

Chapter 2

Biochemical Oxygen Demand (BOD)

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2.1 Introduction

Biochemical oxygen demand (BOD) is a widely used parameter to assess the organic pollution in water systems. This parameter can be detected by the amount of oxygen consumed via microorganisms in aerobic metabolism of organic matter present in the water. The authorized test to analyze biodegradable organic compounds is given by the American Public Health Association Standard Method Committee that is called a 5-day biochemical oxygen demand (BOD₅) test. In this conventional BOD procedure, the analyte is kept in the dark and in properly sealed biological reactors after inoculating with a microbial culture (seed), nutrients, and plenty of oxygen. Afterwards, the amount of oxygen is measured which is consumed by the microorganisms during biological oxidation of organic solutes over a time period of 5 days.¹ Such prolonged analysis makes BOD₅ expensive and requires experienced personnel for reproducible results. This procedure produces good results; however, it cannot be used for rapid analysis such as environmental monitoring and/or process control. The first rapid BOD sensor was proposed by Karube et al. in 1997.² In this approach, microbes were immobilized on a collagen membrane and an oxygen electrode was used as an indicator device. The results were obtained by this method in a short period of time (1 h) and were closely related to BOD estimates (obtained in 5 days).

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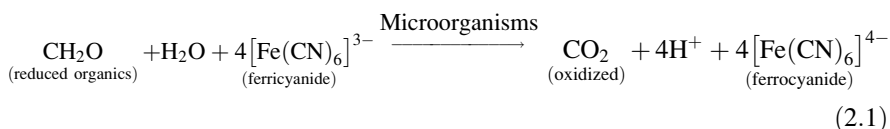
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2.2 Sensors for the Determination of BOD

2.2.1 Ferricyanide-Mediated BOD Sensor

The bacterial catabolism can be monitored with redox mediators. These redox mediators are the species which are able to trap electrons from the redox molecules involved in the electron transport chain.^{3,4} By gaining electrons from the reduced electron transfer chain molecule, the oxidized mediators are reduced. This reduction of mediators is subsequently monitored electrochemically. A biochemical oxygen demand (BOD) sensing method was developed by employing ferricyanide (FC) as a mediator.⁵ This mediator was anchored on an ion-exchangeable polysiloxane. The polysiloxane was synthesized from 3-(aminopropyl) trimethoxysilane by a sol-gel process and ferricyanide was immobilized by ion association and subsequently utilized for electrode modification. Ferricyanide (FC) acts as an efficient mediator for shuttling electrons between the redox centers of reduced bacterial enzymes and the electrode surface.⁶ In the presence of FC as a mediator, electrons derived from the oxidation of organic substrates in aerobic catabolism are transferred to the FC ion which is reduced from ferricyanide to ferrocyanide,⁷ as shown in Eq. (2.1). The reduced form is then re-oxidized to ferricyanide at the working electrode (anode):



FC as a mediator has an advantage over O_2 in designing BOD assays due to its high solubility, linear working range, as well as allowing higher microbial populations. The BOD sensor fabricated by utilizing FC as mediator gives an excellent limit of detection. The results are comparable to the conventional BOD_5 method which proves that the BOD method based on FC is valid for BOD determinations.

2.2.2 Hybrid Material for BOD Sensor

An electrochemical biochemical oxygen demand (BOD) sensor was fabricated by using an organic-inorganic hybrid material.⁸ The hybrid material was synthesized from silica and co-polymerized with poly(vinyl alcohol) and 4-vinylpyridine (PVA-g-P(4-VP)). Afterwards, *Trichosporon cutaneum* strain 2.570 cells were immobilized on the hybrid material. The entrapment of cell strains in extracellular materials provides considerable advantage over free cells such as enhanced metabolic properties and stability. The hybrid materials also protect them from environmental stress and toxicity. The organic-inorganic material provides a biocompatible microenvironment to *T. cutaneum* cells which ensures the long-term viability of

cells proven by confocal laser scanning microscopy (CLSM). The viability of the cells is due to arthroconidia (the state of *Trichosporon cutaneum* when stored) which is produced in the extracellular material.⁹ The arthroconidia state has the ability to resist against environmental stress and toxicity. The proposed sensor by utilizing biocompatible hybrid material could be applied for BOD determinations after activating the arthroconidia in appropriate conditions.

2.2.3 Mediated BOD Sensor

A flow injection biochemical oxygen demand (BOD) analysis system was developed by utilizing a microbial approach.¹⁰ The flow injection technique has advantage over batch analysis due to the ease of rapid and repetitive measurements. A yeast strain was isolated from an activated sludge and was utilized as biological recognition element. This strain was anchored on the substrate made of silica gel particles and packed into a fixed bed reactor. A redox mediator was employed for transporting the electrons from the microbes to the electrode surface. The redox mediators act as electron acceptors from microbes instead of oxygen when organic substrates are decomposed by microbes. Then, these mediators transport the electrons to the electrode surface such as hexacyanoferrate.⁵ The mediators are reduced after accepting the electrons and later are re-oxidized at the electrode surface. The current produced via re-oxidation of the reduced mediator can be related with the concentration of organic contents. Potassium hexacyanoferrate(III) was employed as a mediator. Thus, the mediated BOD sensor device in flow injection mode was fabricated by immobilizing microbes in a reactor which was further coupled to an electrochemical flow cell. The designed detection tool was employed to monitor BOD of *shochu* distillery wastewater (SDW).

2.2.4 Multi-Species-Based BOD Sensor

A BOD detection system containing single-strain microbes shows good stability as well as long lifetime. The single-strain sensors have disadvantages regarding accuracy due to their limited detection capacity for a wide spectrum of substrates. BOD sensors based on multi-species microbes, however, show high detection capacity for a wide spectrum of substrates; but their stability is compromised due to the interference among immobilized multi-species. In the multi-species assay microbial cells are immobilized on a polymer or hydrogel for BOD monitoring systems. If the microbes are immobilized by physical adsorption only, then the activation of the biofilm is very easy but with the disadvantage of limited stability and reproducibility because microorganisms may leak. Proper immobilization of microbes prevents the microorganisms from leaking which provides long-term stability; however, the cross-linked matrix of the hydrogel which entraps the

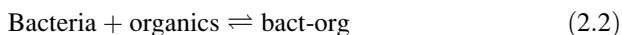
microbes acts as a barrier for the transfer of substrate and oxygen to microbes. Thus, these surfaces required a long activation process.¹¹ The biofilms prepared for the BOD system must be activated¹² before use and this process takes from hours to several days, which is limiting its applicability.

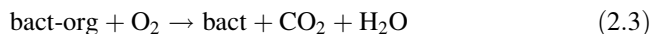
A stable BOD sensor was designed by immobilizing a multi-species BOD seed for wastewater monitoring in a flow system.¹³ The biofilm was synthesized with the BOD seed in an organic–inorganic hybrid material by which the activation time is greatly reduced. The hybrid material was based on a silica sol and co-polymerization was carried out with poly(vinyl alcohol) and 4-vinylpyridine. This organic–inorganic hybrid eliminated problems like cracking and swelling and provided a stable biofilm after immobilizing microbes. The multi-species seed was a commercially available microbial community which was entrapped in the hybrid matrix. The species comprised seven kinds of microbes which were isolated from activated sludge. Thus, immobilizing such multi-species on a hybrid matrix led to a reproducible, long-term stable BOD sensor.

2.2.5 Miniaturized Electrochemical Respirometer

The miniaturization of electrochemical systems is very promising in detecting the analyte of interest. A miniaturized electrochemical respirometer was designed to analyze the organic contents in water samples.¹⁴ This miniaturized device has the ability to monitor the analyte semicontinuously in comparison to other BOD sensors. Thus, the developed sensing tool is based on the concept of a microfluidic respirometer, a microbial fuel cell in an amperometric mode.¹⁵ Thus, it is called a bioreactor—not strictly a biosensor. The BOD detection device contains two twin electrochemical oxygen sensors located in parallel chambers. The protection of electrodes is done by coating with a silicone membrane, and one of them is subsequently modified by an agarose layer containing *Trichosporon cutaneum*, a yeast. The whole system is fabricated by standard microfabrication techniques while the electrochemical oxygen sensors are used to monitor the BOD. The microsystem geometry as well as the coating membranes are optimized to maximize the system output.

The microorganisms consume oxygen in an aerobic process while metabolizing the organic matter present in the sample. In order to understand the function of the miniaturized device, we may assume that the medium is homogeneous. The process can be explained in two steps: At first, bacteria reach an uptake equilibrium with organic matter present in the sample (Eq. 2.2). Then, in a second step they will consume oxygen to metabolize that matter into CO₂ and water (Eq. 2.3):





In the abovementioned equations, bact represents microbial cells without reduced organic matter in their cytosol. The amount of organic matter is represented by organics which will be degraded by microbes by utilizing oxygen. Thus, the amount of oxygen consumed is directly related to the organic contents present in the sample. The term bact-org represents the microbes having reduced organic matter in their cytosol. The respiration of microbial cells is explained by the second equation in which bacteria consume organic matter by utilizing oxygen and produce CO_2 and H_2O . The last part of this miniaturized BOD detection tool is monitoring the amount of oxygen at an electrode expressed in simplified form by Eq. (2.4):



The above mechanism of oxygen detection is rather complex^{16,17} and involves two separate two-electron steps in order to detect the oxygen content by the electrochemical sensor.

2.3 Related Sensors: Bioactivity

Bioactivity sensors (BAS) are related to sensors for the estimation of the BOD, but they are more general in their working concept.^{18–20} They rely on the detection of electroactive metabolites from cultivated biologically active organisms (rather than oxygen in the case of BOD) and may be, therefore, employed under aerobic as well as anaerobic conditions. Bioactivity sensors are useful in quality assessments of wastewaters and may detect the activity of aerobic auto- and heterotrophic biomass, anoxic denitrificants, and anaerobic microorganisms. The working principle is based on a biofuel cell where the electron transfer from the biological component to the anode is the analytically exploitable parameter.

Another assay of bioactivity sensors exploits microorganisms immobilized on electrode surfaces and monitors their activity in dependence on the surrounding conditions.²¹

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Environmental Analysis by Electrochemical Sensors and
Biosensors

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Moretto, L.; Kalcher, K. (Eds.)

2015, XIV, 457 p. 93 illus., 28 illus. in color., Hardcover

ISBN: 978-1-4939-1300-8