Oil Shale, Vol. 14, No. 4 SPECIAL
pp. 518-525

ISSN 0208-198X

https://doi.org/10.3176/oil.1997.4S.08

BOD MEASUREMENT IN PHENOLIC WASTEWATERS BY USING BIOSENSOR BASED ON IMMOBILISED BACTERIA

T. KIKAS
K. TAMMEVESKI
T. TENNO

Institute of Physical Chemistry,
University of Tartu
Tartu, Estonia

The bacterial sensor has been constructed, characterised and used for biochemical oxygen demand (BOD) measurements in phenolic wastewaters. The sensor has been found to be usable as a monitoring device and as a control device in biological treatment systems.

Introduction

Biochemical oxygen demand (BOD) is one of the most generalizing parameters that is used for characterization of the level of contamination of wastewaters. BOD is also of a very important value for controlling optimal input-regime in biological treatment plants. Classical method for BOD measurement takes at least five days, or even 21 days if necessary. Controlling biological systems like biological treatment plants needs continuous information about the quality of incoming and outgoing water and an immediate reaction to appearing differences from the optimal. It is quite obvious that the classical BOD measuring method does not satisfy our demand for information.

One of the most promising ways to get adequate information for time short enough is using specific biosensors. Biosensor is an integrated device which is capable of detecting analyte concentration by using a biological recognition element. Biosensor can be divided into two parts: a biological system and a detecting system that transforms a biochemical signal into a visually observable electrical signal. Electrochemical sensors constructed by coupling intact microbial cells with electrochemical devices offer many unique possibilities for analytical measurements, as

shown by Riedel et al. [1], Karube and Suzuki [2] and Rechnitz and Ho [3]. As phenolic wastewaters are produced in a number of oil-shale processing stages, the evaluation of water quality needs devices which are easy to operate, relatively cheap, and provide fast and continuous measurement.

The amperometric biosensor based on the oxygen decrease measuring principle consists of two parts: an oxygen sensor and a bacteria-agarose membrane.

Substrate reaches the bacterial layer when the sensitive top of the sensor is immersed in the test medium. Bacterial cells produce enzymes which can catalyse the degradation of biological matter (substrate). The metabolites of this degradation are directed into the Krebbs cycle. It means that substrate is metabolized in the bacterial layer by consuming oxygen. The remaining oxygen is reduced at the cathode surface of the oxygen sensor.

The aim of the present research was to construct and characterize a microbial sensor that could be used for the biochemical oxygen demand measurement, first of all, in phenolic wastewaters of oil-shale industry. In the experiment a specially designed oxygen sensor of the Clark type and bacteria *Bacillus subtilis* immobilized in agarose gel film were used. In those cases the activity of the bacteria persisted at least 4 months without a requirement for additional calibrations.

Construction of Oxygen Sensor

The oxygen sensor that was used in the experiment had to meet some specific requirements. Firstly, the sensitive region of the sensor had to be placed at the top of the sensor as it gives additional opportunities to attach polymeric films with immobilized bacteria. It also gives a possibility of using films with different fixed values of thickness. This is of great advantage for studying diffusion processes in polymeric film with immobilized bacteria.

Recently a theoretical model for microbial sensors has been developed by our group [4]. In this model effective parameters are used which makes it possible to introduce the concentrational distribution of oxygen and substrate as a continuous curve. The distribution of oxygen within the biosensor under steady-state conditions is illustrated in Fig. 1. The effective oxygen concentration with respect to x_i is linear in the diffusional layers of the oxygen sensor and nonlinear in the bacteria-agarose membrane due to oxygen consumption in the biochemical reactions of the immobilized bacteria.

A short response time is another requirement for the oxygen sensor to be applicable as a biosensor's base-electrode. If this requirement is not

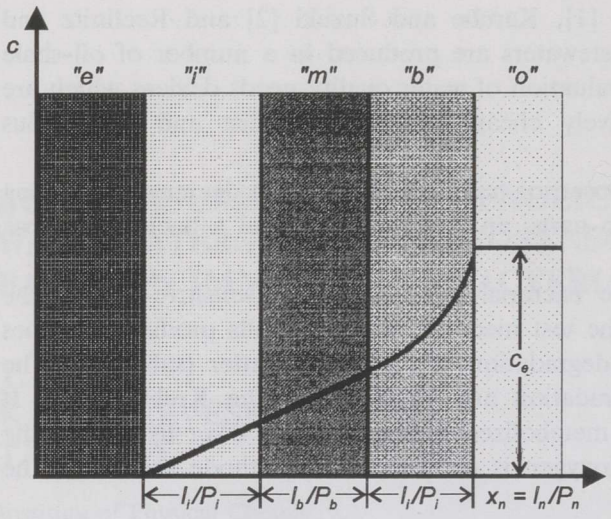

Fig. 1. The distribution of oxygen within the amperometric microbial sensor under steady-state conditions. c_e - effective concentration of oxygen, l_n - thickness of the diffusional layer (n), P_n - permeability of the diffusional layer to oxygen; "*e*" - platinum cathode, "*i*" - electrolyte layer, "*m*" - teflon membrane, "*b*" - bacteria agarose membrane, "*o*" - test medium

met, it has to be taken into account when studying the stabilization of the biosensor's signal. For this purpose a body cell considering these requirements has been designed.

To reduce the response time of the oxygen sensor a specially designed two-electrode Clark-type sensor for the determination of oxygen uptake and a thin membrane (25 μm) were used. Plexiglass was applied as a suitable material for the oxygen sensor's body. 1 M solution of KCl was internal electrolyte. Silver wire with anodically formed AgCl layer served as a reference anode. The working electrode was prepared by thermal decomposition of a solution containing hexachloroplatinic acid. A glass substrate was dip-coated in a solution followed by firing. Five repetitive cycles were enough to prepare a continuous and well-adhesive Pt layer. The sensor's cathode was polarized at -0.7 V with respect to the internal Ag/AgCl electrode.

Characterization of the Bacteria Used

Bacillus subtilis is an aerobic, grampositive, sporulative bacterium with the crosscut diameter of ca 0.6-0.7 μm. *Bacillus subtilis* is a bacterium-anthagonist as it suppresses the growth of other microorganisms. Its spores are especially tolerant to thermal impact. Some spores can survive even boiling for a relatively long period (10 min).

Bacillus subtilis is a strongly thermophilic bacterium with a growth optimum of 37-50 °C and maximum ca 60 °C [5]. It can use most of hexoses (glucose, mannose, fructose etc.), natural disaccharides, biogenic organic acids (citrate, succinate, acetate etc.), water soluble primary alcohols and biogenic aminoacids as sources of carbon [6]. During its life

activity *Bacillus* produces exocellular enzymes (amylases, proteases) that make its growth possible also on natural polymers like starch, proteins and lipids. It is able to decompose gelatine and peptonize milk proteins as well [5].

Metabolism of *Bacillus subtilis* is respirative, but the *Bacillus* can also develop under unaerobic conditions as a facultative anaerobe for the final electron acceptor in the bacteria's respirative metabolism involving molecular oxygen can be replaced by nitrate. Under anaerobic conditions *Bacillus* reduces nitrate to nitrite.

Bacillus subtilis is capable of growing in a relatively wide range of pH - ca 5.5-8.5.

Experimental and Results

The aim of the first part of the experiment was to characterize the oxygen sensor. Response time is one of the limiting parameters for the oxygen sensor. For this purpose the sensitive top of the sensor was attached air tightly into a special pass-flow cell through which argon was run. When the signal had stabilized, the air flow containing ca 20 % of oxygen was led through the cell. Change of the signal and a period of time for its stabilization was observed. The sensor signal was recorded as a voltage difference. The response time of the oxygen sensor after a sudden change in O_2 concentration was ca 20 sec.

To inspect the reproducibility at different O_2 concentrations, the oxygen sensor was calibrated by using gas mixtures with certain oxygen concentrations (Fig. 2).

Dependence of the output signal on temperature was studied as well.

An entrapment technique was used for bacteria immobilization in agarose gel. The prewashed bacterial mass was suspended in 3 ml of phosphate-buffered solution, added to 10 ml of agarose solution in phosphate buffer (0.3 g agarose in 10 ml of buffer solution) and then altogether cast onto a polymeric net of certain thickness. The net was placed between two glass plates till the end of gelatization to achieve a constant thickness of gel membrane. The net with the immobilized bacteria was placed at room temperature in the buffer

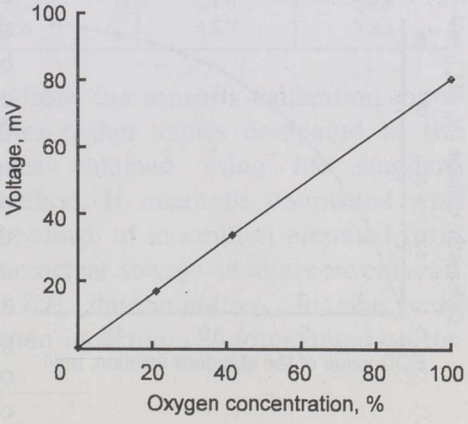

Fig. 2. Signal - O_2 concentration dependence of oxygen sensor

solution. Then a circle with a certain diameter was cut out and attached to the top of the oxygen sensor.

Experiments were carried out in the measurement cell of 150 ml volume at 25 °C. Air-saturated water was used as measurement environment. To eliminate the temperature dependence of the signal the cell was thermostated. A magnetic stirrer was used for stirring the test solution (Fig. 3).

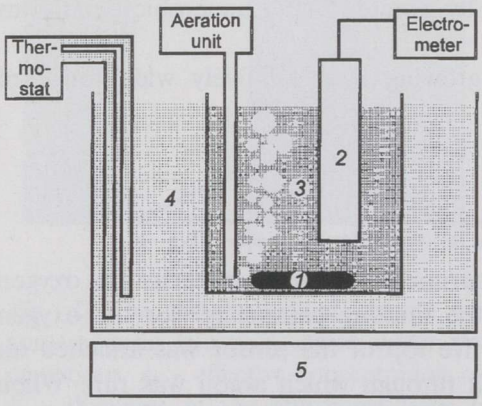

Fig. 3. Measurement cell: *1* - stirring bar; *2* - biosensor; *3* - test solution; *4* - thermostated waterbath; *5* - magnetic stirrer

Preliminary measurements were carried out with BOD standard solution (BOD = =200 mg/l) which contained 150 mg of glucose and 150 mg of glutamic acid in 1 liter of water [7]. Stabilisation of the signal took approximately 15-30 minutes. From these measurements the calibration graph has been drawn up: stabilized signal-BOD concentration dependence (Fig. 4).

To draw up the calibration graph, average outcome voltage values of twelve measurements were used. The BOD_7 value of standard solutions was also checked with the conventional BOD_7 measurement method [8]. Results lay within the allowable error limit (15-20 %). Deviation between different measurements was 5-10 %.

At low BOD values the signal increases significantly, but as the concentration grows, the curve starts to flatten because of saturation of the catalysing capacities of the bacteria. It means that the substrate concentration remains stable inside the biomembrane i.e. velocity of the reaction is limiting step of the process. The biosensor is usable in the concentration range where differences in the signal are considerable. From the calibration graph it can be seen that a biosensor of this particular thickness (0.5 mm) of membrane with immobilized bacteria can be used in the BOD range of 1-15 mg/l. By using

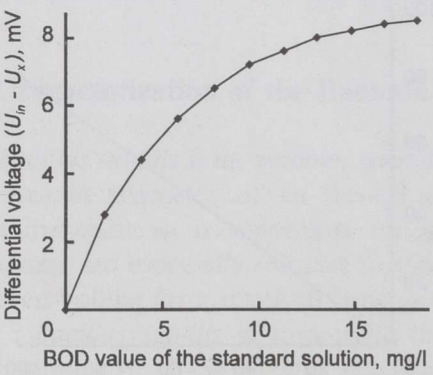

Fig. 4. Calibration graph for the BOD-sensor

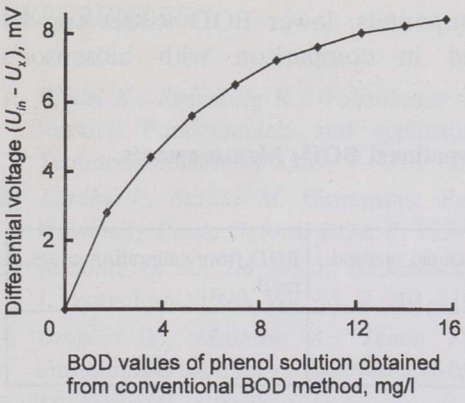

Fig. 5. The dependence of biosensor's differential signal on the BOD value of phenol solution

membranes of 0.3 mm thickness the upper limit can be raised to BOD_7 = 25-30 mg/l.

It was also important to inspect the usability of the biosensor in the phenolic test environment. For this purpose measurements were carried out in a phenol solution (Fig. 5) where BOD_7 had been measured using the conventional method for BOD determination. The results were comparable with those obtained from the calibration graph (Table 1).

In order to evaluate the suitability of the BOD biosensor for testing wastewaters containing phenolic compounds, measurements were carried out with actual wastewaters from the Kohtla-Järve oil-shale industry (Fig. 6) and compared to the results estimated from the calibration graph (Table 2). Parallel measurements were carried out by using the conventional method for BOD_7 detection.

Table 1. **Comparison of Results Evaluated from the Calibration Curve and Obtained by the Conventional BOD_7 Method**

Biosensor's signal, mV	BOD conventional method, mg/l	BOD calibration curve, mg/l
2.8	1.7	2.0
4.4	3.5	3.9
5.6	5.2	5.8
6.5	7.0	7.7
7.2	8.7	9.5
7.6	10.4	11.3
8.0	12.2	13.0
8.2	14.0	14.8
8.4	15.7	16.5

As it can be seen from Table 2 the determination of BOD_7 from the sensor's calibration curve gives higher values compared to the ones obtained using the standard method. It might be connected with the usage of inocculum prepared from municipal sewage in the conventional BOD_7 determination. In the case when inocculum is not adapted on the

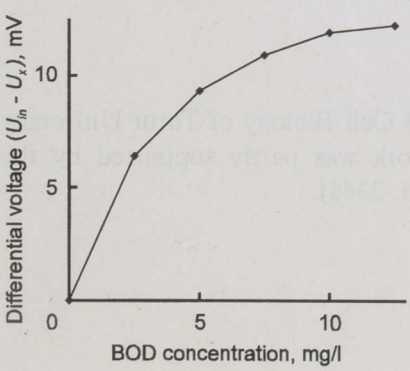

Fig. 6. The dependence of biosensor's signal on the BOD concentration of wastewater

wastewater containing phenolic compounds, lower BOD values can be expected by conventional method in comparison with biosensor's method.

Table 2. **Comparison of Data from Conventional BOD_7 Measurements and Calibration Curve**

Biosensor's signal, mV	BOD from conventional method, mg/l	BOD from calibration curve, mg/l
6.4	2.5	7.0
9.3	5.0	>20

Conclusions

The electrochemical biosensor has been constructed with immobilized bacteria for BOD measurements. It can be widely utilized as a monitoring device. It can also be used as a control device in biological treatment plants.

The main characteristics of the sensor have been studied and found to be satisfactory for monitoring purposes.

The biosensor's main advantages are:

- results can be obtained in 15-30 minutes instead of 7 days required for the conventional method
- it can in principle be used in situ
- it is cheap
- it is easy to handle
- it can be used repeatedly

A main drawback of the device is that BOD cannot usually be measured directly in wastewaters but only in its dilutions where the appropriate BOD range for the biosensor is approximately 1-30 mg BOD/l.

Acknowledgements

We thank the Institute of Molecular and Cell Biology of Tartu University for providing us with bacteria. This work was partly supported by the Estonian Science Foundation (Grant No. 2346).

REFERENCES

1. *Riedel K., Renneberg R., Wollenberger W., Kaiser G., Scheller F. W.* Microbial sensors: Fundamentals and application for process control // J. Chem. Technol. Biotechnol. 1989, V. 44. P. 85-106.
2. *Karube I., Suzuki M.* Biosensors: Practical Approach (Cass A. E. G. ed.). University Press, Oxford. 1990. P. 155-170.
3. *Rechnitz G. A., Ho M. Y.* Biosensors based on cell and tissue material // J. Biotechnol. 1990, Vol. 15. P. 201-218.
4. *Orupõld K., Mashirin A., Tenno T.* Amperometric phenol sensor with immobilized bacteria // Electroanalysis. 1995. V. 7, No. 9. P. 904-906.
5. *Mirzoyeva V. A.* Бактерии группы сенной и картофельной палочек (*Bac. subtilis* и *Bac. mesentericus*). Изд. Акад. Наук СССР.- М, 1959. С. 3-136. [In Russian]
6. *Reizer J., Peterkovsky A.* Sugar transport and metabolism in gram-positive bacteria, 1987. P. 423.
7. Standard Methods for the Examination of Water and Wastewater, 16th Edition. APHA AWWA WPCF- Washington, 1985. P. 525-532.
8. Water Analysis Handbook (Hach Co ed.). Loveland-USA, 1989. P. 453-460.

Presented by J. Kann
Received November 21, 1996