Evaluation of the heavy metal resistant on biosensors for the purpose of biochemical oxygen demand measurements

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EVALUATION OF THE HEAVY METAL RESISTANT ON BIOSENSORS FOR THE PURPOSE OF BIOCHEMICAL OXYGEN DEMAND MEASUREMENTS

EVALUACIJA REZISTENTNOSTI NA TEŠKE METALE BIOSENZORA ZA MJERENJA BIOKEMIJSKE POTREBE ZA KISIKOM

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Abstract: *The biochemical oxygen demand (BOD) sensor based on an immobilized mixed culture of microorganisms in combination with a dissolved oxygen electrode has been developed for the purpose of on-line monitoring of the organic pollution of wastewater. A general problem of all commercial BOD sensors is in measuring samples containing heavy metal ions. The result of this study clearly demonstrates the need to use heavy metal resistant strains, like Alcaligenes eutrophus, while measuring the BOD of waste water contaminated by heavy metal ions.*

Key words: *BOD, waste water, biosensors*

Sažetak: *Suvremeni su senzori za nadzor biokemijske potrebe za kisikom (BOD) bazirani na imobiliziranim mješovitim kulturama mikroorganizama u kombinaciji s kisikovom elektrodom. Generalno veliki problemi se pojavljuju kod svih komercijalnih BOD senzora za on-line monitoring organskog zagađenja otpadnih voda, ako otpadne vode sadrže ione teških metala. Rezultati ovog istraživanja neupitno ukazuju na potrebu korištenja rezistentnih sojeva mikroorganizama, kao što su Alcaligenes eutrophus, za mjerenja BOD indeksa u otpadnim vodama kontaminiranim ionima teških metala.*

Ključne riječi: *BOD, otpadne vode, bio senzori*

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1. Introduction

Environmental monitoring and prevention of pollution are becoming increasingly important. This is particularly true for toxic substances with potential human health risk. The biochemical oxygen demand (BOD) is one of the most widely used and important tests in the measurement of wastewater organic pollution (Riedel, et. al., 1998). The conventional BOD test requires a 5 day incubation period and the test values depend upon the skill of the operator (Eaton, et al., 1995).
Therefore, rapid and reproducible methods are desirable for the BOD test. A very attractive technique for automated wastewater monitoring is the flow-injection analysis in combination with biosensors as the recognition element (Yang, et al., 1997; Yoshida, et al., 2001). The major advantages of these systems are a short contact time between the analyte and the biosensor and the whole system is entirely automated and computer controlled (Reynolds, et. al., 1997; Konig, et al., 1999).
Biochemical oxygen demand sensor, based on an immobilized mixed culture of microorganisms in combination with a dissolved oxygen electrode, has been developed for the purpose of on-line monitoring of the organic pollution of waste water (Preininger, et al., 1994; Chee, et al., 1999). Optimum biosensor design requires effective immobilization techniques which can provides a thin film of biocomponent on the transducer surface in a reproducible manner (Chemnitius, et al., 1996).This biosensors may be defined as an analytical device which combines a biological sensing component with a signal transducer (Amarjeet, et al., 1996).
The biological sensing component provides unique interaction with analyte and . function of the transducer is to convert this signal into a measurable response. Operational stability is one of the important factors to be considered in BOD biosensors (Byung, et al., 2003). Stabile sensor performance over a desired period is essential for a reliable sensor system (Gil, et al., 2003).
A general problem of all commercial BOD sensors consists in measuring samples containing heavy metal ions, which are known to cause inhibitory or toxic effects on microbiological components in commercial BOD sensors (Kim, et al., 2002; Liu, et al,. 2000). To avoid heavy metal interference is important to find resistant microbiological components for measuring the BOD (Sharma & Rogers, 1997).

2. Materials and Methodes

Wastewater was collected from a production process at “Zvečevo dd, Food Industry” Croatia during 2007. The wastewater contained 23± 7.5 mg total nitrogen and 10.9±1.8 mg total phosphorus , respectively. Inorganic nitrogen was less than 5 mg . It was diluted using 50 mM phosphate buffer (pH 7) containing 100 mM NaCl to a designated concentration before being fed into the anode of biosensors. In order too study the influence of heavy metal ions 4mM of cadmium were added into a standard wastewater*.
All measuring experiments were conducted using three separate microbial BOD sensor (Bacillus subtilis, Bacillus licheniformis and Alcaligenes eutrophus) integrated

in a flow trough system ("Strix" plc., Zagreb, Croatia), fig.1. Results are compared with (BOD_5) results which are measured using standard methods (Eaton, et al., 1995).

Fig. 1. Integrated BOD flow trough systema "Strix" plc., Zagreb, Croatia

3. Results and Discussion

Biological recognition element	Sensor BOD	BOD_5	Sensor BOD*	BOD_5 *
Bacillus subtilis,	1210,03 ±3,5%	1201,00±8,5 %	791,03±3,9 %	1202,20±8,6 %
Bacillus licheniformis	1211,00 ±3,9%	1203,03±8,8 %	811,00±3,7 %	1202,93±9,7 %
Alcaligenes eutrophus	1206,05±3,7 %	1198,01±9,1 %	1006,05±4,3 %	1199,31±8,3 %

Table 1: Comparison of BOD values of wastewater samples measured by the sensor and conventional BOD_5

(*4mM of cadmium were added into a standard wastewater)
Biosensors are suitable for many monitoring applications, the biochemical oxygen demand is one of the most widely used and important measurement in the environmental monitoring.
Nevertheless, only a few systems are present on the market today. The reason for this can be found in intrinsic difficulty to preserve working conditions of the biological element.
Table 1. shows the comparison of BOD values of wastewater samples measured by the sensor and those determined by BOD_5 conventional test.
In absence of heavy metal ions sensors were stable and result of measurements were in co-ordinance with BOD_5 test results.
Measurements with a wastewater containing 4mM cadmium ions showed immediately decreased of sensor response by about 15 to 35% these results are in accordance with published results (Byung, et al., 2003).
Operational stability is one of the important factors to be considered in biosensors. A stable sensor performance over a desired period is essential for a reliable sensor system. A typical BOD sensor has a limited stability (Liu & Mattiasson, 2002).
BOD values measured by the sensor showed the standard deviation from ±3,5% to ±3,9% during repeated experiments, and ±3,7% to ±4,3% for test with 4mM cadmium (Table 1).
The reproducibility of previously reported results varied from ±2.4% to ±10% for single strain sensors, (Liu & Mattiasson, 2002).
BOD_5 values measured using conventional methods showed the standard deviation of ±8,3% to ±9,7%, according to the (Eaton, et al., 1995), ±15.4% reproducibility is acceptable in BOD_5 test.

4. Conclusion

Among the analytical systems which are feasible for BOD measuring, biosensors may be the devices of choice, because they offer highly sensitive and cost-effective measurements
The obtained results of this study clearly demonstrates the need to use heavy metal resistant strains while measuring the BOD of waste water which is contaminated by heavy metal ions.
The BOD sensor with Alcaligenes eutrophus had the best heavy metal resistant and operational stability.
Although challenges concerning operational lifetimes, fouling and internal calibration have yet to be investigated.

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