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STABILITY OF A PHENOL-SENSITIVE MICROBIAL SENSOR

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Abstract. A phenol-sensitive microbial sensor was constructed by immobilizing *Pseudomonas putida EST1412* cells on an amperometric oxygen sensor. The stability of the practical microbial sensor was investigated. The influence of the external membrane covering the immobilized bacteria to the long-term stability of the response of the phenol-sensitive microbial sensor is discussed.

Key words: phenol determination, microbial sensor, oxygen sensor, stability.

INTRODUCTION

Electrochemical sensors constructed by coupling intact microbial cells with electrochemical devices offer a large number of possibilities for analytical measurements as shown by Riedel et al. [1], Karube & Tamiyra [2], and Rechnitz & Ho [3]. Microbial sensors may prove useful for environmental monitoring. Corcoran & Rechnitz [4] discussed the advantages of using cells rather than enzymes in biosensors to enhance enzymatic activity in the cells, and stressed their low cost, self-regeneration, and longer service life.

Concern over pollution risk to the environment has highlighted the need for easy to operate, low cost, and fast continuous measurement of phenols. The aerobic degradation of phenol by specific microorganisms leads to new possibilities for developing biosensors for phenol determination on the basis of an oxygen sensor.

Leypold & Gough [5] showed theoretically that the imposing diffusional limitations on the enzymatic reaction can lead to improvements in sensor performance in various ways, such as increasing the sensitivity to the substrate, extending the range of linearity, decreasing the sensitivity to biocatalyst inactivation, etc. The influence of the additional diffusional restrictions of the external membrane on the characteristics of the

microbial sensor can be also investigated theoretically on the basis of the mathematical model introduced by the present authors earlier [6].

The work described here focuses on the stability of the developed phenol-sensitive microbial sensor. The influence of the external membrane covering the immobilized *Pseudomonas putida* cells on the stability of the microbial sensor is discussed.

EXPERIMENTAL

Phenol degrading bacteria *Pseudomonas putida EST1412* were obtained from the Institute of Molecular and Cellbiology of the University of Tartu. Cells were grown on a shaker at 30 °C in 200 ml sterile minimum medium with trace elements. Phenol was used as carbon source at 5 mmol/l. Microorganisms were collected at an exponential growth phase by centrifugation 5000 p/min for 5 min. The cells were washed twice and resuspended in 0.1 M phosphate buffer (pH = 7.2). The suspension of bacteria was mixed with agarose solution so that the mixture obtained agarose concentration 2% (wt/v).

The polyethylene membrane of the oxygen sensor constructed at the University of Tartu was covered with the bacteria–agarose mixture. The bacterial membrane was additionally covered with 2% agarose gel. The scheme of the microbial sensor is illustrated in Fig. 1.

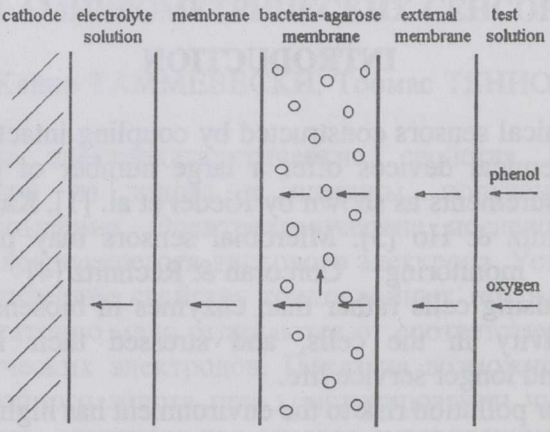

Fig. 1. Scheme of the microbial sensor.

The steady state responses of the microbial sensor based on oxygen sensor to phenol in 0.025 M phosphate buffer (pH = 6.9) solutions at 25 °C were studied. The test solutions were continuously aerated with air and stirred to maintain a constant dissolved oxygen concentration. When not in use the prepared sensors were held in 0.025 M phosphate buffer (pH = 6.9) at 4 °C.

RESULTS

The current of the microbial sensor in the buffer solution was recorded as a baseline current I_0. When the sample solution containing phenol was added into the system, phenol permeated through the membrane and was assimilated by microorganisms. It caused consumption of oxygen by the immobilized microorganisms and resulted in a decrease in the amount of dissolved oxygen on the polyethylene membrane of the oxygen sensor. The steady state response indicates that the consumption of oxygen by microorganisms and that diffused from the test solution to the membrane were in equilibrium. The steady state current I depended on the phenol concentration in the test solution. The sensor response δI was expressed as the relative difference between the two steady state currents $(I_0 - I)/I_0$.

The establishment of the additional diffusional restrictions to the system by covering the bacterial membrane with an external agarose membrane was accompanied by an improvement of the stability of the microbial sensor. Figure 2 shows the long-term stability of the response of the microbial sensors at phenol concentration 0.116 mmol/l over the period of 50 days. The response of the microbial sensor with the external membrane over this period was found to be fairly constant with a standard deviation 1.2%.

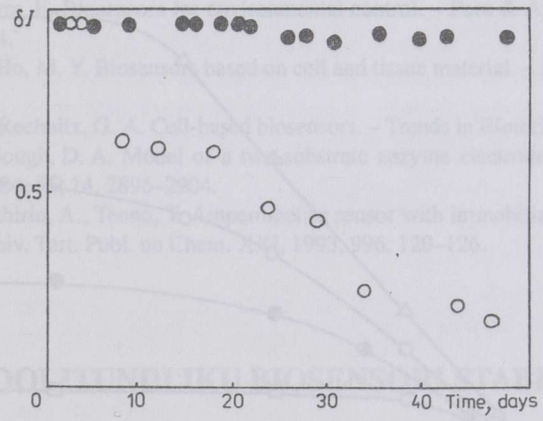

Fig. 2. Stability of the response δI of the microbial sensor with (●) and without (○) an external membrane at phenol concentration 0.116 mmol/l.

Figures 3 and 4 show the stability of the calibration curves of the microbial sensors. The decreasing readings obtained with the sensor without an external membrane can be explained by the influence of the inactivation of the immobilized bacteria in the sensor in time. The response of the microbial sensor was found to be constant over the period of three months when the bacterial membrane of the sensor was covered with an external agarose membrane. The external membrane increased the stability of bacteria and also decreased the influence of the inactivation of biocatalysts to the biosensor characteristics. The results in Figs. 2–4 show that the microbial sensor gave good sensitivity and reproducibility only when it had the external membrane on the bacterial membrane.

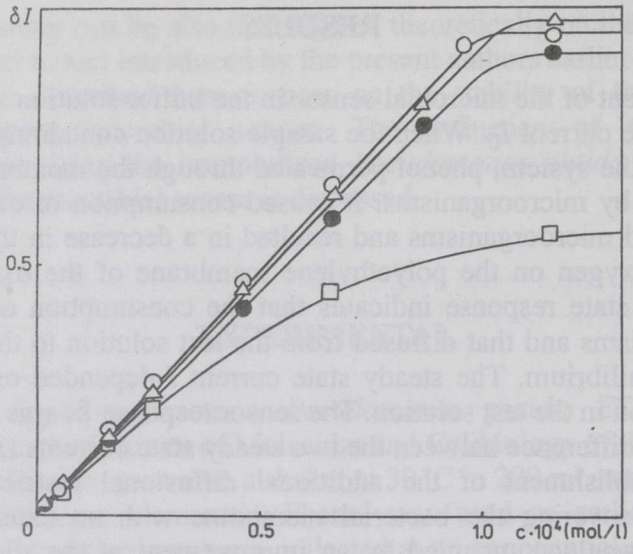

Fig. 3. The dependence of the response δI of the microbial sensor with an external membrane on the phenol concentration in test solution: Δ – 1 day, ○ – 1 month, ● – 3 months, and □ – 4 months after the immobilization of bacteria.

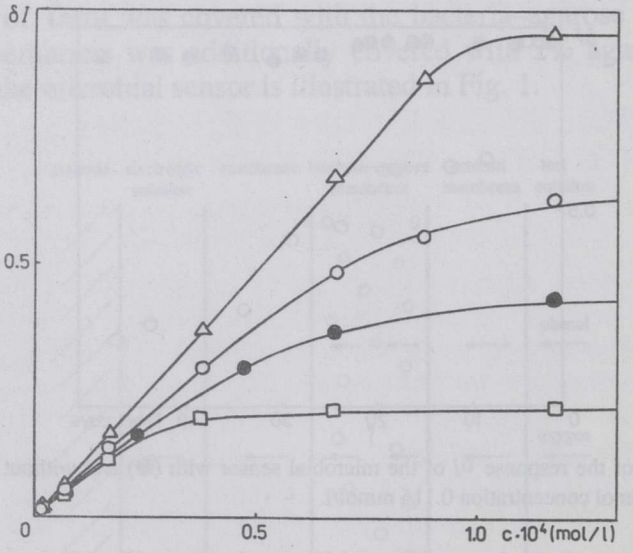

Fig. 4. The dependence of the response δI of the microbial sensor without an external membrane on the phenol concentration in test solution: Δ – 1 day, ○ – 8 days, ● – 29 days, and □ – 44 days after the immobilization of bacteria.

CONCLUSIONS

The amperometric biosensor with immobilized bacteria appears to be quite promising for the estimation of the phenol concentration in waste

waters. It is necessary to cover the bacterial layer with an external membrane. In addition to the influence of the external diffusional layer on the linearity and sensitivity of the sensor and also on the response time, it guarantees good long-term stability of the developed phenol-sensitive microbial sensor.

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FENOOLITUNDLIKU BIOSENSORI STABIILSUS

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Fenooli degradeerivate bakterite *Pseudomonas putida EST1412* immobiliseerimisega amperomeetrilisele hapnikuandurile valmistati keskkonna monitooringus vajalik mikroobsensor fenooli kontsentratsiooni määramiseks heitvetes. Käesolevas töös uuriti fenoolitundliku sensori stabiilsust ning välise membraani tähtsust mikroobsensori stabiilsuse tagamisel.

СТАБИЛЬНОСТЬ ФЕНОЛ-ЧУВСТВИТЕЛЬНОГО БИОСЕНСОРА

Кайа ОРУПЫЛЬД, Тоомас ТЕННО

Исследован биосенсор для определения концентрации фенола. Сенсор сконструирован на основе амперометрического датчика кислорода. Фенол-чувствительный слой сенсора был получен нанесением иммобилизированных бактерий *Pseudomonas putida EST1412* на полимерную диффузионную мембрану кислородного датчика. Изготовленный фенол-чувствительный биосенсор испытывался на стабильность в заданном интервале времени. Установлена зависимость стабильности биосенсора от параметров его дополнительного внешнего диффузионного слоя.