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Review Article

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Review of Electrochemical Biosensors for Microbial Detection in Food Industry

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Abstract Food safety and nutritional quality monitoring are crucial to maintain public health. Microbial hazards may enter food at any point during production and storage processes. Standard analytical procedures for safety and quality analyses are laborious, time intensive and high maintenance. Alternatively, biosensors are good substitutes for agro-food diagnostics which provides reliable and rapid detection of pathogens. In this article we provide an overview of different types of electrochemical biosensors like potentiometric, amperometric and impedimetric biosensors to detect food borne pathogens. The systematic study of variable signals of electrochemical biosensor enables us to provide accurate real time information about presence of pathogen in food sample.

Keywords Agro-food diagnostics, Potentiometric biosensor, Amperometric sensor, Impedimetric biosensor, Food borne pathogens

1. Introduction

Foodborne pathogens pose a major threat of infectious life-threatening diseases all around the globe. Advancement of a rapid sensing mechanism with high sensitivity is vital to avoid such outbreaks by timely recognition of the infectious foodborne pathogens [1]. The conventional analytical techniques for quality and safety analyses are very tedious, time consuming and require trained personnel, therefore there is a need to develop quick, sensitive, and reliable techniques for quick monitoring of food quality and safety. In this connection biosensor is an appropriate alternative to conventional techniques. Biosensor devices are emerging as one of the foremost relevant diagnostic techniques for food, clinical and environmental monitoring due to their rapidity, specificity, ease of mass fabrication, economics and field applicability [2]. The electrochemical biosensor is an analytical device that transduces biochemical events such as enzyme-substrate reaction and antigen-antibody interaction to electrical signals (e.g., current, voltage, impedance, etc.) [3,4,5] This review focuses on advances in the detection of common toxin producing, food borne pathogens *Escherichia coli*, *Salmonella*, and *Bacillus cereus* using electrochemical biosensors.

Amperometric detection of *Escherichia coli*

First isolated in 1982, Shiga toxin-producing *Escherichia coli* O157: H7 has become an important food and waterborne pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) in humans. An enterohemorrhagic bacterial strain, *E. coli* O157: H7 infects the alimentary tract and induces abdominal cramps with hemorrhagic diarrhea. Transmission of *E. coli* O157: H7 occurs via the faecal-oral route after consumption of contaminated, undercooked liquids and foods [6,7,8].

Amperometry is based on the measurement of the current resulting from the electrochemical oxidation or reduction of an electroactive species. It is usually performed by maintaining a constant potential at Pt, Au or C based working electrode. (2) Clark oxygen electrodes represent the basis for the simplest forms of amperometric biosensors, where a current is produced in proportion to the oxygen concentration. This is measured by the reduction of oxygen at a platinum working electrode in reference to an Ag/AgCl reference electrode at a given potential [9,10].

The amperometric biosensor for detection of *E. coli* utilizes a combination of the amperometric technology principle along with a substrate-enzyme complex. If a horseradish peroxidase (HRP) enzyme was conjugated with an antibody specific for E. coli O157:H7, the conjugated antibody would work as a biological receptor for E. coli O157:H7 bacteria. Once E. coli O157:H7 binds to the antibody conjugated with HRP, hydrogen peroxide could be added causing a product to be formed, namely oxygen (see reaction below). This oxygen formation would be able to be detected with a Clark electrode and could be correlated to the bacterial concentration [11].

$$H_2O_2 \xrightarrow{HP} O_2 + 2H \qquad \text{HP = Horseradish peroxidase}$$

Impedimetric detection of *Salmonella*

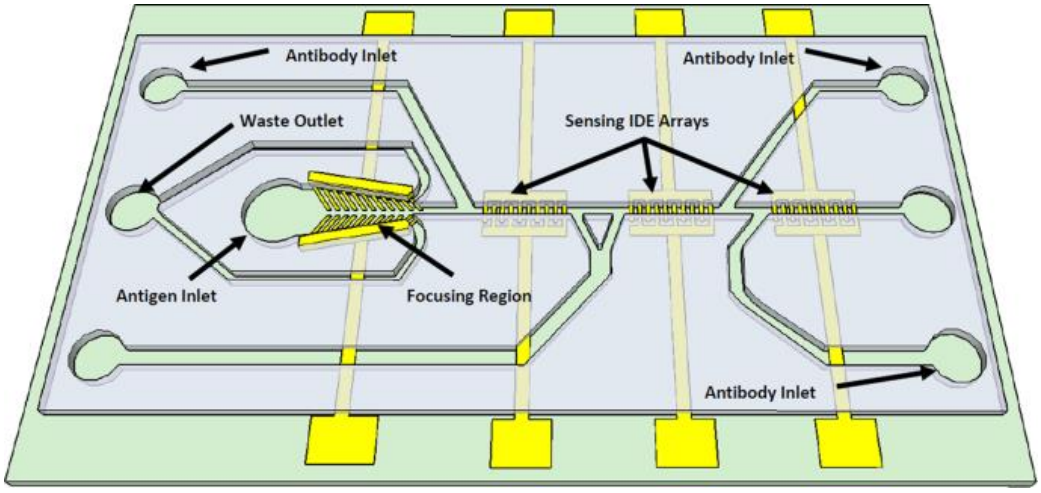
Isolated first from pig intestine in 1884 the *Salmonella* bacteria is a gram-negative, motile, hydrogen sulphide producing, an acid-labile facultative intracellular microorganism that infects humans, causing clinical infections with different clinical features like gastroenteritis, enteric fever, bacteremia and a chronic carrier state [12].

Impedance technique, as one kind of the electrochemical biosensors, has been proved to be a promising method for foodborne pathogenic bacteria detection. Generally, the impedance detection techniques can be classified into two types depending on the presence or absence of specific bio-recognition elements. The first type works by measuring the impedance change caused by binding of targets to bioreceptors (antibodies and nucleic acids) immobilized onto the electrode surface, while the detection principle of the second type is based on metabolites produced by bacterial cells as a result of growth [13].

The impedance-based biosensor for rapid and simultaneous detection of *Salmonella* serotypes B, D, and E consists of a focusing region, and three detection regions. The cells focusing was achieved using a ramp down electroplated vertical electrode pair along with tilted thin film finger pairs that generate p-DEP forces to focus and concentrate the bacterial cells into the centre of the microchannel and direct them toward the detection region.

The detection regions consist of three interdigitated electrode arrays (IDEA), each with 20 pairs of fingers coated with a mixture of anti-*Salmonella* antibody and crosslinker to enhance the adhesion to IDEA. The impedance changes as the target *Salmonella* binds to the antibody.

The biosensor has showed excellent performance as proven by the detection of a single *Salmonella* serotype B, and simultaneous detection of two *Salmonella* serotypes B and D with a limit of detection (LOD) of 8 Cells/ml in ready-to-eat turkey samples [14].



3D schematic of the impedance biosensor showing the focusing IDE array and three sets of the IDE arrays for three sensing regions. Each of the region could be used for sensing different serotypes of *Salmonella* without causing any cross-contamination.

Potentiometric detection of *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive facultative aerobe that can grow in the absence of oxygen by fermentation or by using an alternative electron acceptor. Staphylococcal food-borne disease (SFD) is one of the most common food-borne diseases worldwide resulting from the contamination of food by preformed *S. aureus*. Symptoms of SFD include nausea, vomiting, and abdominal cramps with or without diarrhoea [15,16].

Potentiometric biosensors are based on the measurement of oxidation and reduction potential of an electrochemical reaction. Thus, a pH-meter consists of an immobilized enzyme membrane surrounding the probe, where the hydrogen ions are produced or absorbed by the catalysed reaction. Potentiometric biosensors include the use of ion-selective electrodes to convert the biological reaction into an electrical signal [17].

A potentiometric nanobiosensor technique using selective patterns was used for *Staphylococcus aureus* exotoxin detection. A molecular framework and polymer were produced using methacrylic acid (MAA) monomers, which formed covalent bonds between MAA monomers to produce a white polymer. In addition, hydrogen bonds formed between the amino acids of the exotoxin and the MAA functional groups, which functioned as selective sites for the polymer. The results showed that the molecular framework polymer (MFP) in the designed potentiometric biosensor was able to detect an exotoxin density up to 10^{-3} M at 68 nm of synthesized molecularly imprinted polymer (MIP) [18].

Summary and future outlook

The detection of pathogens is becoming more important in food safety and nutrition quality monitoring. A major challenge in pathogen diagnostics is the rapid and accurate characterization of antibiotic resistance of infecting species or strains in different environments to prescribe appropriate antibiotics to patients and regions at the early stages of infection. Although these sensors have shown excellent performance, further research is needed. Not only do researchers need to simplify sample preparation, but also need to enable sensors to detect target analytes in samples at very low concentrations. Furthermore, real-time monitoring needs to be done on-site. In the future, with the emergence of new nanomaterials, microfluidics, and molecular biology techniques, more new electrochemical biosensors will be designed with higher detection performance to ensure food safety and quality monitoring.

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