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Bioelectronic tongue and multivariate analysis: A next step in BOD measurements

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ABSTRACT

Seven biosensors based on different semi-specific and universal microorganisms were constructed for biochemical oxygen demand (BOD) measurements in various synthetic industrial wastewaters. All biosensors were calibrated using OECD synthetic wastewater and the resulting calibration curves were used in the calculations of the sensor-BOD values for all biosensors. In addition, the output signals of all biosensors were analyzed as a bioelectronic tongue and comprehensive multivariate data analysis was applied to extract qualitative and quantitative information from the samples. In the case of individual biosensor measurements, most accurate result was gained when semi-specific biosensor was applied to analyze sample specific to that biosensor. Universal biosensors or biosensors semi-specific to other samples underestimated the BOD_7 of the sample 10–25%. PLS regression method was used for the multivariate calibration of the biosensor array. The calculated sensor-BOD values differed from BOD_7 less than 5.6% in all types of samples. By applying PCA and using three first principal components, giving 99.66% of variation, it was possible to differentiate samples by their compositions.

1. Introduction

Biochemical oxygen demand (BOD) is a widely used parameter in environmental analysis to monitor wastewater treatment processes. Measured BOD values characterize the oxygen required for the biochemical degradation of organic material and the oxygen used to oxidize inorganic material (APHA, 1985). The method is a universal one for analyzing samples from various sources and requires no expensive equipment. However, it is time consuming and labor intensive and therefore, not suitable for process monitoring in wastewater treatment systems where fast results are required. To overcome this limitation, several faster methods based on biosensors have been developed.

Since the announcement of the first BOD biosensor (Karube et al., 1977), many different biosensor designs have been applied. Most reported BOD biosensors are biofilm-type microbial biosensors in which membrane containing immobilized microorganisms is coupled with a Clark-type dissolved oxygen sensor (Lin et al., 2006). These biosensors rely on measuring a change in bacterial respiration rate, which is expressed by the decrease in measured oxygen concentration and proportional to the BOD of the sample solution (Liu et al., 2004). However, the BOD values measured with biosensors are

Abbreviations: ANOVA, Analysis of Variance; BOD, Biochemical Oxygen Demand; BOD_7, 7 day biochemical oxygen demand; CMC, carboxyl-methyl-cellulose; OECD, Organization for Economic Cooperation and Development; PCA, Principal Component Analysis; PC, Principal component; PLS, Partial Least Squares; Sensor-BOD, BOD measured with biosensor.

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not identical to the conventional BOD values in all cases. The BOD biosensors can normally give responses only to fast and easily assimilable compounds in the samples, whereas BOD measured by the conventional method is the sum of the oxygen used to oxidize both the easily assimilable compounds and biodegradable polymers (e.g. starch and proteins) (Liu et al., 2004). Although some microorganisms can assimilate a wide range of organic compounds, the range provided by a single or two microorganisms would still be inadequate to cope with the wide range of different wastewaters (Tan and Wu, 1999). Wastewaters, especially industrial wastewaters, contain various specific refractory compounds. During the short measuring time of a biosensor microorganisms are not able to degrade these compounds and thereby, lower sensor-BOD values will be measured. However, it is possible to minimize the discrepancies between different methods by selecting suitable microbial strains as biological recognition elements (Dhall et al., 2008; Liu and Mattiasson, 2002).

Better correlation between sensor-BOD and BOD_7 in analysis of industrial wastewaters can be obtained by using semi-specific microbes. Semi-specific microorganisms differ from universal microorganisms by their substrate spectra. As universal microorganisms use a limited substrate spectrum common to a majority of aerobic heterotrophic microorganisms, semi-specific microorganisms can additionally biodegrade some refractory compound or group of compounds specific to that particular microorganism. Thereby, semi-specific microbes are able to oxidize refractory compounds found in industrial wastewaters, which would be undetected by universal biosensors, thus better correlation between sensor-BOD and BOD_7 can be achieved. Functionality of semi-specific biosensors for different kinds of industrial wastewater has been demonstrated (Kibena et al., 2012; Raud et al., 2010, 2012a,b; Raudkivi et al., 2008). Nevertheless, biosensors based on semi-specific bacteria do not excel in wastewaters containing refractory compounds toward which they do not have semi-specific properties and thereby, underestimate the BOD value to an extent of those compounds (Raud et al., 2012b). Therefore, any single semi-specific biosensor is advantageous only in the case of the wastewater with known specific content toward which the biosensor is semi-specific. This limitation could be overcome if several different semi-specific biosensors were incorporated into an array – a bioelectronic tongue.

The electronic tongue is an analytical system applied to liquid analysis formed by a sensor array in order to generate multidimensional information, plus a chemometric processing tool to extract meaning from these complex data (del Valle, 2010). Various sensor arrays based on chemical sensors have been applied for wastewater analysis (Bourgeois et al., 2003; Campos et al., 2012; Stuetz et al., 1999). The area of sensor arrays based on microbial biosensors is however less researched. Sakaguchi et al. (2003, 2007) applied a single luminescent recombinants of *Escerichia coli* in biochip which enabled to measure various BOD values simultaneously. Biosensor-array based on several *E. coli* mutants was applied for simultaneous detection of different saccharides (Aivasidis et al., 2002) and König et al. (2000) used two different microbial strains for BOD and naphthalene detection. The main criteria for application of sensors in a sensor array are low selectivity and high cross-sensitivity (partial specificity) (Vlasov et al., 2005). In case of the BOD biosensor array, it can be achieved by slightly different substrate spectra of microorganisms used in different biosensors. Due to the universal substrate spectrum of microorganisms, BOD biosensors are sensitive to variety of organic compounds in wastewater sample. In addition to that, biosensors based on semi-specific microorganisms have wider and partially specific substrate spectra. Thus, they can also detect and measure specific refractory compounds. By combining variety of semi-specific and universal microbial biosensors into a biosensor array, a complex signal containing information about various components in the medium can be obtained (Vlasov et al., 2008).

The properly trained electronic tongue is capable of recognizing qualitative and quantitative composition of complex samples (Vlasov et al., 2005) which can be extracted by employing suitable multivariate analysis techniques to process the signals generated by an array of biosensors (Hines et al., 1999; Scott et al., 2006; Skov and Bro, 2005). Among various methods Principal Component Analysis (PCA) is the most common and versatile method for data projection. It is widely used in data analysis to display groupings among sets of samples (Hibbert, 1998; Hruškar et al., 2010). PCA reduces dimensionality of the sensor array data to a few principal components, which contain most of the variation in the data and thereby patterns can be separated from each other using simple scatter plots. To implement quantitative analysis, the Partial Least Squares (PLS) regression method is widely used for multi-determination applications (del Valle, 2010).

The aim of this work is to use an array of different semi-specific and universal biosensors as a biosensor array to determine BOD in complex industrial wastewaters. Comprehensive multivariate data analysis was applied to biosensors' signals to investigate possibilities to differentiate wastewater samples according to their composition and to gain more precise BOD estimation in various samples.

2. Materials and methods

2.1. Chemicals

All chemicals except otherwise noted were analytical grade. Phosphate buffer solution of pH 6.86 ($Na_2HPO_4 \cdot 12H_2O$, 6.90 g l^{-1}; $NaH_2PO_4 \cdot H_2O$, 7.04 g l^{-1}) were used.

2.2. Standard solution

Synthetic wastewater according to the recipe of Organization for Economic Cooperation and Development (OECD) (peptone, 1.6 g l^{-1}; meat extract, 1.1 g l^{-1}; urea, 0.3 g l^{-1}; K_2PO_4, 0.28 g l^{-1}; NaCl, 0.07 g l^{-1}; $CaCl_2 \cdot 2H_2O$, 0.05 g l^{-1}; $MgSO_2 \cdot 7H_2O$, 0.02 g l^{-1}) (OECD, 1984) was chosen as a standard solution to calibrate BOD biosensors. The BOD_7 of standard solution was measured as 2000 mg l^{-1}.

2.3. Microorganisms

Semi-specific and universal bacterial cultures used in previous studies and described in Table 1 were selected for construction of biosensors.

Table 1 – Microorganisms used for biosensors construction and their main characteristics.

Biosensor	Microorganisms	Substrate spectra description	Reference
P75	*Pseudomonas fluorescens*	Universal, low specificity	(Raud et al., 2010, 2012a,b; Raudkivi et al., 2008)
P69.1	*Aeromonas hydrophila*	Semi-specific to fat	(Raud et al., 2012a)
P67	*Pseudomonas putida*	Semi-specific to phenol	(Raudkivi et al., 2008)
R17.1	*Escerichia coli*	Semi-specific to lactose	(Raud et al., 2010)
CE22.1	*Bacillus subtilis*	Semi-specific to cellulose	(Raud et al., 2012b)
HTK158a	*Paenibacillus* sp.	Semi-specific to cellulose	(Raud et al., 2012b)
17.3	*Microbacterium phyllosphaerae*	Semi-specific to milk and phenol	(Kibena et al., 2012)

2.4. *Preparation of cell suspension*

The bacteria were grown under aerobic conditions in a rotating shaker (Sanyo Orbital Incubator, Sanyo) at 30 °C in a Luri-Bertan medium (tryptone, 10 g l^{-1}; yeast extract, 5 g l^{-1}; NaCl, 5 g l^{-1}). 2 ml of culture medium was inoculated and incubated for 14 h; subsequently the cell suspension was subcultured into a 150 ml of culture medium and incubated 8–14 h until gaining late exponential phase of growth. Optical densities of the bacterial suspensions were measured with spectrophotometer (HP 4853, $\lambda = 600$ nm) to assure cells deriving from the late exponential phase of growth.

The bacterial suspension was centrifuged (Jouan CR3) at 4000 r/min for 15 min at room temperature and the supernatant was decanted. To circumvent the culture medium from getting into the membrane, the cells were washed twice with phosphate buffer solution and centrifuged at the same conditions. The washed bacterial paste was used immediately to prepare the microbial membranes.

2.5. *Immobilization*

Mixture of 180 mg agarose and 7.5 ml phosphate buffer was heated at 70 °C until complete melting of agarose. The mixture was cooled down to 45 °C and 900 μg of the previously prepared cell paste was added. After mixing polypropylene net discs (Scrynel, PP 500 HD) were imbued with the homogenous agarose suspension. Until hardening of the agarose suspension, the resulting membranes were placed between two glass plates and even force was applied to gain certain and even thickness of membranes. During the service life the membranes were maintained at 4 °C in a phosphate buffer solution.

2.6. *Pre-conditioning*

After immobilization the membranes were placed in a phosphate buffer solution dosed with OECD synthetic wastewater (BOD_7 of solution 30 mg l^{-1}) for pre-conditioning up to 14 days until the sensor output signal remained stable.

2.7. *Equipment*

The biosensor measuring system was constructed as described in previous papers (Kibena et al., 2012; Raud et al., 2010, 2012a,b). Clark-type dissolved oxygen probe (WTW, CellOx 325, Germany) was used as an electrochemical transducer. Biosensor output signal was registered with measuring module (WTW, InoLab 740, Germany) and detected and transformed using MultiLab Pilot program. Computer was used to save and analyze the data. Aeration unit and magnetic stirrer (KEBO-LAB MR 2002) were used in order to ensure saturation of measuring solution with oxygen. Membrane containing immobilized microorganisms was attached to the dissolved oxygen probe with special insertion ring and a holder.

2.8. *Experimental procedure*

The measurements were carried out at room temperature (22 ± 2 °C) in a beaker containing 50 ml of previously aerated phosphate buffer solution. Before measurements the membranes were held in a room temperature for 1 h in order to adapt immobilized microorganisms with room temperature. The biosensor was placed into the aerated measuring solution and after an initial stable current was reached (I_0), certain amount of substrate was added. The substrate was inserted into the measuring solution using step-by-step adding method in order to change the BOD_7 of measuring solution gradually with each addition. During addition of substrate the initial and end-point steady state signal (I_S) was recorded. Experiment was carried out until the output from oxygen sensor was zero or did not change after addition of substrate into the measuring solution.

2.9. *Calibration*

All calibration measurements with biosensors were conducted using steady state method described in previous paragraph. For single biosensor measurements, the OECD synthetic wastewater was used as a calibration solution. The BOD_7 of standard solution was measured as 2000 mg/l. Partial least squares (PLS) regression was chosen as a multivariate calibration method and it was conducted using six sensors and three latent variables. The initial dataset was divided into calibration and test datasets - one third of measurements were used for calibration and two thirds were used as a test dataset to test the PLS model. The dataset consisted of measurement results gained from analyzing OECD synthetic wastewater and OECD synthetic wastewater spiked with different refractory compounds. The composition of previously mentioned samples is described in Section 2.10. Four

parallel measurements on every BOD_7 value were considered for redacting calibration graphs for single sensor analysis and when conducting PLS calibration.

2.10. Wastewater samples

Experiments were conducted with several synthetic wastewaters. Combined synthetic wastewaters simulated wastewaters from different industries (meat, dairy, oil shale and paper industry) and consisted in OECD synthetic wastewater and an additional refractory compound, characteristic to specific industrial wastewaters.

The combined wastewater with fatty substances was produced by dissolving components of OECD synthetic wastewater in a saturated solution of fat. As fat has low solubility in water, half-sized quantities were used in order to achieve the proportion of fat in the BOD_7 of combined synthetic wastewater of approximately 15–20%. Additionally combined wastewaters dosed with either milk, phenol or CMC were used, by adding 1 ml milk with fat content of 2.5%, 0.168 g phenol or 1 g CMC, respectively, into 1000 ml of OECD synthetic wastewater. Conventional BOD_7 test was conducted with all samples according to APHA standard (APHA, 1985).

2.11. Statistical analysis

Normalized signal response at certain BOD_7 value was used as a biosensor response in all calculations. The biosensor output signal was normalized using formula 1:

$$NSR = \frac{I_0 - I_S}{I_0} \quad (1)$$

Where *NSR* is normalized signal response, I_0 is the initial steady state signal at zero BOD_7 value and I_S is the steady state signal at certain BOD_7 value. Normalized signal response was plotted against BOD_7 value and calibration graphs were constructed which were used to calculate sensor-BOD values of different individual sensors. Normalized signal responses of each sensor which were scaled and centered and used for multi-sensor analysis.

Factorial ANOVA was conducted using three factors – sensor, sample and BOD_7, to determine the significant difference between different biosensors. Factor BOD_7 was used in 9 levels (0, 5, 10, 15, …, 40 mg/l), factor sensor had 7 levels indicating different biosensors and factor sample had 5 levels indicating different samples. 4 parallel measurements were considered in analysis. Scheffe test was used in *Post hoc* analysis to locate the difference between biosensors. PCA and PLS were used for multi-sensor analysis. For PLS the initial dataset were divided into calibration and test datasets – one third of measurements were used for calibration and two thirds were used as test dataset to test the model.

Data was gathered using Microsoft Excel 2003 and all calculations were made using GraphPad Prism 5 (GraphPad software Inc., San Diego, USA) and Statistica 8 (Statsoft Inc., Tulsa, USA) software. Significance level $\alpha = 0.05$ was used in all analysis.

3. Results and discussion

3.1. Measurements with individual sensors

All individual sensors were calibrated using the OECD synthetic wastewater. In order to investigate the performance of individual biosensors in wastewaters containing refractory compounds, different synthetic industrial wastewater samples were used. Fig. 1 illustrates the agreement between BOD_7 and sensor-BOD values measured in these wastewater samples using different semi-specific biosensors.

Majority of individual sensors underestimate the BOD_7 of synthetic industrial wastewaters by 10–25%, which is approximately the extent of additional BOD_7 introduced into the OECD synthetic wastewater with the refractory compound. However, biosensors that have semi-specific properties toward that certain refractory compound outperform non-specific biosensors and biosensors semi-specific to other refractory compounds. Thus, in the synthetic wastewater spiked with milk, closer results to the BOD_7 values were gained with biosensor R17.1 which overestimated the BOD_7 of sample by 11%, while other biosensors underestimated it up to 23%. Similar result can be seen in fat spiked synthetic wastewater using biosensor P69, phenol spiked synthetic wastewater using biosensor P67, and CMC spiked synthetic wastewater using biosensors CE22.1 and HTK158a. The non-specific biosensor P75 underestimated BOD_7 in all synthetic industrial wastewater samples up to 25% that was approximately the proportion of additional BOD_7 introduced into the sample with the refractory compound.

Lower sensor-BOD values are caused by the partial assimilation of refractory compounds which remained undetected (Kumar et al., 2010), while the portion of BOD_7 resulting from OECD synthetic wastewater was detected. Both, non-specific and semi-specific microorganisms used in biosensors were unable to assimilate refractory compounds toward which they were not semi-specific. Therefore, the refractory compounds in those wastewater samples remained inassimilable causing underestimation of BOD_7. Generally, semi-specific biosensors are more suitable to conduct industrial wastewater analysis than universal biosensors. When using single biosensor for analysis of industrial wastewaters containing refractory compounds, it is essential to have prior information about wastewater source and constitution in order to select the suitable biosensor and thus, gain accurate results.

3.2. ANOVA and post hoc analysis

The differences between BOD values measured with different biosensors were verified using ANOVA ($p = 0.000$). Analysis confirmed that all factors (sample, sensor and BOD_7) have significant influence on the measured sensor-BOD values e.g. the measured sensor-BOD depends on the actual BOD_7 value of the sample, composition of the sample, and the sensor used to analyze it. Subsequently, Scheffe test as *post hoc* analysis was used to specify the differences between biosensors for different wastewater samples (Table 2).

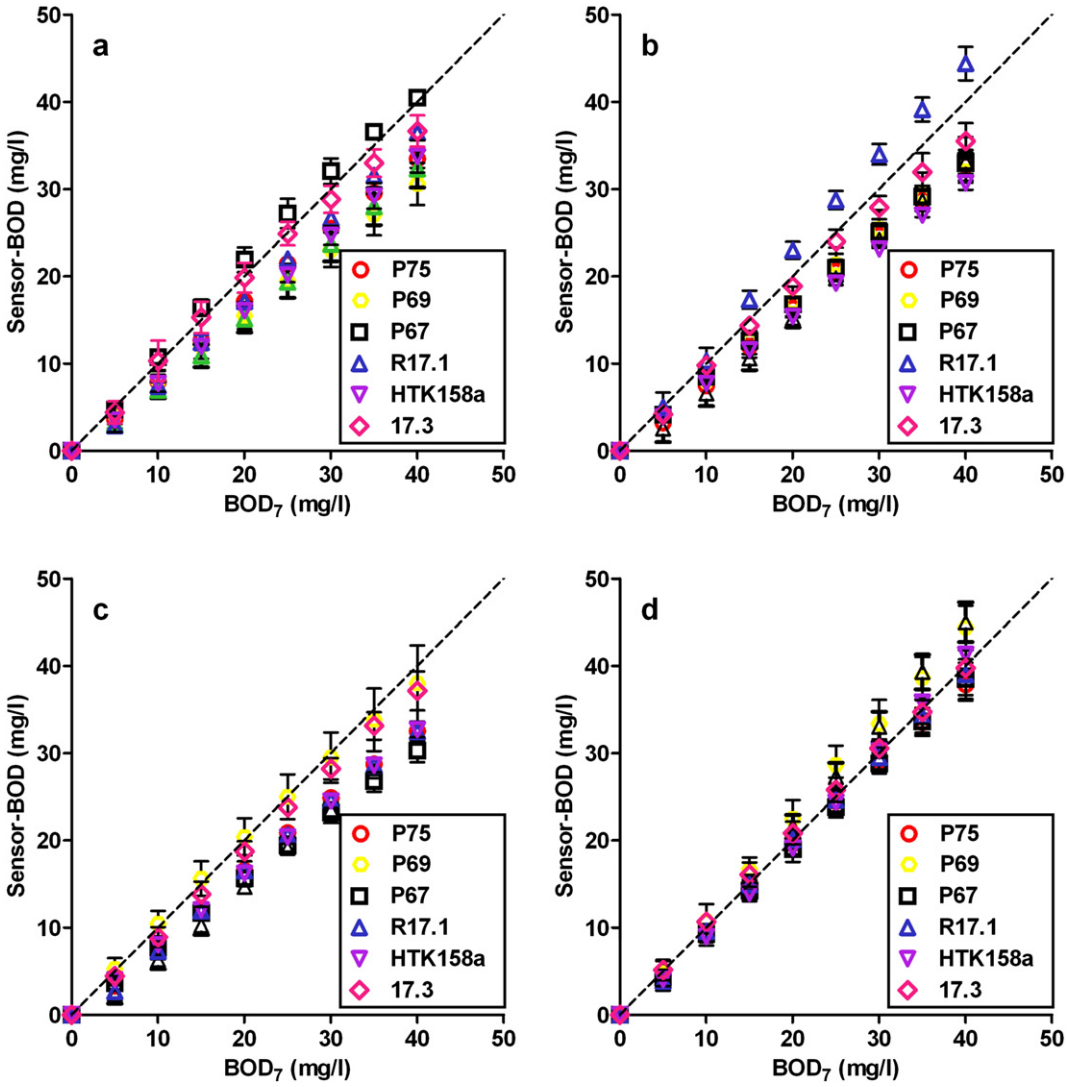

Fig. 1 – Comparison of BOD_7 and sensor-BOD of spiked OECD synthetic wastewater measured with single individual sensor: A – OECD phenol; B – OECD milk; C – OECD fat; D – OECD CMC. 1/1 correlation between sensor-BOD and BOD_7 is shown with the center-line on the graph.

Post hoc analysis revealed statistically different sensor-BOD values between semi-specific biosensor and other biosensors when specific sample corresponding to a semi-specific biosensor was analyzed. The results from biosensors R17.1 and 17.3, both designed for dairy industry, were higher and differed from most other biosensor's results in milk spiked sample. Similarly, sensor-BOD measured with biosensors P69 and 17.3 in fat spiked sample, and P67 and 17.3 in phenol spiked sample were different from the results gained with other biosensors. Thus, it can be concluded that there is indeed qualitative information present in the collective signal of the selected semi-specific biosensors.

In case of CMC spiked sample, the sensor-BOD values measured with biosensor CE22.1 and HTK158a, designed for analysis of cellulose rich wastewaters from pulp and paper industry, were similar to three and five other biosensors, respectively. Smaller differences from other biosensors could be caused by lower biodegrading capabilities of microorganisms toward CMC which is barely detectable even with BOD_7 analysis (Raud et al., 2012b). Since the results from these biosensors were similar in analysis of all sample types only one of them was used in further multivariate analysis.

Although bacterial culture used in biosensor 17.3 was initially isolated and tested as a semi-specific toward lactose and milk, higher sensor-BOD values were measured with it in fat and phenol spiked samples as well. The latter can be explained by a broader substrate range of used microorganisms that were partially able to assimilate a wider range of refractory compounds. Thus, somewhat better estimation of BOD was achieved with biosensor 17.3 compared with non-specific biosensor but lower than semi-specific biosensors in the samples spiked with corresponding refractory compounds.

Non-specific biosensor P75 measured lower sensor-BOD values in all the samples. Its sensor-BOD values differed from sensor-BOD values of semi-specific biosensors when the wastewater samples corresponding to those semi-specific biosensors were used.

Table 2 – *p*-Values from Scheffe test conducted as a *Post hoc* analysis. Values smaller than 0.05 (marked bold) indicate statistically relevant differences between the results of biosensor pairs.

Sample	Sensor	P75	P69	P67	R17.1	CE22.1	HTK158a	17.3	
OECD + Fat	P75		0.853	**0.000**	1.000	0.794	1.000	**0.000**	OECD + Phenol
	P69	**0.000**		**0.000**	**0.042**	1.000	0.999	**0.000**	
	P67	0.997	**0.000**		**0.000**	**0.000**	**0.000**	**0.001**	
	R17.1	1.000	**0.000**	0.999		**0.027**	0.987	**0.002**	
	CE22.1	0.959	**0.000**	1.000	0.999		0.999	**0.000**	
	HTK158a	1.000	**0.000**	0.999	1.000	0.978		**0.000**	
	17.3	**0.000**	0.872	**0.000**	**0.000**	**0.000**	**0.000**		
OECD + CMC	P75		1.000	1.000	**0.000**	1.000	0.999	**0.000**	OECD + milk
	P69	**0.000**		1.000	**0.000**	0.939	0.662	**0.020**	
	P67	1.000	**0.000**		**0.000**	0.988	0.857	**0.004**	
	R17.1	1.000	**0.000**	1.000		**0.000**	**0.000**	**0.000**	
	CE22.1	**0.000**	0.999	**0.000**	**0.010**		1.000	**0.000**	
	HTK158a	1.000	**0.000**	0.999	1.000	0.082		**0.000**	
	17.3	0.593	**0.005**	0.437	1.000	0.779	0.999		

3.3. *Principal component analysis*

PCA was used to test whether it is possible to distinguish different samples by their composition and BOD_7 values using simultaneous analysis of several biosensor signals. PCA is a major linear technique used to reveal groupings among sets of cases (Hibbert, 1998). By using PCA, multivariate data can be explored, reducing their noise, without loss of information, besides the possibility of assessing the significance of individual sensors (Riul et al., 2010). In the current study, average sensor signal of six biosensors from analysis of five different synthetic wastewater samples were used in PCA analysis to extract qualitative information of sample composition. Sensors used in analysis were P67, P69, R17 17.3, P75 and HTK158a.

Fig. 2 shows PCA score plots when three first components, giving 99.66% of total variation, were used. Measurements at smaller BOD_7 values are clustered closely together but as the BOD_7 increases, the measurements are located farther away from the starting point. Score plot of PC1 versus PC2 (Fig. 2a) illustrates how BOD_7 can be described using PC1 which values change from negative to more positive as the BOD_7 of solution increases. In score plot of PC2 versus PC3 (Fig. 2b), different types of samples detach farther from the center in different directions as the BOD_7 increases. The discrimination of samples by constituents is easier in case of average or larger BOD_7 values since they are located farther from the starting point. The point density in the fringe area of the figure is smaller and points are located in the different sections of the plot as the composition of the sample changes.

In Fig. 2a, the measurements with samples containing phenol and fat have positive PC2 values while other samples have negative PC2 values. In addition, milk or phenol spiked wastewaters also have either positive or negative PC3 values (Fig. 2b), respectively, which enables better discrimination of these samples. Other types of wastewaters and wastewater with no additives were clustered somewhat closer to each other which make their discrimination more difficult. Clustering of points from these types of samples might have been caused by relatively small difference in the sample constitution. The samples used for measurements differed from each

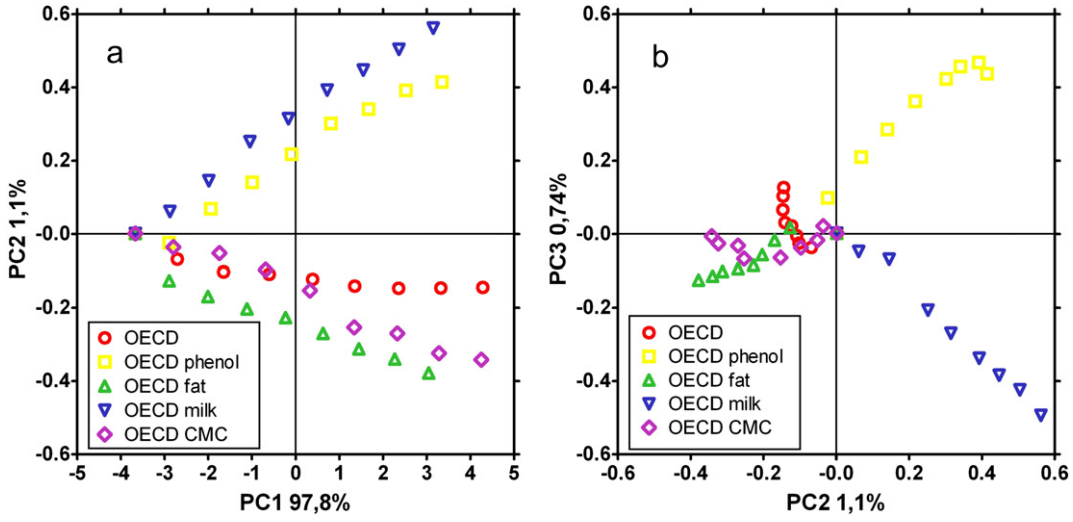

Fig. 2 – Plots of principal components using 6 biosensors showing separation of different synthetic industrial wastewater samples.

other by 15–20% of BOD_7 which was the content of additional refractory compound introduced to the OECD synthetic wastewater.

3.4. *Multivariate calibration*

PLS regression was chosen as a multivariate calibration method because of its usefulness in predicting a set of dependent variables from a large set of independent variables (Hruškar et al., 2010). PLS analysis was conducted using six sensors and three latent variables. The initial dataset were divided into calibration and test datasets – one third of measurements were used for calibration and two thirds were used as test dataset to test the PLS model.

The results indicate that by using simultaneously several semi-specific biosensors, corresponding to different substrate spectra, it is possible to gain more precise BOD estimation than by using any single biosensor. Fig. 3 shows the correlation of BOD_7 and calculated sensor-BOD values of different synthetic wastewater samples. Calculated sensor-BOD values differed from BOD_7 in case of all samples less than 5.6% at different BOD_7 values and average underestimation and standard deviation were less than 1% and 3.4%, respectively. The proposed approach of multivariate analysis and biosensor array measurements overcomes the information deficiency in wastewater analysis when inflows from various industrial and domestic sources are mixed in the wastewater treatment plant. As previously indicated, the analysis with a single semi-specific biosensor requires prior information about the composition of the wastewater in order to select suitable biosensor. Furthermore, multivariate analysis takes into account the diverse signals from all semi-specific biosensors and thereby, more precise results are obtained in case of all samples.

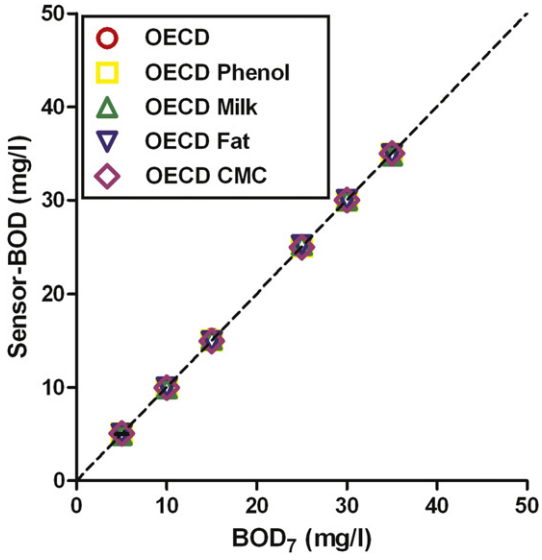

Fig. 3 – Comparison of BOD_7 and sensor-BOD when PLS was used to estimate the latter. 1/1 correlation between sensor-BOD and BOD_7 is shown with the center-line on the graph.

4. Conclusions

Different universal and semi-specific biosensors were used individually and as an array to analyze synthetic wastewater samples spiked with refractory compound specific to a certain industry. Generally suitable semi-specific biosensors enabled to gain most accurate results in industrial wastewater specific to that certain biosensor. On the other hand, semi-specific biosensor with wrong specificity toward wastewater does not improve the accuracy of the results and underestimates the BOD of sample. Therefore, to gain accurate results with a single biosensor, it is vital to have a pre-knowledge of the sample composition and origin to select the suitable sensor. This limitation was overcome when different biosensors, semi-specific to different compounds, were used as an array and multivariate data analysis was applied for data analysis to extract qualitative and quantitative information. Simultaneous use of array of different semi-specific biosensors gives broader substrate spectra in comparison with random single biosensor and by using PLS, considerably better estimation of BOD in any wastewater was achieved. The signal from array of semi-specific biosensors contains qualitative information which could be extracted by using three first principal components of PCA. By applying PCA, it was possible to differentiate samples by their compositions.

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