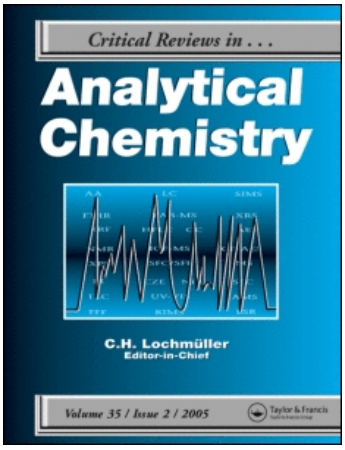

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DETERMINATION OF BOD IN PHENOLIC WASTEWATERS AND A STUDY OF BIODEGRADATION OF PHENOLIC COMPOUNDS

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Introduction

The removal of biodegradable compounds is a major concern in biological treatment plants. 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 measurable electrical signal. Electrochemical sensors constructed by coupling intact microbial cells with electrochemical devices offer many unique possibilities for analytical mesurements, 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. Recently a theoretical model for microbial sensors has been developed in our group [4].

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 Clark-type oxygen sensor and bacteria *Bacillus subtilis* immobilized in agarose gel film were used. In those cases the activity of the bacteria persisted at least 5 months without a requirement for additional calibrations.

In addition, measurements were carried out to study the biodegradation of phenolic compounds (phenol and p-cresol). BOD_7 was estimated by the conventional method and the rate of removal of phenolic compounds was followed by liquid chromatography (HPLC).

Results and discussion

Measurement of BOD with an electrochemical biosensor

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 anaerobic conditions as a facultative anaerobe for the final electron acceptor in the bacteria's respirative metabolism involving molecular oxygen can be replaced by nitrate. *Bacillus subtilis* is capable of growing in a relatively wide range of pH - ca 5.5 - 8.5.

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 polymerization to achieve a constant thickness of gel membrane. The net with the immobilized bacteria was placed at room temperature in the buffer 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 (see Fig. 1.).

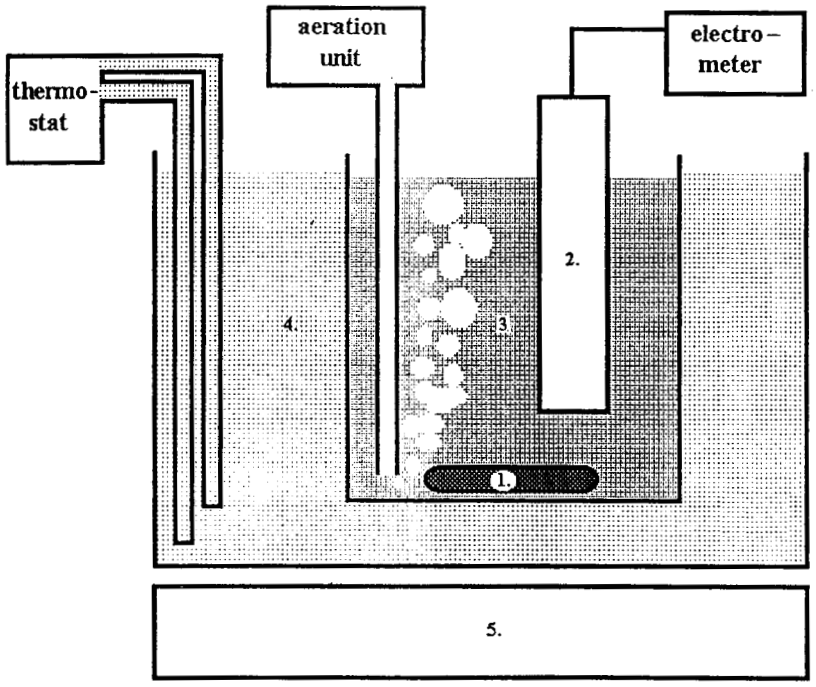

Figure 1. Measurement cell. 1. stirring bar; 2. biosensor; 3.test solution; 4. thermostated waterbath; 5. magnetic stirrer

Preliminary measurements were carried out with BOD standard solution (200 mg BOD/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. To draw up the calibration graph, average outcome voltage values of twelve measurements were used. The BOD level of standard solutions was also checked with the conventional BOD_7 measurement method [8]. Results lay within the allowable error limit (15-20%).

At low BOD concentrations the signal has a significant rise, but as the concentration values grow, the curve starts to flatten because of saturation of the catalysing capacities of bacteria. It means that the substrate concentration remains stable inside the biomembrane i.e. velocity of the reaction is limiting stadium 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 BOD/l. By using membranes of 0.3 mm thickness the upper limit can be raised to 25 - 30 mg BOD/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 the solution of phenol (Fig. 2.) whose BOD_7 had been measured using the conventional method for BOD determination.

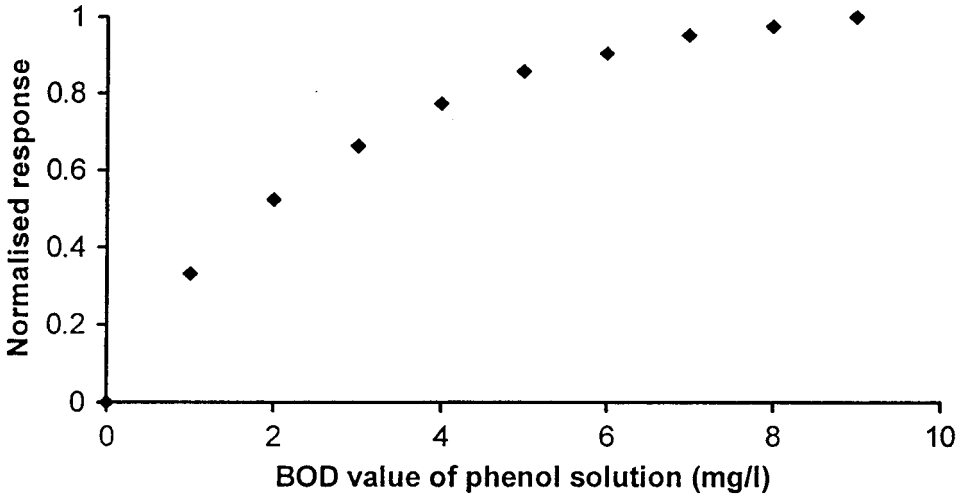

Figure 2. Biosensor's signal dependence on the BOD value of phenol solution.

In order to evaluate the suitability of the BOD biosensor for testing wastewaters containing phenolic compounds, measurements were carried out with wastewaters from the Kohtla-Järve oil-shale industry.

Biodegradation test of phenolic compounds

Measurements were performed to study the biodegradation of phenolic compounds more deeply. For the conventional determination of biological oxygen demand (BOD_7) the sample was diluted with water containing inorganic nutrients needed for the biochemical processes to occur. As the test solution that contained only phenol and p-cresol was not a natural one, it was necessary to add 1 ml of seeding to 1 liter of the solution. Seedings were taken from different wastewaters. The dissolved oxygen level was determined by an electrochemical oxygen sensor after every 2 - 4 h during the first days of experiment, but later the interval between measurements increased. In each sample where the oxygen content was measured, the concentrations of phenol and p-cresol were also determined by using liquid-liquid extraction followed by chromatographic detection. A 100 ml sample was acidified with sulphuric acid (1 ml/l) to pH 2 and then extracted with one 20 ml and three 10 ml portions of

diethylether. For all extractions separating funnels were shaken for 10 min and two phases were allowed to separate for 10 min. The organic phases were collected and 2 ml of acetonitrile were added to avoid losses by evaporation. Then the extract was evaporated to 2-3 ml in the water bath. The sample was redissolved in 10 ml of acetonitrile. The sample was analysed by the HPLC using UV detection. The experiments were performed by using an INKROM liquid chromatograph with an isocratic pump. The separation of the compounds was achieved by using an HP C_{18} Hypersil ODS (250 mm × 4 mm, 5 μm) analytical column. The eluent used was a mixture of 1 % acetic acid and acetonitrile (66:34).

For one set of measurements the seeding originated from municipal sewage and for another one from the sludge of the aeration basin of the wastewater treatment plant.

Figure 3 presents the data of biodegradation measurements of phenolic compounds. It can be seen that during the first 2 - 3 hours the concentration of phenol and p-cresol decreases slightly, followed by a long period when the concentration is almost constant. The plateau corresponds to the period of adaptation during which the organisms get accustomed to the conditions of environment. A remarkable decrease in the phenol concentration is observed just after about 20 h of lag period. The decrease in the oxygen concentration of the test solution occurs simultaneously. Less than 30 hours were needed to remove all phenol and p-cresol as tested by the HPLC (see Fig. 3). Further decrease in the O_2 concentration is most probably connected with the removal of degradation products of phenol and p-cresol.

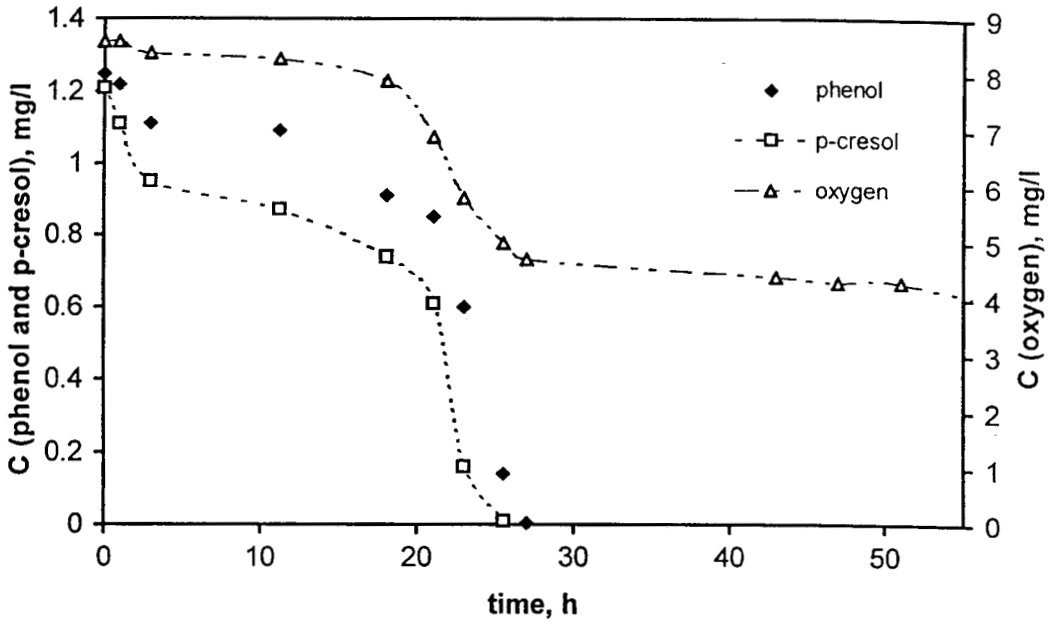

Figure 3. Biodegradation of phenol and p-cresol using seeding from municipal sewage.

The biodegradation of phenol and p-cresol was also studied by using microorganisms from the activated sludge of wastewater treatment plant whose influent contained phenolic compounds. The decrease in the phenol concentration followed the same trend found for the previous seeding taken from municipal wastewater. It should be mentioned that for phenol the lag period lasted up to 10 h followed by a gradual decrease in concentration. Rapid degradation of phenol commenced at ca 25 h. For p-cresol there was no essential degradation during the first 20 h. The differences observed in the course of the degradation process can be explained by taking into account different adaptability of various microorganisms towards phenol and p-cresol.

Conclusions

The electrochemical biosensor has been constructed with immobilized bacteria for BOD measurements in phenolic wastewaters. 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 biosensors main advantages are: results can be had in 15 - 30 minutes instead of 7 days required in conventional method; it can be roughly used in situ; it is cheap and easy to handle and 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 mgBOD/l.

The biodegradation of phenolic compounds was studied. On the basis of BOD_7 and HPLC data it was found that the removal of phenol and p-cresol by micoorganisms takes ca 25-30 h.

Acknowledgements

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