BIOSENSOR PRINCIPLES AND APPLICATIONS

edited by
Loïc J. Blum
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Series Introduction

Bioprocess technology encompasses all of the basic and applied sciences as well as the engineering required to fully exploit living systems and bring their products to the marketplace. The technology that develops is eventually expressed in various methodologies and types of equipment and instruments built up along a bioprocess stream. Typically in commercial production, the stream begins at the bioreactor, which can be a classical fermentor, a cell culture perfusion system, or an enzyme bioreactor. Then comes separation of the product from the living systems and/or their components followed by an appropriate number of purification steps. The stream ends with bioproduct finishing, formulation, and packaging. A given bioprocess stream may have some tributaries or outlets and may be overlaid with a variety of monitoring devices and control systems. As with any stream, it will both shape and be shaped with time. Documenting the evolutionary shaping of bioprocess technology is the purpose of this series.

Now that several products from recombinant DNA and cell fusion techniques are on the market, the new era of bioprocess technology is well established and validated. Books of this series represent developments in various segments of bioprocessing that have paralleled progress in the life sciences. For obvious proprietary reasons, some developments in industry, although validated, may be published only later, if at all. Therefore, our continuing series will follow the growth of this field as it is available from both academia and industry.

W. Courtney McGregor

Preface

A strong demand exists for improving the control and automation of industrial processes and for monitoring specific analytes in hospital critical care services, environmental control, or even defense. Following key parameters in real time remains difficult, and on-site analysis, which is an alternative to time-consuming conventional analysis performed in central laboratories, is raising a great amount of interest.

New generations of sensors able to directly provide direct and immediate information on the composition of their surroundings are promising tools in this area. Among them, biosensors, which can be thought of as highly sophisticated chemical sensors incorporating some kind of biological material in a sensing layer intimately associated with a transducer, are very attractive. Based on the highly specific and sensitive biomolecular recognition of target analytes, they provide a transduced signal, generally electrical, that can be correlated, after calibration, to the concentration of the analyte present in a complex medium.

Enzyme electrodes are the archetype of the first generation of biosensors, and some of them are now commercially available.

New generations of biosensors are emerging. They are based on novel and promising transducers such as field-effect transistors or optoelectronic devices. Efforts have been made by different groups to improve the selectivity and sensitivity of the sensing layer, to explore new concepts in transduction modes, and to miniaturize both the probes and related smart-signal processing systems.

Even though the literature published on this subject during the last two decades is abundant, it is still quite difficult to develop a clear idea of the future of such devices by reading randomly gathered papers.

Our aim in preparing this book was to ask authors—all active in the field and selected for their expertise—to contribute chapters based on experimental facts, presenting the state of the art in their domain that can serve as a reliable basis for other researchers.

As a matter of fact, the main bottleneck in such an interdisciplinary area is the common language that has to be developed to bridge the gap between readers from different disciplines. For this purpose, we asked authors to be didactic and comprehensive using examples from their own work and presenting major and

relevant contributions they have identified in their field. Although it is particularly difficult in such a book to avoid overlapping as well as omissions, emphasis in each chapter is on the principle of measurement, the biological material used, the description of transducers, and characteristics and performances of the biosensors thus designed.

In our opinion, groups gathering specialists of biomolecular engineering, microelectronics, optronics, computer sciences, and automation capable of developing a comprehensive interdisciplinary approach may soon claim a decisive leadership in the field.

Finally, our hope is that reading *Biosensor Principles and Applications* will boost the constitution of such groups and will encourage young scientists to join this challenging and promising area in the near future.

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BIOSENSOR PRINCIPLES AND APPLICATIONS

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What Is a Biosensor?

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DEFINITION AND BACKGROUND

The abundant literature that can be related to the keyword *Biosensor* proves without doubt that the field is attractive. It is an interdisciplinary area for which sharp limits cannot be defined easily. The concept of biosensor has evolved; for some authors it is a self-contained analytical device that responds selectively and reversibly to the concentration or activity of chemical species in biological samples. No mention is made here of a biologically active material involved in the device; thus any sensor physically or chemically operated in biological samples can be considered a biosensor. This definition is obviously too broad but may involve, for instance, microelectrodes implanted in animal tissues, like brain. For most of the authors in this book the association of a biological sensing material with a transducer is compulsory, and even if different definitions are given, a biosensor can be simply defined, in a first approach as a device that intimately associates a biological sensing element and a transducer.

The first biosensor described, even if the term was not used at the time, was the combination of the Clark amperometric oxygen electrode serving as transducer and the enzyme glucose oxidase as sensing element for glucose monitoring. In 1962 Clark and Lyons (1) took advantage of the fact that an analyte like glucose could be enzymatically oxidized with, in parallel, consumption of the coreactant O_2 or the appearance of a product, H_2O_2, which could be electrochemically monitored. The enzyme, retained by a perm-selective membrane,

thus added to the amperometric detector a high selectivity that could not be obtained without the bioelement.

During the following decade a lot of effort was devoted to obtaining bioconjugates for enzyme immunoassays. Various methods for enzyme immobilization were also described, including adsorption, entrapment in a gel lattice, covalent binding trough activated groups on the support, or the use of a cross-linking reagent (2).

In 1967 Updike and Hicks (3) gave the name *enzyme electrode* to a device comprising a polyacrylamide gel with entrapped glucose oxidase coating an oxygen electrode for the determination of glucose. Besides amperometry, potentiometric electrodes were also proposed by Guilbault and Montalvo in 1969 (4).

Since the early 1970s various combinations of biological material associated with different types of transducers gave birth to the larger concept of "biosensor." As a matter of fact, as exemplified in Figure 1, a biosensor associates a bioactive sensing layer with any suitable transducer giving a usable output signal. Biomolecular sensing can be defined as the possibility of detecting analytes of biological interest, like metabolites, but also including drugs and toxins, using an affinity receptor (enzymes being the simplest and historically the first employed), which can be a natural system or an artificial one mimicking a natural one, able to recognize a target molecule in a complex medium among thousands of others.

To obtain a usable output signal that can be correlated with the amount or concentration of analyte present in the medium, multiple events must take place sequentially. Briefly, a first chemical or physical signal consecutive to molecular recognition by the bioactive layer is converted by the transducer into a second signal, generally electrical, with a transduction mode that can be electrochemical, thermal, optical, or based on mass variation. The selective molecular recognition of the target molecule can theoretically be achieved with various kinds of affinity systems, for example (but not exclusively),

Enzyme for substrate
Antibody for antigen
Lectin for sugar
Nucleic acid for complementary sequence

The first problem we must face is the degree of bioamplification obtained when molecular recognition occurs. If the bioactive molecule present in the sensing layer is a biocatalyst a reaction takes place in the presence of the specific target analyte, and a variable amount of coreactant or product may be either consumed or produced, respectively, in a short time depending on the turnover. Biocatalysis thus corresponds to an amplification step generating a chemical signal.

In contrast, the use of antibodies for the detection of antigens is not normally a

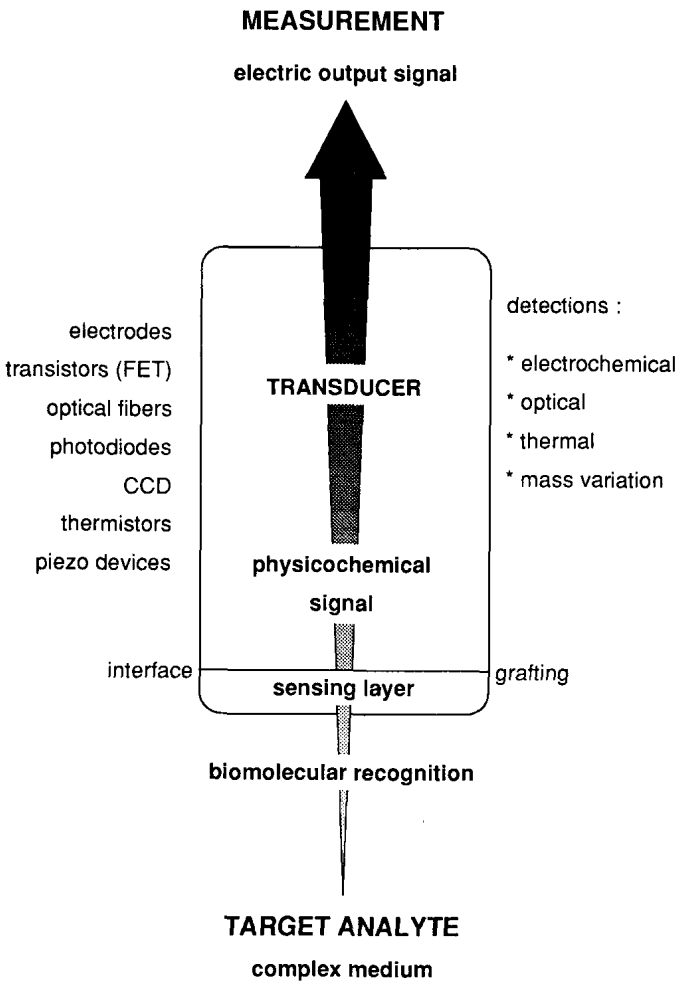

Figure 1 Configuration of a biosensor.

biocatalysis phenomenon, and different approaches can be considered. A bioconjugate involving a bound enzyme can be prepared, and the presence of the target antigen is determined through the related enzyme reaction. Conventional detection has already been described with enzyme electrodes. New approaches are now being intensively explored, and antibody-antigen binding can be sensed directly through transducers sensitive to mass variation for example.

Another key point to which attention must be paid is the intrinsic specificity of the biological material involved in the recognition process. Some enzymes, for instance, may be strictly specific, like urease, or highly specific, like glucose