new ones. Consequently, the period of stable sensor performance can be prolonged.

As demonstrated in Table 1, operational stability of previously reported BOD biosensors varies from 7 up to 140 days depending upon the above-mentioned factors. For comparison of the sensor operational stability, it is therefore recommended to take these variables into consideration.

As an alternative to those long-term stable BOD sensors, disposable BOD sensors and sensor structures with an easy replacement of the biofilm could be interesting approaches. In the case, biosensor or biofilm can be changed in a frequent, regular mode. Consequently, this makes the BOD biosensors being able to benefit a wide detection range by using the mixed cultures as biological recognition elements without worrying about the stability limitation.

4. Techniques to improve agreement between BOD_{st} and BOD_5

BOD_{st} is not identical to BOD_5 in all cases, it is therefore considered to be a new parameter for environmental process monitoring. However, it is possible to improve the correspondence between BOD_{st} and BOD_5 by the following techniques.

4.1. Selection and induction of suitable microbial strains as the biological recognition element

BOD sensors are aimed at being capable to rapidly analyse a sample of complex constituents with relatively low selectivity. Thus, the sensor shall respond to all kinds of biodegradable organic solutes in the sample. A biological recognition element with high detection capacity for a wide substrate spectrum is therefore needed. Since a single microbial species only has a specific substrate spectrum, it may only assimilate a proportion of organic substrates in the wastewater and cannot achieve the requirement given above. As described previously, combination of two identified microbial strains or utilisation of multi-strains with different substrate spectra has been applied to meet this requirement. A desired microbial consortium can be formed by continuous cultivation of a mixed microbial inoculum (e.g. activated sludge) on the wastewater to be analysed. A better detection capacity for the substrate spectrum to be analysed can be expected since automatic screening of desired microbial strains will be carried out [9].

4.2. Selection of suitable calibration solution

The microbial BOD sensors must be calibrated to enable a comparison with the conventional BOD test. Selection of a proper calibration solution is considered to be one of the key issues for obtaining a good agreement between the BOD_{st} and BOD_5. Although the GGA solution has been widely used as a reference for the conventional BOD test, it was realised that this standard solution was not suitable for the microbial BOD sensors. The reasons can be (1) it is unstable due to rapid microbial contamination, (2) the glutamic acid reaction of microbes is decreased in presence of glucose, due to glucose repression, and (3) since it only consists of two simple components, a calibration will not be reliable when analysing real wastewater samples with a complex mixture of constituents.

Synthetic wastewaters with more complex constituents therefore have been tested for calibrating the BOD biosensors. Tanaka et al. [60] tested different recipes until the combination became close to water quality analyses of secondary effluent from wastewater treatment plants in Japan. Liu et al. [9] chose a synthetic wastewater according to a recipe from the Organisation for Economic Cooperation and Development (OECD). In comparison with calibrating in GGA solution, a remarkable improvement on agreement between the BOD_{st} and BOD_5 was achieved. Table 3 lists the reported synthetic wastewaters and solutions for calibrating BOD biosensors.

An artificial standard solution can be properly designed to calibrate a BOD sensor for analysing wastewaters that have similar composition to the standard solution, but might not be suitable for applying to other wastewaters in different composition. Design of a calibration solution should therefore be given special attention in each particular case. Consequently, Liu et al. [9] introduced the concept of normalised sensor response in order to study the correlation of calibration solutions and sample solutions. The normalised sensor response of a suitable calibration solution must be similar to that of the wastewater sample to be analysed.

4.3. Pre-incubation of the sensor

It has been noticed that pre-incubation by immersing the BOD sensor into a solution with the desired substrates for a few hours before sample analysis can increase the sensitivity and improve agreement between the BOD_{st} and conventional BOD_5. This microbial adaptation period is used to induce the necessary enzymes for assimilation of desired compounds. Riedel et al. [28] reported a significant improvement in agreement between the BOD values estimated by a pre-incubated microbial sensor and those determined by the conventional 5-days method for wastewaters from the fermentation and chemical industry, as well as for OECD synthetic wastewater. In contrast, the BOD_{st} obtained by non-pre-incubated sensor shows a large difference to the BOD_5. Tan et al. [30] pre-incubated a mixture of two identified microbial strains with various organic solutes before preparation of a biofilm. The pre-incubation time was varied up to a maximum length of 6 days. It was observed that the pre-incubation process significantly enhanced the biodegradation capacity of the microbial system in the sensor, and therefore improved agreement between the BOD_{st} and BOD_5. Correlation of the biodegradation capacity and the pre-incubation time for various organic solutes showed in a similar curve pattern, i.e. a lag phase followed by a sharp rise to a maximum value after about 5 days. Furthermore, the pre-incubation time required for full activation of microbes is longer with smaller initial cell population [24]. Yang et al. [20] even developed a robot to repeat a serial pre-conditioning steps over 24 h including a procedure of immersing in a phosphate buffer for 30 min, incubating in a target sample for 5 min and washing in water for 1 min.

4.4. Pre-treatment of sample

In order to let the BOD biosensor give responses to presence of biodegradable polymers as well, it is possible to carry out a sample pre-treatment step by acid hydrolysis or an enzymatic step. This hydrolysis will in most cases cause an increase of BOD_{st}, because the polymers are split into their monomers, such as monosaccharides, amino acids, glycerol and fatty acids. The immobilised microbes can then assimilate these

Table 3
Solutions for calibrating the BOD biosensors

Composition of calibration solutions (mg/l)	References
GGA standard solution: D-glucose (1 5 0) and glutamic acid (1 5 0)	[7,23,27,28,31,34,42,51,53]
OECD synthetic wastewater: peptone (15.0), beef extract (11.0), urea (3.0), NaCl (0.7), $CaCl_2 \cdot 2H_2O$ (0.4), K_2HPO_4 (2.8) and $MgSO_4 \cdot 7H_2O$ (0.2)	[9]
AWW synthetic wastewater: nitrohumic acid (4.246), tannic acid (4.175), sodium ligninsulphonate (NaLS) (2.427), gum arabic (4.695) and sodium laury sulphfate (LAS) (0.942)	[26,41]
Beef extract (1.802), peptone (2.703), nitrohumic acid (4.246), tannic acid (4.175), lignin sulphenic acid (2.427), sodium lauryl sulphate (0.942), gum arabic (4.695) and minerals	[60]
Alanine (3 0 0) and glutamic acid (3 0 0)	[37]
Phenol or hydroxybutyric acid (100 ppm)	[61]
D-glucose (4 5 8)	[29,32]
L-lactate	[38]
Glycerol	[62]
Fructose	[14]

easily degradable substrates during the limited sensor measuring time.

Kwong et al. [62] integrated an acid hydrolysis step (6 M HCl at 100°C) into the analysis procedure using the ARAS BOD apparatus (Dr. Lange GmbH, Germany). Agreement between the BOD_{st} and BOD_5 was improved for the municipal wastewater samples. Reiss et al. [63] incorporated two columns filled with the immobilised amyloglucosidase and α-amylase into the flow system of a commercial BOD sensor (Prüfgerätewerk Medingen GmbH, Germany). Because starch will be degraded enzymatically before analysis, such a sensor system is suitable to measure starch-containing wastewater. In contrast, the BOD apparatus without enzyme columns could not give any response to the starch content in the sample.

Another interesting approach is pre-ozonation of refractory organic compounds in wastewater samples. The hydroxyl radical generated by ozone self-decomposition was used as oxidant to split up organic compounds. In most cases the ozonation of the sample caused an increase of BOD_{st} [40].

However, when acid hydrolysis and ozonation are used, the BOD_{st} value may be overestimated because of the unspecific splitting of organic compounds. In contrast, the specific degradation of samples with enzymes is a better choice [44].

5. BOD biosensors based on different configuration and principles

5.1. The respirometer-type BOD biosensors

The respirometer-type BOD biosensors are actually specifically designed respirometers for short-term BOD measurement. Respirometry is measurement and interpretation of the respiration rate of activated sludge. Although different measuring principles are available, a common feature to all respirometer-type BOD sensors is in a bioreactor configuration, i.e. a small reactor where activated sludge and target organic substrates are brought together [64].

There are two measuring techniques available, *viz*. the batch methods and continuous methods. Typical feature of batch methods is that the BOD is calculated from a respirogram obtained after the addition of a wastewater sample to a respirometer, which can be either a closed respirometer or an open aerated one. In continuous methods, the wastewater sample is continuously fed into a flow-through reactor having a volume of maximum some litres. One approach is to measure the difference of the oxygen consumption between two parallel continuous sludge reactors, where one of the reactors is fed with influent sample while the other is fed with tap water. The influent BOD is calculated from DO and BOD mass balances over the reactors. Another approach is based on the measurement of three different oxygen uptake rates of the activated sludge: endogenous, instantaneous (momentary) and actual respiration rate. Here, the endogenous respiration rate is defined as the oxygen uptake rate of sludge free of readily biodegradable matter. The instantaneous rate is the oxygen uptake rate measured when sludge directly flows from the aeration tank through a respiration metre. The actual respiration rate is the real oxygen uptake rate in the aeration tank [5,65].

Based on the respirometric principle, BOD sensor systems in bioreactor configuration are also reported by using identified microbial strains, such as *Bacillus polymyxa* [66], *Trichosporon cutaneum* [36,67] and a mixture of *Rhodococcus erythropolis* and *Issatchenkia*

orientalis [14]. One benefit of having a bioreactor configuration is that it is very simple to change the transducer without affecting the microbial system, whereas it is almost impossible in case of biofilm configuration. Moreover, with the same substrates and the same microbial strains, the BOD sensors in bioreactor configuration give a more stable performance [36].

5.2. BOD biosensors based on other measuring principles

Except for respirometric principle, BOD biosensors based on other measuring principles are also reported. Karube et al. [7,83] developed a biofuel cell sensor for BOD_{st} estimation. The measuring principle is based on the fact that the current generated by the biofuel cell results from the biooxidation of the hydrogen or formate produced form organic compounds by hydrogen producing bacterium, *Clostridium butyricum*, under anaerobic condition.

An innovative technique is the use of a luminous bacterium, *Photobacterium phosphoreum*, for BOD sensor fabrication. The measuring principle is based on the relationship between the intensity of luminescence produced by the bacterium and the cellular assimilation of organic compounds from a wastewater sample [68,69].

Teutscher [70] and Hassapis [71] described a method for continuous BOD measurement by dynamically adjusting the dilution ratio of fresh water to the monitored wastewater in order to maintain a constant rate of oxygen consumption in a bioreactor. The BOD was assessed by monitoring the changes in the dilution ratio. A commercial BOD apparatus, BIOX−1010 (STIP Isco GmbH, Germany), is designed using the same principle.

BOD estimated by measuring acidic compounds released from microbial oxidation of substrate with oxygen was reported. Both a pH sensitive electrolytic-effect-type transistor (pH-ISFET) [72] and a surface photovoltage (SPV) device have been used to fabricate BOD sensors [73].

In general, BOD estimation based on normal microbial respirometric principles is influenced by the amount of DO concentration in the sample, where oxygen is the terminal electron acceptor. However, certain redox-active substances can be reduced by certain microorganisms, thereby bypassing the oxygen reduction step. It can serve as electron shuttling between microorganisms and electrode. Therefore, mediator-type BOD biosensors were developed to overcome the intrinsic problem of these BOD methods based on aerobic respirometric principle. Yoshida et al. [74] described a mediator-type BOD sensor using potassium hexacyanoferrate(III) [HCF(III)] as an electroactive compound for detecting the metabolic reactions of immobilised *Pseudomonas fluorescens* biovar V. Alternatively, Trosok et al. [75] demonstrated the applicability of using an immobilised yeast strain and ferricyanide as an electron mediator for rapid analysis of BOD in wastewaters.

The BOD of wastewater sample can be determined also by measuring the metabolic heat released during oxidation of organic substrates by microbes and converting the heat to a proportional potential signal [76–78].

6. Commercial BOD instruments

The first commercial microbial BOD apparatus was produced by Nisshin Denki (Electric) Co. Ltd. in 1983. Subsequently, a few more BOD sensors have been developed and marketed by various manufacturers. Sensor configurations in both biofilm- and bioreactor-types are available. Table 4 lists some of the commercial BOD instruments and their characteristic parameters. All of these instruments have automatic sample injection and calibration with a standard solution with defined BOD. It should be noticed that the information presented here is only a snapshot of the commercially available equipments due to the limited sources of valid data.

Practical experiences by applying these BOD instruments to real wastewater samples have been reported as well. It is important that these commercial BOD instruments can be used to provide analytical data comparable to those obtained from the conventional BOD test, and perform the analysis outside the well-controlled laboratory.

Praet et al. [36] compared BOD_{st} estimated by BOD sensor 7842-Microbe Sensor Series (DKK Corporation) to BOD_5 values. Comparable BOD estimations are difficult to realise for real wastewaters that change in composition from one sample to another. The choice of the calibration solution and its composition dramatically influence the quality of analysis. However, Marty et al. [37] reported that good correlation was observed for the influent and effluent of the municipal wastewater and domestic landfill leachates with a BOD value up to 500 mg/l.

Iranpour et al. [79] studied the correlation between the BOD_{st} estimated by BOD-2000 (Nisshin Electric & Co. Ltd., Japan) and conventional BOD test. Under well-controlled laboratory conditions, this BOD apparatus performs excellent analyses on filtered samples of a primary influent. However, modifications are needed to obtain a system with a better durability and less labour requirement for field service.

Kwong et al. [62] estimated BOD of water samples collected in the marshes by the conventional BOD test and ARAS BOD sensor (Dr. Lange GmbH, Germany).

Table 4
Commercially available BOD instruments

Model	BOD−2000 BOD−3000	DKK™ BOD sensor 7842	BODypoint	BSBmodul	ARAS	BIOX−1010[c]	RODTOX 2000	BOD-BioMonitor	QBOD metre & EZ-BOD metre[e]	RACOD™ metre
Manufacturer	Nisshin Denki & Central Kagaku Co. Ltd., Tokyo, Japan	DKK Corporation, Japan	Aucoteam FmbH, Berlin, Germany	Prüfgerätewerk Medingen GmbH, Dresden, Germany	Dr. Lange GmbH, Berlin, Germany	STIP Isco GmbH, Groß-Umstadt, Germany	Kelma, Belgium	LAR Analytik & Umweltmesstechnik GmbH, Berlin, Germany	Bioscience, Inc., Bethlehem, USA	USFilter, Vineland, NJ, USA
System configurations	Biofilm type, flow-through system	Biofilm type, flow injection with 3 ml measuring chamber	Biofilm type, flow-through system	Biofilm type, flow-through system	Biofilm type, with 2 ml stirred measuring chamber	A bioreactor combined with a dilution system	Respirometer type (BOD & toxicity analyser)	Respirometer type	Respirometer type (bioreactor)	Respirometer type
Microbial species	*T. cutaneum*	*T. cutaneum*	*T. cutaneum, C. parapsilosis*	*T. cutaneum, C. parapsilosis*	*R. erythropolis* + *I. Orientalis*	Bacteria isolated from wastewater	Activated sludge	Activated sludge	Activated sludge	Activated sludge
Measuring time (min)	20−40	5	<1	3 or <1	1−3	3−15	20–40	3−5	20[d], 15−60[c]	10 (high range) or 30 (low range)
Measuring ranges (mg/l BOD)	0−100, 0−200, 0−500[a]; 3−1000[b]	0−60	5−500	0−22 or 2−33	2−300	5–1500, 20−1500, 20−100000	0−500000	0−50, 0−200000	0.5−300 or 0.5−5000[d]	100–4000 (high range) or 0–100 (low range)
Working Stability (days)	30[a,b]		30	30	30		14		60−90[d]	
Precision (±%)	3[a,b]	5	<10	<10	<5	3	<5		10 (low range)[d] 5 (high range)[d]	
Calibration Standard	GGA	GGA	Glucose	Glucose	Glycerol		Stabilised wastewater of the plant being monitored			
	[37,48,52, 79,80]	[36,37,80]	[37,48]	[36,48,52, 63,81]	[36,48,79]	[79] (http://www.isco.com, 2/01/2002); (http://www.envitech.co.uk, 6/15/2001)	[82] (http://www.kelma.com/rodtox.html, 6/15/2001)	[79] (http://www.lar.com/bod.htm, 6/15/2001)	(http://www.bioscienceinc.com, 6/15/2001).	(http://www.usfilter.com, 1/02/2002)

[a] BOD−2000.
[b] BOD−3000.
[c] Measuring principle based on dynamic dilution of two gear pumps depending on the O_2 respiration of microbes.
[d] QBOD metre.
[e] EZ-BOD metre.

Good correlation was observed after enzymatic pre-hydrolysis of macromolecules in the samples.

7. Conclusion

BOD biosensors show potential application for rapid estimation of biodegradable organic matter in wastewaters. A short response time is the major advantage of using the BOD biosensor system since it opens the possibility for on-line monitoring and process control. Several BOD biosensor systems have even been developed commercially. However, there still remain restrictions of the present BOD biosensors, and consequently these weaknesses limit the industrial applications. In general, these weaknesses include: (1) insufficient reliability for wastewater samples with varied compositions or high content of polymers, (2) insufficient resistance to various toxic compounds in the wastewater (3) lacking robustness for field service, (4) complicated requirements of maintenance, (5) restrictions due to the lack of standardisation and legislation in most countries. There is no doubt that further research and developments are required to overcome the problems mentioned above. New technologies, new biomaterials and optimisation of biosensor systems will lead to improved BOD biosensors. Furthermore, miniaturisation of the BOD sensor systems and development of portable systems should be encouraged for field service and process monitoring. Finally, investigations on practical experiences by applying the BOD biosensors to field service should be promoted for the aim of wide industrial applications.

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