CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Journal home page: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

ELECTROCHEMICAL BIOSENSOR FOR FOOD BORNE PATHOGENS: AN OVERVIEW

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https://doi.org/10.34302/crpjfst/2020.12.2.1

Article history:
Received:
4 February 2020
Accepted:
20 May 2020

Keywords:
Food;
Pathogen;
Electrochemical;
Biosensor;
Nanomaterial.

ABSTRACT

Food safety is very significant for community fitness issue, as at present food borne diseases widespread and increasing public health issue all over the world. The fast and specific detection of food borne pathogens needed to control and avoid human food borne infections. Biosensors are fast and low price method of food borne pathogen detection. It uses the distinctive properties of biological and physical materials to identify a target molecule and effective transduction of an electronic signal. Many biosensors have been discovered, viz., electrochemical biosensor, optical biosensor and mass based biosensor. In this study, we review electrochemical biosensors for detection of food born pathogen. Electrochemical biosensors have many advantages over other biosensor such as the possibility to operate in disorganized media, sensitivity of instrument, and small size. Electrochemical biosensor are of different kinds like potentiometric, amperometric, potentiometric, impedimetric, or conductometric based upon different transducing elements used in it. From last few decade nanotechnology has arisen as a favorable field for solving food safety problems in terms of detecting contaminants. The nanomaterials used into electrical sensors to make them appropriate to reach over low detection limit, high sensitivity, and multi detection abilities.

1. Introduction

Food-borne pathogens are very diverse in nature and keep producing major public health problems all over the world. Therefore, food safety is very important issue for consumers and food industry. The globalization of food causes to changes in the dietary behaviors and changes in the food production, consumption and distribution because of these changes in food habits new safety issue arrived (Sankarankutty, 2014). Foodborne diseases produced by pathogens result in recurring intestinal swelling, chronic kidney infections, mental retardation, joints problems, impaired vision, and even death (Hoffmann *et al.*, 2015). Therefore, the food safety is very important and World Health Organization has promoted food safety as follows: "from farm to plate make food safe" on World Health Day, 2015 (WHO, 2015). Traditional methods for the recognition of toxins are sensitive and inexpensive, but they need many days to produce results. Conventional methods for recognition of pathogen detection are needs more manpower, low sensitivity and specificity, and need trained users (Fournier *et al.*, 2013). On the other side biosensors gives results very quickly with high sensitivity (Yang *et al.*, 2008). They can be used in different areas like in food industry for detection of pathogen and additives, environmental monitoring, clinical diagnoses and biodefense due to their high sensitivity, selectivity and fast response (Thakur and Ragavan, 2013). Biosensors are an aid to the food industry as they are intelligent combination of biological component and technical component to find physical and chemical changes and to transmit it in to form of data. The implementation of nanotechnology increases the importance of biosensor in the field of pathogen detection. The sensitivity of the biosensor increased by using of nanomaterials such as magnetic Nano-particles (MNPs), carbon nanotubes (CNTs), Nano rods (NRs), and quantum dots (QDs). This review describes application of electrochemical biosensor for detection of food borne pathogens.

2. Electrochemical biosensors

The electrochemical biosensor is more popular than other biosensors due to its advantages such as low cost, high sensitivity and selectivity and small size for the detection of food-borne pathogens (Palchetti and Mascini 2008). This biosensor uses electrochemical transduction that typically measure conductivity or impedance changes, sensing methodology for electrochemical biosensor. Electrochemical biosensors uses chemical reaction comprising immobilized biomolecules and target analyte which effect measured electrical properties of solution such as an electric current or potential by producing or consuming ions (Zhang *et al.*, 2008). Block diagram for electrochemical biosensor given in figure 1. Accordingly, which type of transducer used electrochemical biosensor can categorized in amperometric biosensor, potentiometric biosensor and impedimetric biosensor.

The detection methods usually depend on the electrochemical properties of a particular electrode surface. Electrochemical methods include a reference, counter, and a working electrode. Reference electrode is make by silver chloride and put at a distance from the reaction-site to maintain a stable potential while sensing element work like a transducing element (Yunus, 2018). A counter electrode made contact in between electrolytic solution and electrode surface to supply current to the working electrode.

Milk used in this study obtained from Ege University Menemen Research and Application Farms. Beneo (Mannheim, Germany) Nutriz, rice bran formula obtained from Artisan Gida San. For the preparation of rice milk, 13.6 g of rice bran diluted in 100 mL of water. MYE 96-98 starter culture for yoghurt production containing *S. thermophilus* and *L. bulgaricus* was obtained from Maysa Gida San. In addition to the yoghurt culture, *Lactobacillus gasseri* ATCC 4963 and *Bifidobacterium longum* DSM Lafti B22 strains were used. Filling and packaging were done with packages obtained from Ege University Faculty of Agriculture Menemen Farms and Ege University Faculty of Agriculture Department of Dairy Technology.

2.1. Amperometric biosensors

Amperometric biosensors are universal way for finding the food pathogens. Transducer of amperometric biosensor measure the amount of electric current produced at constant potential between working and reference electrode (Sharma *et al.*, 2013). The equipment for amperometric biosensor contains three electrodes, a voltage source and a device for measuring current (Arora *et al.*, 2018). Equipment for amperometric biosensor displayed in figure 2. The amperometric biosensors make usage of Clarks oxygen electrode, which determines the quantity of oxygen (present in the analyte) reduced. It usually depends on an enzyme system that catalytically converts analyte into product that can oxidized at working electrode. Commonly Horseradish peroxidase (HRP) and alkaline phosphatase enzymes are used (Zourob *et al.*, 2008). Various types of amperometric biosensor are present for detection of food pathogen; examples are DNA based, immunosensor, and microbial metabolism based biosensor. A list of amperometric biosensors for detection of food pathogen presented in table 1.

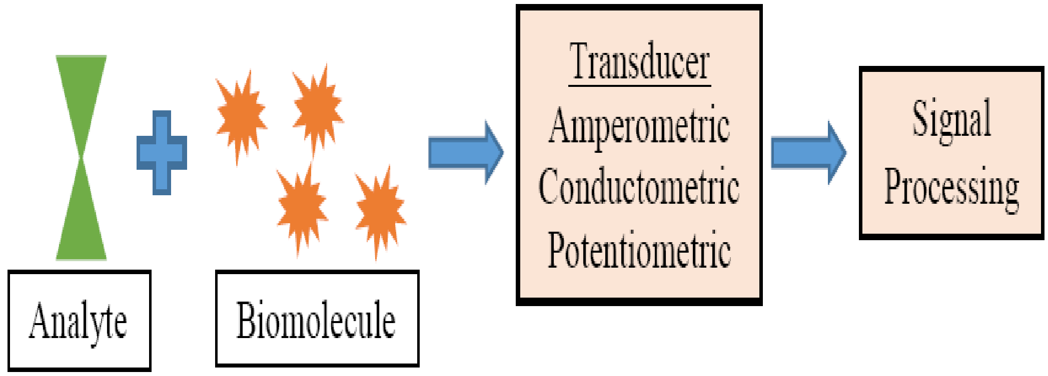

Figure 1. Electrochemical Biosensor

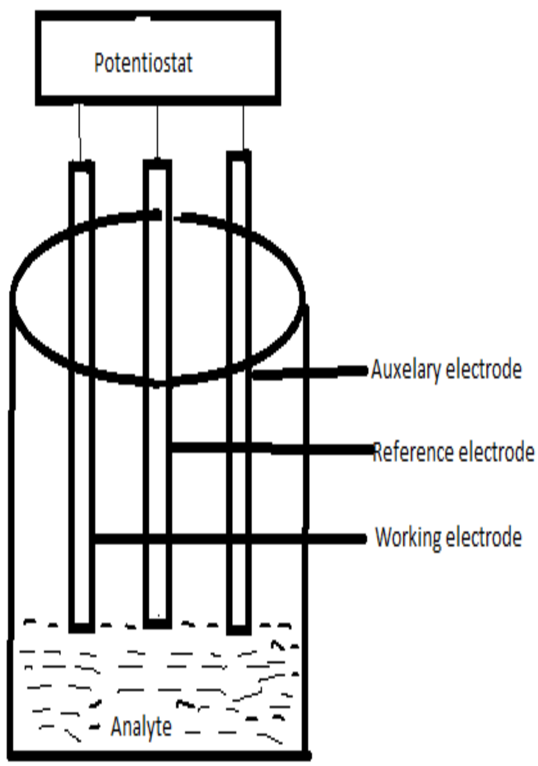

Figure 2. Amperometric Biosensor

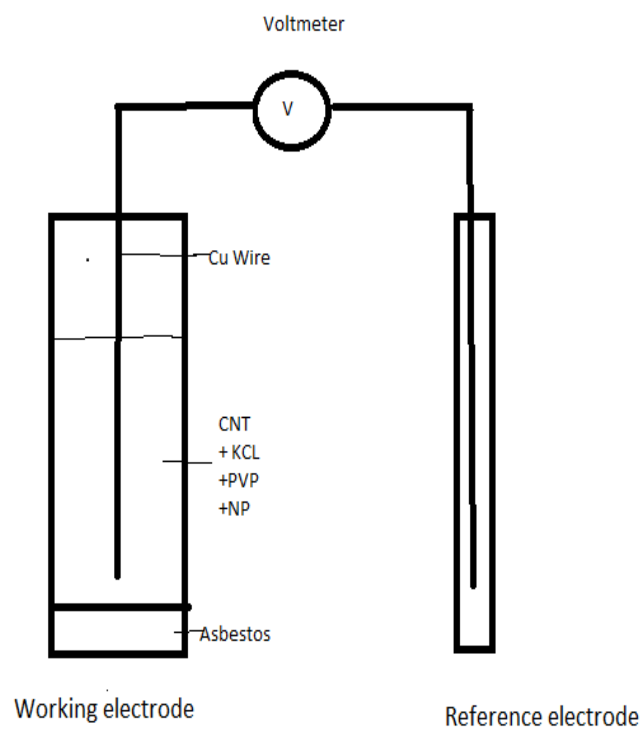

Figure 3. Potentiometric biosensor

Table 1. Amperometric biosensor for detection of food pathogens

Food sample	Pathogen	Bioreceptor	Electrode	LOD	Reference
Food sample	*Salmonella pullorum*	Antibody	Screen printed electrode (SPE) modified GNP	89 CFU/mL	Fei *et al.*, 2016
PBS	*Streptococcus agalactiae*	Antibody	Screen-printed carbon electrodes	10 CFU/mL	Vásquez *et al.*, 2016
Skim & whole milk	*Salmonella typhimurium*	Polyclonal antibody	Gold electrode	10 CFU/mL	Alexander *et al.*, 2018
Milk	*L. monocytogenes*	HRP-labeled antibody	Novel multiwalled carbon nanotube	1.07×10^2 CFU/mL	Lu *et al.*, 2016
Food sample	*E. coli* O157:H7 *S. aureus*	Antibody	Gold electrode	1×10^{-12} mol/L	Fernandes *et al.*, 2014
Food sample	*E. coli*	Biotinyl Antibody	Saturated calomel electrode	$3x10^1 – 3.2x10^6$ CFU/mL	Li *et al.*, 2013
Blue-berry	*L. monocytogen*	Antibody	Gold nanoparticle modified screen printed carbon electrode	2 log CFU/g	Davis *et al.*, 2013
Milk	*Staphylococcus aureus*	Antibody	DropSens screen-printed gold electrodes	1 CFU/mL	De Avila *et al.*, 2012
Milk	*E. coli*	Antibody	Photo-lithographic gold	100 cells/mL	Laczka *et al.*, 2011
Food sample	*S. aureus* nuc gene	Antibody	Gold electrode	3.23×10^{-14} mol/L	Sun *et al.*, 2015

2.1.1. Amperometric DNA based biosensors

A DNA biosensor is device in which oligonucleotide incorporate with a known sequence of bases, either combined within or closely connected with the electrode (Zourob *et al.*, 2008). There are various kind of electrochemical DNA sensors developed for detection of the bacterial nucleic acid. A DNA based amperometric nanoparticle biosensor was developed by Fernandes *et al.*, (2014) for detection *of E. coli* O157:H7 and the nuc gene of *S. aureus.* This DNA sensor provide very low detection limit for both pathogen that is 1×10^{-12} mol/L. This DNA based amperometric biosensor gives high specificity and selectivity in detection of target DNA. Another use of the electrochemical DNA biosensor includes detection of *S. aureus* gene sequence in the concentration range of 1.0×10^{-13}-1.0×10^{-6} mol/L with LOD of 3.23×10^{-14} mol/L (Sun *et al.*, 2015).

2.1.2. Amperometric immunosensors

In immunosensors microorganism detected by using antibodies that are immobilized on electrode surface or magnetic beads. This is one of the successful techniques for pathogen detection; various researchers reported immunosensors for detection of food pathogen. Laczka *et al.* (2011) reported immunosensor for the recognition of *E. coli* in a microfluidic system joined with immunomagnetic beads with LOD of 100 CFU/mL in milk. Lu *et al.*, (2016) established an amperometric immunosensor with LOD of 1.07×10^{2} CFU/mL by immobilization of HRP antibody against *Listeria monocytogenes* onto the surface carbon nanotube fibers.

2.1.3. Amperometric microbial metabolism based biosensor

Metabolic metabolism based biosensor uses specific marker enzyme for detection purpose (Arora *et al.*, 2011). Various researchers reported microbial metabolism biosensors for detection of pathogens in food. These biosensors commonly used for analysis of water samples to recognize coliform by their metabolic product released enzyme β-D-glucuronide, lucuronoso-hydrolase (GUS) and β-d-galactosidase (Arora *et al.*, 2018). Neufeld and coworkers (2003) developed an amperometric microbial metabolism based biosensors for quantification of coliform *E. coli* K-12 using bacteriophage immobilized on screen-printed carbon electrodes with the sensitivity of 1 CFU/100 mL of sample. Togo *et al*. (2007) developed a bacteria-based biosensors for GUS detection by immobilization of *Moraxella* species. Metabolic product of GUS enzyme of *E. coli* to *p*-nitrophenol (PNP) and D-glucuronic acid by *Moraxella* shows the occurrence of *E. coli*.

2.2. Potentiometric biosensors

Potentiometric biosensors uses the ion selective electrodes to find the potential of a solution based on particular relation with ions in the solution. These biosensors measure the electrical potential between working and reference electrode. The potential of reference electrode not change during the entire period of measurement while working electrode undertakes some change in its potential even for minor changes in concentration of analyte (Ahmed *et al.*, 2014). Equipment for potentiometric biosensor shown in figure 3. This technique is not very common for detection of food pathogen. A list of potentiometric biosensor given in table 2.

2.3. Conductometric biosensors

Conductometric biosensors use transducers, which measure deviation of the ionic strength of a solution that changes flow of current (Karunakaran *et al.* 2015). Conductometric biosensors have no need of reference electrode so it have miniaturization possibilities and low cost. These biosensors have advantages of low cost, small size (Salek-Maghsoudi *et al* 2018) and real-time monitoring (Bettazzi *et al.* 2017) but disadvantage of low sensitivity. Hnaiein *et al.* (2008) reported conductometric biosensor for finding of *Escherichia coli* with LOD of one CFU/mL. Tahir and Alocilja (2004) developed a conductometric biosensor for the detection of *E. coli* O157:H7 and *Salmonella* with an LOD of 81 CFU/mL. Pal *et al.* (2008) also reported a conductometric biosensor for *B. cereus* in different foods.

2.4. Impedimetric biosensors

Impedimetric biosensors developed by addition of impedance with biological recognition element (Yang and Bashir, 2008). This method is one of the oldest methods for detection of microorganism. G.N. Stewart in 1899 developed first impedimetric biosensors for detection of microorganism. Impedimetric transduction to find various kinds of foodborne pathogens. These biosensors have advantages of unobstructed measurement of the molecule of interest, with no need for the enzymatic analyte and ability of multiple detection (Ahmed *et al.*, 2014). However, they have disadvantages of reproducibility and problems with nonspecific binding. Various researchers reported impedimetric biosensors for detection of food born pathogen. A list of impedimetric biosensors used for food pathogen are presented in table 3.

3. Commercially available electro-chemical biosensor for finding pathogen in foods

Although there are very large no of publication on biosensor for detection of pathogen but very few are commercially available. Table 4 shows list of commercially available electrochemical biosensor for detection of food pathogens. There are some limitations such as low lifespan of biological component, mass production and not easy to use. However, by application of nanotechnology in biosensor these problems can be solved in near future, as biosensors have unique ability in terms of sensitivity, specificity and quick response. There are costs and technical issue that can slow the commercialization of new systems.

Table 2. Potentiometric biosensor for detection of food pathogens

Sample	Pathogen	Bioreceptor	Electrode	LOD	Reference
Pig skin	*Staphylococcus aureus*	Aptamer	Single-walled carbon nanotubes	$8x10^2$ CFU/mL	Zelada *et al*., 2010
Milk, Fruit juice	*E. coli*	Aptamer	Single-walled carbon nanotubes	26 CFU/mL in juice and 6 CFU/mL in milk.	Zelada *et al*., 2010
Food sample	*Staphylococcus aureus*	DNA Aptamers	Carbon nanotube aptamer based electrode	Single CFU/mL	Hernandez *et al*., 2014
Food sample	*Staphylococcus aureus*	DNA Aptamers	Carbon nanotube aptamer based electrode	Single CFU/mL	Cao *et al*., 2009
Food sample	Sulfate-reducing bacteria	None	Glassy carbon electrode	$2x10^{-2}$ to $3x10^7$ CFU/mL	Wan *et al*., 2010
Lettuce, carrots	*E. coli*	Antibody	LAPS	10 cells/mL	Ercole *et al*., 2003

Table 3. Impedimetric biosensor for detection of food pathogens

Food sample	Pathogen	Bioreceptor	Electrode	LOD	Reference
Milk	*Listeria innocua*	Bacteriophage endolycin	Gold screen printed electrode (SPE)	10^5 CFU/mL	Tolba *et al.*, 2012
Whole milk	*E. coli* O157:H7	Antibody	Alumina	83.7 CFU/mL	Joung *et al.*, 2013
Ground beef, cucumber	*E. coli* O157:H7	Antibody	Gold Nano particle	1.5×10^4 and 1.5×10^3 CFU/mL	Wang *et al.*, 2013
Fat free milk	*S. typhimurium*	Antibody	GNP and poly (amidoamine)-multi walled carbon nanotubes	10^3 CFU/mL	Dong *et al.*, 2013
Milk	Gram +ve bacteria, selectively *L. monocytogen*	Antimicrobial peptide	Interdigitated gold microelectrodes	10^3 CFU/mL	Etayash *et al.*, 2014
-	*Listeria monocytogenes*	DNA Aptamer	Platinum interdigitated array microelectrodes	5.39 ± 0.21 CFU/mL	Sidhu *et al.*, 2016
ground beef, chicken	*E. coli* O157:H7, *Salmonella typhimurium*	Antibody	Screen- printed interdigitated microelectrode	2.05×10^3 CFU/g and 1.04×10^3 CFU/mL	Xu *et al.*, 2016
PBS	*E. coli* O157:H7	Antibody	-	100 CFU/mL	Wan *et al.*, 2016

Table 4. Commercially available biosensor for detection of food pathogens

Name of device	Type of Biosensor	Manufacturer	Target compound	Sample
Midas Pro	Amperometric	Biosensori SpA, Milan, Italy	Food pathogen	Food sample
Bactometer	Impedimetric	Bactomatic Inc., Princeton, USA	Food pathogen	Food sample
Bactometer	Impedimetric	Biomerieux, France	Food pathogen	Food sample
Bac Trac	Impedimetric	Sy-Lab, Austria	Food pathogen	Food sample
Malthus ATanalyzer	Impedimetric	Malthus Instruments	Food pathogen	Food sample
Malthus 2000	Potentiometric, conductometric, field Effect	Malthus Inc., Stoke-on-Trent, UK	Food pathogen	Food sample

Analyte 2000TM	Electrochemical	Research International Ltd.	*E. coli* O157:H7	Hamburger
Malthus systems	Electrochemical	Malthus Instruments Ltd.	*E. coli* O157:H7, Fungi, Yeast	Shell fish
RABIT	Electrochemical	Don Whitley Scientific Ltd.	Food pathogens	Vegetables
BioflashTM system	Electrochemical	Innovative Biosensors Inc.	*E. coli* O157:H7	Lettuce
Biosensor	Electrochemical	Michigan State University's, USA	*E. coli* O157:H7, *Salmonella*	Meat
Biosensor	Electrochemical	Massachusetts Institute of Technology. USA	*E. coli* O157:H7	Lettuce (Canary)
Biosensor	Electrochemical	Georgia Research Tech Institute, USA	*Salmonella* and *Campylobacter*	Pork industry

4. Nanotechnology in electrochemical biosensors

Application of nanotechnology in biosensor give advantage of increased sensitivity and selectivity, quick response and minimum cost of production. Four types of nanomaterial are very popular in electrochemical biosensor: these are GNP, graphene, carbon nanotube and photonic crystals. Many researcher developed electrochemical biosensor incorporated by nanomaterial for detection of pathogen. Ma *et al.*, (2014) developed electrochemical biosensor with gold nanoparticles (AuNPs) for detection of *Salmonella typhimurium* in pork with a detection limit of 3 CFU/mL. Zelada *et al.*, (2012) made potentiometric biosensor with carbon nanotube for detection of *S. aureus* in pigskin. A list of electrochemical biosensor incorporated with nanomaterials given in table 5.

Table 5. Biosensor using nanotechnology for detection of food pathogens

Biosensor	Food sample	Pathogen	Nanomaterial	LOD	Reference
Potentiometric biosensor	Pig skin	*S. aureus*	Carbon nanotube	800 CFU/mL	Zelada *et al.*, 2010
Electrochemical biosensor	Pork	*Salmonella typhimurium*	AuNPs	3 CFU/mL	Ma *et al.*, 2014
Electrochemical biosensor	Milk and infant formula	*Bacillus cereus*	AuNPs	10 CFU/mL	Izadi *et al.,* 2016
Impedimetric biosensor	Spiked food	*Salmonella typhimurium*	Copolymer	-	Sheikhzadeh *et al.*, 2016
Electrochemical biosensor	Food sample	*Salmonella enteritis* and *Bacillus anthracis*	Au NPs and MNPs	0.5 ng/mL and 50 pg/mL	Zhang *et al.*, 2010
Electrochemical immunosensor	Food sample	*E. coli* O157:H7	Carbon screen printed	3.47×10^3 CFU/mL	Dou *et al.*, 2013

5. Conclusions

Biosensors are the tools for detecting pathogen of foods. Electrochemical biosensors have lots of advantage over other methods like small size, low cost, easy to handle, high sensitivity. In the last decade, various new technique discovered. The application of nanotechnology in biosensor make it great tool for finding impurities of foods. Although biosensors have benefits over old-style methods, yet there are several problems in its development such as on site monitoring. Until now, few biosensors are commercially available for this purpose. It is expected that in future electrochemical biosensors, information technology will be included to help food industries and customers. Application of real time monitoring, nanoparticle and nanotechnology in electrochemical biosensor will be a great tool for detection of germs and pathogens in the foods.

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