Biological Acoustic Sensors for Microbial Cell Detection

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O. I. Guliy, B. D. Zaitsev, A. A. Teplykh, and I. A. Borodina

Abstract

One of the most popular areas in microbiology is the development of fast and sensitive methods for the detection of bacteria based on electrophysical analysis. The paper demonstrated the capabilities of various electroacoustic biological sensors for detection and identification of microbial cells. These sensors are based on the following main elements: the piezoelectric resonator with a longitudinal electric field, the piezoelectric resonator with a lateral electric field, the acoustic delay line with inhomogeneous piezoactive acoustic waves, and the delay line using a slot acoustic mode. They can conduct cell detection and identification of bacteria using immobilized microorganisms or directly in cell suspension. The principle of operation of such sensors is based on the registration of the interaction of microbial cells with specific antibodies, bacteriophages, and mini-antibodies. The sensitivity range of microbial cell detection is 10^3–10^8 cells/ml with the suspension conductivity of 5–50 μS/cm. At that the analysis time varies from 5 min to several hours. The presented possibilities of electroacoustic biological sensors for the detection of bacteria are focused on the clinical use of *onsite* as a personalized diagnostic device. The possibility of rapid detection of microflora allows timely diagnosis of the disease and timely medical assistance. In general, acoustic biological sensors form a wide class of detection systems and are very promising for use in microbiology, medicine, and veterinary medicine for solving the problems of detection and identification of bacteria.

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11.1 Introduction

Methods for the detection of microbial cells are widely used in such fields of biology and medicine as the diagnosis of unknown microorganisms, the assessment and monitoring of the physiology and functioning of cells, the study of cellular waste products such as enzymes and antibiotics, and the development of new drugs.

Classical approaches such as microbiological and biochemical tests, methods of genetic engineering, and immunological methods are used to detect and identify microorganisms. These methods for the determination of bacteria, based on the cultivation of microorganisms with subsequent microbiological and biochemical analysis, are characterized by laborious and time-consuming use of expensive equipment. Another limitation of immunological methods and the method of polymerase chain reaction (PCR) is associated with the impossibility of screening a huge amount of material for the preliminary determination of bacteria.

Spectroscopy, fluorescence, and bioluminescence methods are also effective for the determination of microorganisms (Van Emon et al. 1995; Basile et al. 1998; Yousef 2008). However, they also require the use of expensive equipment and the involvement of highly qualified specialists.

Increasing attention of specialists is directed to the development of new express methods of indication of bacteria. In the development of modern methods for the diagnosis of microorganisms, two main directions of development can be distinguished.

The first direction is the development of the complicated automated systems. However, this way means the use of expensive equipment and the involvement of highly qualified specialists.

The second direction is connected with the development of new approaches that allow the rapid analysis of microbial cells, for example, using biosensor devices (Von Lode 2005; Hu et al. 2014). With the advent of biosensors, the traditional approaches to methods for determining microorganisms are changing significantly. The development of biosensor microbial cell detection technologies will be extremely useful for the early detection of diseases and the timely provision of medical care (Chandra 2016). The interest in biosensor systems for the detection of bacteria in aqueous solutions is due to their simplicity, cost-effectiveness, and relatively high sensitivity (Griffiths and Hall 1993; Li et al. 2010).

Obviously, new sensory technologies for the detection of microorganisms must have high sensitivity, reliability, and be able to get results in a short period of time. Therefore, among the electrophysical methods of analysis, electroacoustical methods are of particular interest (Andle and Vetelino 1994; Don et al. 2016).

The distinctive features of electroacoustic methods of the determination of microbial cells are:

– The possibility of analysis in solutions with a high content of ions, since their presence distorts the analytical signal
– The possibility of multiple use and cleaning of the liquid container from the sample being measured
– The short time of analysis

The principle of the operation of acoustic biological sensors is based on the registration of the biospecific reactions in a liquid suspension contacting with the surface of the piezoelectric. The interaction of the bacterial cells and specific agent causes a change in the conductivity of the suspension. When it contacts with the surface of the piezoelectric resonator, these changes lead to both a shift in its resonant frequency and a change in the frequency dependences of the electrical impedance (Zaitsev et al. 2012). Upon contact of the suspension with the surface of the piezoelectric waveguide with a propagating acoustic wave, the indicated change in conductivity leads to a change in the insertion loss and phase of output signal of the sensor (Borodina et al. 2018). All these effects are very sensitive for the detection of bacterial cells in suspension. It is obvious that the development and application of electroacoustical technology for the determination of bacteria will be extremely useful for the early detection of diseases.

So the chapter will discuss the main types and principles of operation of biological electroacoustic sensors. The main focus is pointed to the description of electroacoustic sensors for the detection of bacteria, which are oriented on the clinical use of *onsite* as a personalized diagnostic device. On the basis of literature data and our own experimental studies, the capabilities of the electroacoustic analysis method will be shown and compared with other ones.

11.2 Sensors Based on Piezoelectric Resonators with a Longitudinal Electric Field

Sensors based on piezoelectric resonators with a longitudinal electric field have been widely used for several decades. Such a sensor represents a piezoelectric plate with two metal electrodes deposited on each side of it. An active film containing immobilized microorganisms (usually bacterial cells) is applied directly to the electrode on one side of the sensor. Upon contact with a suspension containing any specific reagents (bacteriophages or antibodies), a layer of protein mass builds up on the active side of the resonator, which leads to a shift in the resonant frequency and a decrease in Q-factor. The distinctive features of such sensors are their reusability and low cost. However, these sensors have two significant drawbacks. Firstly, they do not allow the analysis of cells directly in suspension without applying the active layer of immobilized microorganisms. This is due to the fact that upon contact with a suspension, the parameters of the resonator respond only to changes in

the mechanical properties of the fluid and practically do not respond to changes in its electrical properties. Secondly, the analysis time which leads to the appearance of the noticeable protein load on the resonator are several hours. Nevertheless, such sensors have been actively used for several decades (Ermolaeva and Kalmykova 2006; Ermolaeva and Kalmykova 2012).

The studies (Olsen et al. 2006; Ripp et al. 2008) show the possibility of using the affine-selective filamentous bacteriophage as a probe for detecting *S. typhimurium* cells using an acoustic biosensor based on a quartz resonator with a longitudinal electric field. The introduction of bacterial cells on the surface of the quartz resonator leads to a change in the resonant frequency, which is recorded by the device. The biosensor allows the detection of cells with a minimum number of 10^2 cells/ml, and the result is recorded in time less than 180 s.

By immobilization of antiviral antibodies on the surface of a piezoelectric resonator with a longitudinal electric field, an immunosensor was developed for the selective determination of herpes viruses in human blood (Koenig and Graetzel 1994) and biosensor for the detection of viruses in natural and artificial reservoirs (rivers, sewers, and wastewater) without prior processing of the analyzed substrate (Bisoffi et al. 2008).

11.3 Sensors Based on Piezoelectric Resonators with a Lateral Electric Field

Over the past two decades, the piezoelectric resonators with a lateral electric field have attracted particular attention from researches developing the biological sensors (Pinkham et al. 2005; York et al. 2005; Wark et al. 2007; Handa et al. 2008; Vetelino 2010).

Unlike conventional resonators with a longitudinal electric field, these sensors have electrodes only on one side of the piezoelectric plate, and the acoustic wave propagates mainly in the space between the electrodes. In this case, the sensitive surface bordering the liquid or the biochemical sample under study remains free, and the studied object does not contact the electrodes. The metallization-free surface allows the sensor to respond not only to the mechanical properties of the sample under study (viscosity) but also to its electrical properties (conductivity, permittivity).

Based on the lateral electric field resonators, there are two types of acoustic biosensors for the detection and identification of bacterial cells – sensors using bioreceptor films and sensors directly contacting with the suspension.

11.3.1 The Sensors Using the Bioreceptor Films

Sensors of this type use a bioreceptor film, which is fixed on the free surface of a piezoelectric plate. Biological material, for example, a suspension containing the object under study, is in contact with the biosensor through the specified film. The

biological interaction between the measured object and the bioreceptor leads to a corresponding change in the analytical signal (resonant frequency or electrical impedance).

Traditionally, such sensors use active layers with immobilized bacterial cells. Due to the contact with a suspension containing any specific reagents (bacteriophages or antibodies), a layer of protein mass builds up on the active side of the resonator, which leads to a shift in the resonant frequency.

The paper (York et al. 2005) describes a sensor based on such a resonator, using anti-rabbit IgG and *E. coli* as test objects. The NH_2 film was deposited on the free surface of the resonator using a special vacuum technique, and then antibodies sensitive to the above antigens were immobilized. Experiments have shown that the interaction of a sensitive coating with an antigen solution resulted in a change in the resonant frequency of the order of 20–30 ppm in 5–8 h.

The biological sensor based on a resonator with a lateral electric field for the study of pesticide (organophosphates), contained in vegetables and fruits, was described in Pinkham et al. (2005). The free surface of the resonator has been covered with polyepichlorohydrin film, which is selectively sensitive to phosmet ($C_{11}H_{12}No_2PS_2$). It was shown that the shift of the resonant frequency with an increase in the phosphate concentration exceeded the frequency shift in a conventional quartz resonator with a longitudinal electric field by almost 30%. The analysis time in both cases was ~ 40 min.

The possibility of developing a sensor based on a resonator with a lateral electric field for determining the content of saxitoxin in water is described in Wark et al. (2007). The free side of the resonator was covered with a special multilayer sensitive film. The shift of the resonant frequency varied in the range of 0–20 ppm with a change in the concentration from 0 to 200 μM. The analysis time was ~ 2–3 min.

The possibility of developing a biological sensor based on a resonator with a lateral electric field with a sensitive multilayer film was shown in Vetelino (2010). The interaction of a sensitive coating with a suspension of *E. coli* cells resulted in a change in the resonant frequency within 20 ppm in 6 h.

It should be noted that sensors using active coatings have a number of significant drawbacks: a long detection time (several hours) and the impossibility of multiple use of the same active layer. After the first experiment, it is necessary to remove the used active film and apply a new one. These facts significantly limit the possibility of sensors using film bioreceptors.

11.3.2 The Sensors Directly Contacting with Suspension

Sensors of this type are free from the above disadvantages. They record the change in the physical properties of a biological liquid or suspension without the use of active films. These changes lead to a corresponding change in the analytical signal (resonant frequency or electrical impedance). A biological sensor based on a resonator with a lateral electric field, which allows the detection and identification of bacterial cells without an active reagent directly in the liquid phase, is described in

Handa et al. (2008). This possibility was demonstrated by the detection of *S. typhimurium* cells using the specific bacteriophages.

It has been pointed above that acoustic sensors based on piezoelectric resonators with a lateral electric field have an advantage over other types of sensors. But the main difficulty in designing such resonators is the suppression of unwanted oscillations in order to ensure a high-quality factor for the selected resonant frequency. In this regard, the choice of the optimal shape of the electrodes and their exact orientation relative to the crystallographic axes of the plate and the edges of the crystal were traditionally used (Wark et al. 2007). However, the realization of this approach involves a number of technical difficulties.

Another approach to the problem of suppressing unwanted oscillations in a resonator with a lateral electric field by partially covering the electrodes with a damping layer allowed to develop a new biological sensor described in Zaitsev et al. (2012). The sensor is designed to detect bacterial cells directly in the liquid phase by registering specific interactions such as "bacterial cells–antibodies," "bacterial cells–bacteriophages," and "bacterial cells–mini-antibodies." Next, we focus on a brief description of the design of this sensor and on the features of the experiments.

Figure 11.1 shows the scheme of the biosensor used, containing a resonator with two rectangular electrodes on X-cut lithium niobate plate and a liquid container. Studies have shown that for the indicated orientation of the plate, a stable resonance is observed on a longitudinal wave propagating along the X axis and excited by lateral components of the electric field. The aluminum electrodes with a width of 5 mm and a length of 10 mm were deposited on a lithium niobate plate through a special mask in a vacuum. The frequency dependences of the real and imaginary parts of the electrical impedance of the resonator were measured using a precision LCR meter 4285A (Agilent Company, USA). The quality factor of such resonator turned out to be ~ 630 at a resonant frequency of ~ 6.6 MHz.

The idea of experiments with an electroacoustic sensor is based on the fact that the physical properties of a cell suspension after adding the bioselective agents (antibodies, bacteriophages, or mini-antibodies) change, which allow to detect and identify the microbial cells in the sample under study. The general scheme of the experiments is presented in Fig. 11.1.

11.3.3 Interaction "Cells–Antibodies"

Registration of the interaction of microbial cells with specific antibodies (Abs) is an important condition for their successful use for the detection of cells. The principle of interaction "antigen–antibody" is widely used in sensory systems to identify microorganisms (Gascoyne et al. 1997; Vaughan et al. 2001; Yousef 2008).

To study the interaction of antibodies with microbial cells by the developed acoustic sensor, polyclonal antibodies (pAbs) and microbial cells of *Azospirillum brasilense* Sp7 with different concentrations in suspension were used (Guliy et al. 2013). The frequency dependences of the real and imaginary parts of the electrical impedance were measured before and after the addition of specific Abs (Fig. 11.2a

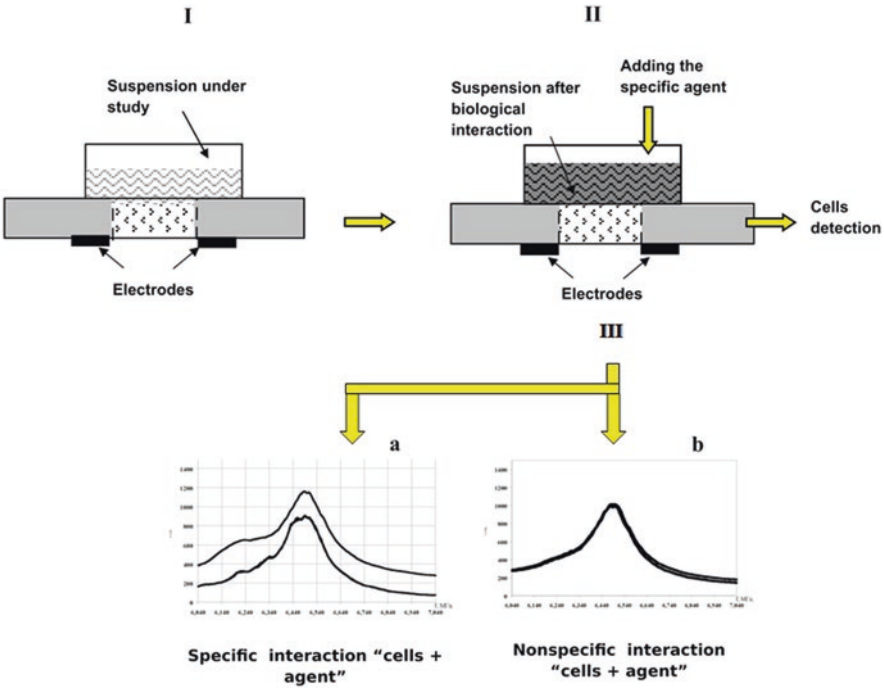

Fig. 11.1 General experimental scheme
The process of the measurement consists of the following steps:
I – a suspension cells is placed in the liquid container and the analytical signal of the sensor is measured
II – the specific/nonspecific (for studied cells) antibodies/or bacteriophages/or phage mini-antibodies are added in liquid container and the analytical signal is measured
III – the obtained results are analyzed and this allows to make conclusion about the presence of the cells under study in suspension:
III (**a**) if the specific agent interacts with the cells, the analytical signal being recorded for suspensions of cells with specific agent (*curve 1*) and without them (*curve 2*) significantly differs
III (**b**) if the specific agent do not interact with the cells, the analytical signal being recorded for suspensions of cells with specific agent (*curve 1*) and without them (*curve 2*) is practically the same

and b). Additionally, the transmission electron microscopy (TEM) identification of the interaction of *A. brasilense* Sp7 cells with the used antibodies labeled with colloidal gold was carried out. In the micrograph (Fig. 11.2e), one can see that the antibodies obtained interact with the *A. brasilense* Sp7, and the accumulation of the marker occurs over the entire cell surface. It has been found that an increase in the electrical conductivity of a cell suspension during their interaction with specific antibodies is due to the adsorption of antibodies on the cell surface (Guliy et al. 2013). Also it was shown that the interaction of *A. brasilense* Sp7 cells with specific antibodies in the presence of extraneous microflora (*E. coli* BL-Ril cells) leads to a change in the abovementioned recorded dependencies (Fig. 11.2c, d). The results of

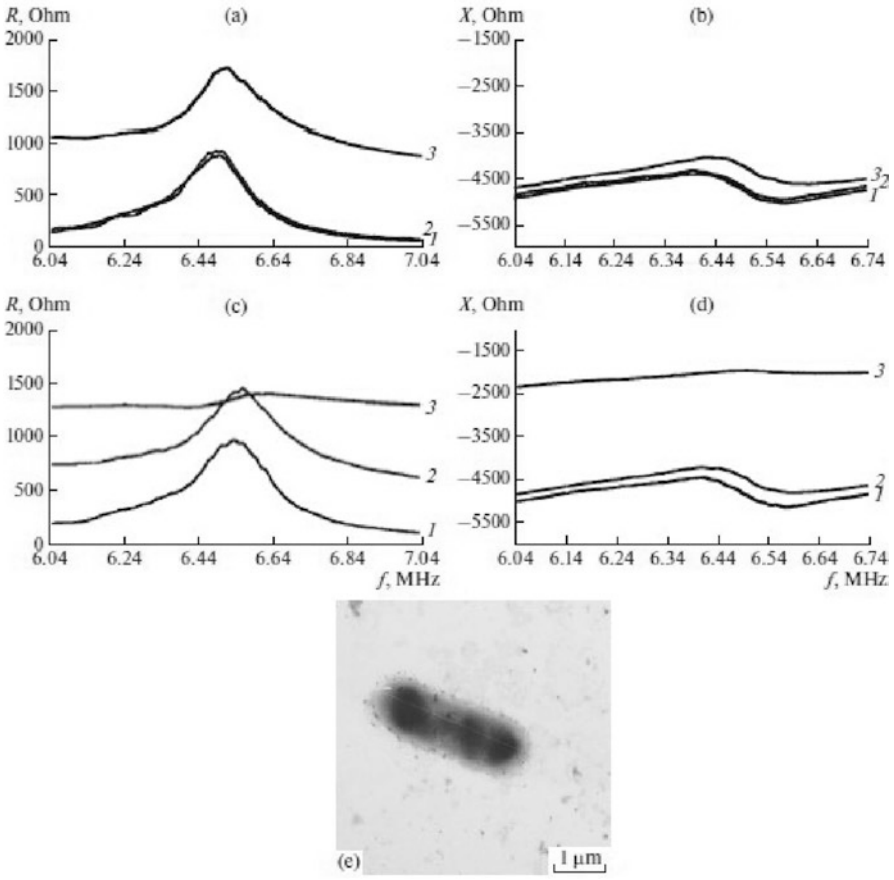

Fig. 11.2 The frequency dependences of the real (**a**) and imaginary (**b**) parts of the electrical impedance of the resonator (the cell contains 10^4 cells/mL) for the interaction between cell suspension of *A. brasilense* Sp7 and specific antibodies. The frequency dependences of the real (**c**) and imaginary (**d**) parts of the electrical impedance of the resonator for the interaction of a mixed cell suspension containing *A. brasilense* Sp7 and *E. coli* BL-Ril and Abs specific to *A. brasilense* Sp7: (1) distilled water; (2) cell suspension without Abs; (3) cell suspension with Abs; (**e**) a TEM image of *A. brasilense* Sp7 cells labeled with the conjugate of protein A and colloidal gold after reaction with the Abs

electroacoustic analysis are in good agreement with the results of electron microscopy.

Therefore, the use of an acoustic method for the detection of microbial cells by registering a specific interaction "bacterial cells–antibodies" has great prospects for analysis of microbial cells with a lower detection limit of ~ 10^4 cells/ml.

11.3.4 Interaction "Cells–Bacteriophages"

Bacteriophages have a certain selectivity of interaction with the cells surface, and they are very accurate indicators that determine the species and type of bacteria. Therefore, they are widely used in medical practice to identify bacteria secreted from the body of the patient and infected objects of the environment (Jung et al. 1999; Summers 2005).

In this regard, the methods of phage indication and phage identification of cells *Bacillus subtilis* in food raw materials and food were developed (Yuan et al. 2012). A method of identifying *Pseudomonas mallei* bacteria of glanders pathogens using *P. pseudomallei* bacteriophages is widely used (Manzeniuk et al. 1994). Method of microbial cell detection based on specific binding to bacteriophages was developed for pathogenic cells such as *Bacillus cereus* and *Clostridium perfringens* (Kretzer et al. 2007) and also other bacteria (Chatterjee et al. 2000; Low et al. 2005; Schmelcher 2008).

Despite the fact that the methods of phage typing of microbial cells are quite specific, simple in formulation, and generally accessible, they require a lot of time (from 48 h to 5 days). Therefore, the development of alternative methods for the detection of bacteria using bacteriophages with a shorter analysis time is very important.

The experiments investigating the possibility of an acoustic sensor for the detection of microbial cells *Escherichia coli* during their interaction with specific bacteriophages were conducted in Guliy et al. (2015). Also as a model system, the cells *A. brasilense* Sp7 for infection by bacteriophage FAb-Sp7 were used in Guliy et al. (2017). The scheme of the experiment was similar to the case of the interaction the microbial cells with specific antibodies (see Fig. 11.1).

As an example Fig. 11.3 shows the dependences of the real part of the electrical impedance for cells *A. brasilense* Sp7, which are infected with the specific bacteriophage FAb-Sp7. The number of cells in the liquid container was 10^6 (a) and 10^4 (b) cells/ml, respectively. As a result, it has been shown that the developed sensor allows distinguishing the cases of infection of bacterial cells by specific bacteriophages from the control experiments without such infection (Guliy et al. 2015, 2017).

To confirm the results of electroacoustic measurements, an electron microscopic study of the interaction of the bacteriophage FAb-Sp7 with microbial cells *A. brasilense* Sp7 was performed (Fig. 11.3c). The presented data shows that bacteriophages are uniformly adsorbed on the entire surface of the bacterial cell. To improve the perception of the obtained data, Fig. 11.3d shows electron microscopic image of the bacteriophage FAb-Sp7.

It was also shown that a significant change in the physical properties of the suspension, leading to a change in the analyzed frequency dependencies of the real and imaginary parts of the electrical impedance, occurred even in the presence of extraneous microflora. This means that the sensor detects the infection of bacterial cells by specific bacteriophages even in the presence of extraneous microflora.

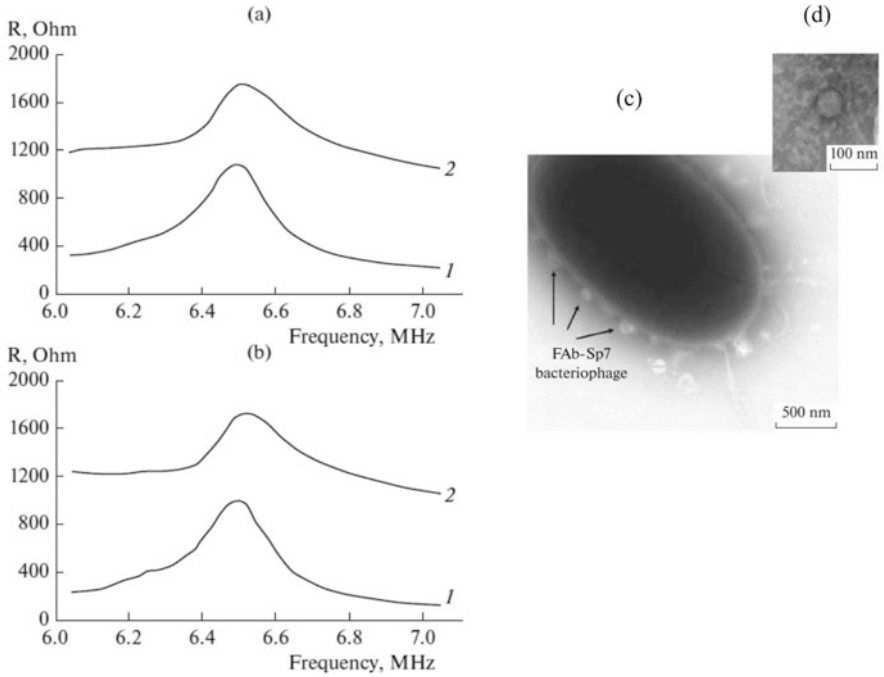

Fig. 11.3 The dependence of the real part of the electrical impedance of the resonator for bacterial suspension of *A. brasilense* Sp7 infected by FAb-Sp7 bacteriophage for the number of cells in the container: 10^6 cells/mL (**a**) and 10^4 cells/mL (**b**). Dependencies (1) and (2) refer to the suspension before and after addition of bacteriophages, respectively. (**c**) A TEM image of *A. brasilense* Sp7 cells at their interaction with the FAb-Sp7 bacteriophage; (**d**) the sidebar contains an image of the FAb-Sp7 bacteriophage

Thus, changes in the parameters of the cell suspensions under the action of bacteriophage occur only if the microbial cells under study are sensitive to bacteriophages. So the electroacoustic sensor allows distinguishing situations when infection of bacterial cells by specific bacteriophages occurs from the control experiments when such an infection is absent. It also allowed determining the spectrum of lytic activity of bacteriophages (Guliy et al. 2015). Based on numerous experiments and statistical data processing, an approximate criterion was developed for the presence of specific interaction of the bacteriophages and cells in the analyzed suspension. It consists in the following: the change in the module of the electrical impedance of the sensor should not be less than ~ 10% when a minimum number of bacteriophages are added to the cell suspension (five phage particles per cell) (Guliy et al. 2015, 2017). The results show the possibility of creating a rapid method for detecting microbial cells when they are infected with a specific bacteriophages and determining their bacteriophage resistance.

11.3.5 Interaction "Cells–Mini-antibodies"

Traditionally, biological components used to identify cells are polyclonal and monoclonal antibodies (Abs), which are widely used in the production of diagnostic test systems. However, Abs, obtained by phage display technology, also can be used to determine microbial cells (Smith and Petrenko 1997; Petrenko 2003, Petrenko and Sorokulova 2004; Williams et al. 2003; Paoli et al. 2004; Nanduri et al. 2007).

We have obtained phage-displayed mini-antibodies (mini-Abs) by phage display technology to whole cells of *A. brasilense* Sp245 and investigated their interaction with cells *A. brasilense* Sp245 with help of this sensor. It has been found that the frequency dependences of the real and imaginary parts of the impedance for the cell suspensions with mini-Abs and without them are very different. It should be noted that during the measurements no changes in the resonator impedance were observed after ~ 10 min, i.e., the impedance change time was fairly short. These measurements recorded the final state of the suspension, since repeated experiments led to the same results (Guliy et al. 2012).

These studies have shown the possibility of cell detection using specific mini-Abs, and the limit of detection is approximately 10^3 cells/ml. The degree of change in the characteristics of the resonator depends on the concentration of cells, which opens up the possibility of carrying out not only qualitative but also quantitative analysis of bacteria.

11.4 Sensors Based on the Use of Inhomogeneous Piezoactive Acoustic Waves

11.4.1 Sensors Using the Active Films with Immobilized Microorganisms

Sensors based on the surface acoustic waves are widely used to measure various physical quantities and chemical composition of objects contacting with the waveguides. In order to develop liquid and biological sensors, basically, Love waves with shear-horizontal polarization, characterized by low attenuation due to the absence of radiation losses, are used (Gaso et al. 2012). The principle of operation of sensors based on surface acoustic waves is that the measured parameter affects the wave propagation velocity, which in turn causes changes in the time interval between the input and output signals or in the phase of the output signal.

In acoustic biosensors, the recognizing reagent is usually a macromolecule immobilized inside a membrane chemically bound to a surface that contacts the solution of the analyte. A specific chemical reaction takes place between the reagent and the substance under study. This can be either direct interaction of the reagent with the analyte, as in the case of an antigen–antibody reaction, or catalytic interaction of the immobilized enzyme with the analyte leading to the formation of an easily identifiable product. Piezoelectric sensors register a mass change of the order of 10–12 ng. Such biosensors contain an enzyme immobilized on a surface of

piezocrystal, an antibody, an antigen, or a DNA (RNA) strand. The presence of a detectable agent entering into an enzymatic immune response or forming a hybrid molecule with an immobilized bioobject is evidenced by the formation of the corresponding complex on the piezocrystal, which changes the wave velocity. With the help of a piezoelectric biosensor with an immobilized antigen, it can be detected antibodies in serum diluted 1000 times.

The first experiments using Love waves for biochemical research were described in Kovacs et al. (1992) and Gizeli et al. (1992). In 1997, acoustic devices based on Love waves were used to study the real-time antigen–antibody interaction in liquid media (Harding et al. 1997).

A two-channel biosensor on the Love wave was developed for simultaneous detection of *Legionella* and *E. coli* cells with the help of antibodies (Howe and Harding 2000). The detection limit for both types of bacteria was 10^6 cells/ml at 3 h analysis time.

An immunosensor on the Love wave for the detection of viruses or bacteria in liquids (in food and process water, beverages, etc.) in real time is also developed and described in Tamarin et al. (2003). The authors used monoclonal antibodies against bacteriophage M13 immobilized on the surface of the waveguide and showed the possibility of determining bacteriophage M13.

In has been shown that a sensor with a Love wave, containing a layer of zinc oxide, is promising for use as immunosensor (Kalantar-Zadeh et al. 2003). The authors successfully controlled the process of adsorption of rat immunoglobulin G.

A sensory platform based on immobilized cells and a sensor using a Love wave for detecting heavy metals in a liquid medium was also successfully used (Gammoudi et al. 2010, 2011). The acoustic delay line was included in the generator feedback circuit, and the generation frequency was recorded in real time. A polydimethylsiloxane chip with a liquid chamber was in contact with a waveguide with a propagating acoustic wave. Bacteria (*E. coli*) were fixed in the form of bioreceptors on a sensitive sensor surface coated with a multilayer polyelectrolyte using a simple and efficient electrostatic self-assembly procedure between layers. The response of the sensor in real time to various concentrations of cadmium and mercury ions was investigated. It turned out that the detection limit was 10–12 mol/l. The analytical signal of the sensor depended on changes in the viscoelastic properties of the fluid associated with changes in the metabolism of bacteria.

A similar principle with immobilized cells was also used to determine ions of heavy metals (Tekaya et al. 2012). In this case, the immobilization of microalgae *Arthrospira platensis* was used as a receptor.

An innovative method of detecting *E. coli* using a sensor with a Love wave and the antibodies immobilized on the surface of the waveguide with the threshold of detection of 10^6 bacteria/ml is described in Moll et al. (2007). The same group of researchers demonstrated a multifunctional immunosensor on the Love wave for the detection of bacteria, viruses, and proteins (Moll et al. 2008). They successfully detected bacteriophages and proteins up to 4 ng/mm^2 and *E. coli* bacteria up to 5×10^5 cells in a volume of 500 µl with good specificity and reproducibility. The authors stated that whole bacteria can be detected no later than 1 h.

In the work of Andrä et al. (2008), a sensor for determining the lipid specificity of human antimicrobial peptides is described. In this case, the membranes were attached to the sensor surface, imitating the cytoplasmic and outer bacterial membranes.

The resonators on the surface acoustic wave were also used as biological sensors. For example, it has been shown that the formation of the biofilm of bacteria due to the interaction with an antibiotic on the surface of a piezocrystal can be controlled with high sensitivity by measuring the resonant frequency of a resonator on a surface acoustic wave (Kim et al. 2016). However, the total time of biofilm growth and its removal was 48 h, during which a change in a temperature could lead to an uncontrolled change in the resonant frequency.

11.4.2 Sensors Based on the Use of Surface and Plate Piezoactive Acoustic Waves Without Immobilized Microorganisms

Another approach is the development of sensors for the analysis of bacteria without the immobilization of microorganisms.

In this regard, a sensor using the Love wave based on the aptamer, which allowed detecting small biomolecules, was developed (Schlensog et al. 2004). This biosensor has an advantage over immunosensors, since it does not require the production of antibodies against toxic substances.

A biosensor based on the Love wave was developed to detect pathogenic spores at the level of infections by inhalation (Branch and Brozik 2004). Monoclonal Abs with a high degree of selectivity were used to determine anthrax spores under water conditions. The authors claim that using the developed method, whole cells can also be detected.

An acoustic sensor for the microbial cell detection using the plate acoustic waves is described in Zaitsev et al. (2001). It represented a two-channel delay line made of a Y-X plate of lithium niobate 0.2 mm thick (Fig. 11.4). Two pairs of interdigital transducers (IDT) for excitation and reception of an acoustic wave with shear-horizontal polarization in each channel were applied by the photolithography method. One of the channels of the delay line was electrically shorted by depositing a layer of aluminum on the surface between the IDTs; the second channel remained electrically free. The liquid container was fixed on the surface of the plate between the IDTs. The volume of the suspension container was ~ 5 ml. The specified sensor was connected to 4-port meter of S parameters 5071C (Agilent Technologies, USA).

With the help of this sensor, an experimental study of a specific interaction of the types “bacterial cells–bacteriophages,” “bacterial cells–antibodies,” and “bacterial cells–mini-Abs” directly in suspension with different initial electrical conductivities was carried out. As an example Fig. 11.5 (a, b) presents the time dependencies of insertion loss of sensor (a) and phase of output signal (b) before and after adding the specific mini-Abs in the cell suspension for electrically open (1) and electrically shorted (2) channels, respectively. Figure 11.6 (a–f) shows the dependencies of the change in insertion loss ($\Delta\alpha$) (a, c, e) and phase of output signal ($\Delta\Phi$) (b, d, f) on

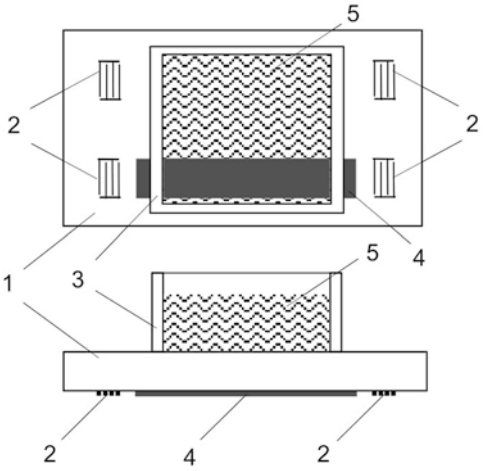

Fig. 11.4 The scheme of acoustical sensor with two channels: 1, plate of Y-X lithium niobate; 2, interdigital transducers; 3, container for liquid; 4, aluminum film; 5, cell suspension

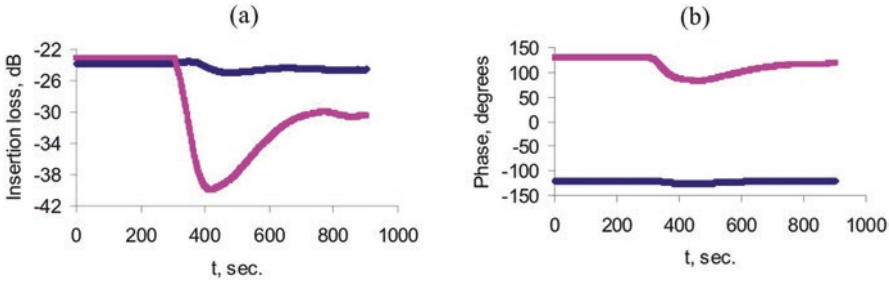

Fig. 11.5 The time dependencies of insertion loss (**a**) and phase of output signal (**b**) before and after adding the specific mini-Abs in cell suspension for electrically open (1) and electrically shorted (2) channels, respectively. Mini-Abs were added at the point of time t = 300 s

the conductivity of the buffer solution (σ) at specific interaction "bacterial cells *A. brasilense* Sp245–mini-Abs" for the various cell concentrations for electrically short and open channels. One can see that these changes for electrically shorted channel are significantly less in comparison with electrically open channel. This confirms the earlier statement that a specific interaction increases the conductivity of the cell suspension. The mini-Abs interaction specificity was controlled by electron microscopic identification of the interaction of *A. brasilense* Sp245 with CG-labeled mini-Abs (Fig. 11.6g). One can see that the labeled mini-Abs interacted with *Azospirilla* and the CG label was distributed throughout the cell surface (Guliy et al. 2018).

The value of the minimum concentration of cells, for which their detection is possible, turned out to be 10^4 cells/ml for each type of the interaction. At that the initial conductivity of the buffer solution was changed in the range 2–50 μS/cm.

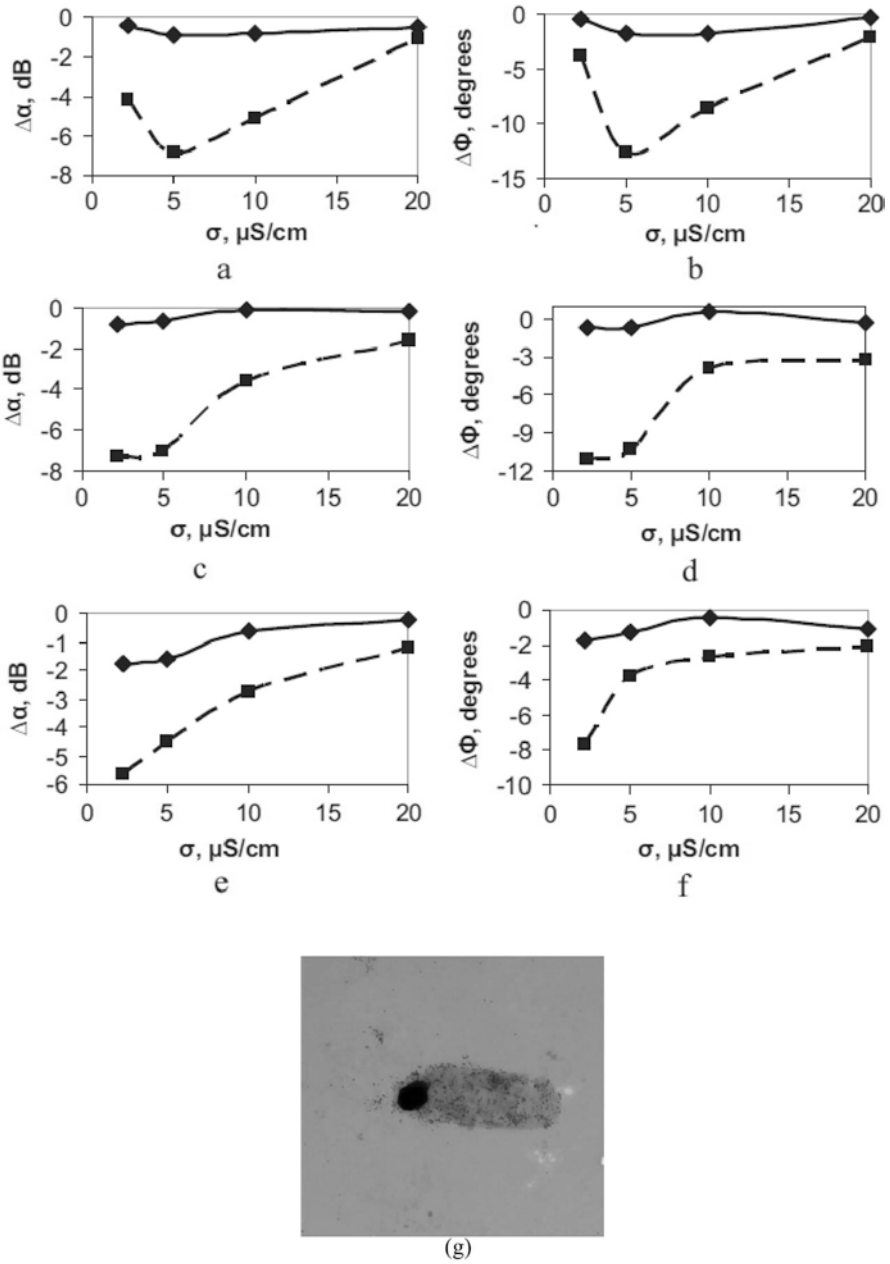

Fig. 11.6 The dependencies of the change in insertion loss (Δα) (**a**, **c**, **e**) and phase of the output signals (ΔΦ) (**b**, **d**, **f**) on the conductivity of the buffer solution (σ) at specific interaction "bacterial cells *A. brasilense* Sp245–mini-Abs" for cell concentrations of 10^4 cells/ml (**a**, **b**), 10^6 cells/ml (**c**, **d**), and 10^8 cells/ml (**e**, **f**). Dotted and solid lines correspond to electrically open and shorted channels, respectively

TEM of bacterial cells *A. brasilense* Sp245 marked by conjugant of antiphage antibodies after interaction with phage antibodies (×10,000) (g)

11.5 Noncontact Sensor Based on a Slot Acoustic Mode

As already noted, over the past few years, acoustic biological sensors with good speed and sensitivity for detecting bacterial cells directly in the liquid phase have been developed. But it should be noted that in most articles describing sensors, there is practically no data on the conditions for cleaning the sensors from the spent biological sample (Turner et al. 1987; Gaso et al. 2012; Chen and Cheng 2017).

However, the known methods of cell detection have a significant drawback associated with the need for thorough cleaning the surface of the piezocrystal after contact with the investigated suspension. Often this requires the use of special methods to remove residues of previous biological samples and control the quality of cleaning, which can lead to damage to the sensor elements and, as a result, distortion of the analytical signal. This problem could be solved using contactless analysis methods, in which the container with the investigated suspension is isolated from the sensor surface. To solve this problem, a contactless biological sensor was developed (Borodina et al. 2018) based on a slot mode in a single-channel delay line with a shear-horizontal acoustic wave of zero order. In accordance with Fig. 11.7, a delay line based on a Y-X piezoelectric plate of lithium niobate (1) with a thickness of 200 μm was used as the main element of the sensor for contactless examination of bacterial cells in conducting suspensions. Two interdigital transducers (2) were applied to the surface of the plate to excite and receive an acoustic wave with a shear-horizontal polarization of zero order at a center frequency of ~ 3.5 MHz. A liquid container (5) with a volume of 1.5 ml was placed above the acoustic delay line between the IDTs. The bottom of the cell was made of a plate of Z-X lithium niobate (4) (Borodina et al. 2013). A fixed gap between the surface of the delay line and the bottom of the liquid container was provided by means of strips of aluminum foil with a thickness of 8 μm. To study the specific interaction of the bacterial cells with the polyclonal antibodies, the sensor was connected to the meter of S-parameters E5071C ("Agilent," USA). This meter operated in the regime of measuring the

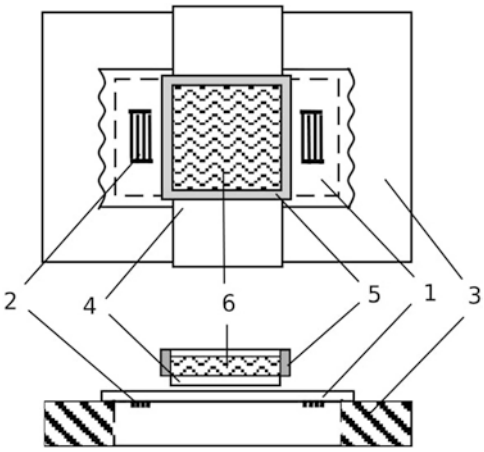

Fig. 11.7 The scheme of the contactless sensor: 1, piezoelectric plate of Y-X $LiNbO_3$; 2, IDTs; 3, the holder of the plexiglass; 4, piezoelectric plate of Z-X $LiNbO_3$; 5, liquid container; 6, suspension of the cells under study

frequency dependence of the insertion loss of the sensor. It has been found (Borodina et al. 2013) that such structure is characterized by the clearly pronounced resonant peaks on the frequency dependence of the insertion loss associated with the excitation of the slot mode.

As an example Fig. 11.8a shows the frequency dependence of the insertion loss of the sensor loaded with a buffer solution with a conductivity of 20 μS/cm (Borodina et al. 2018). Figure 11.8b presents the same dependencies of a sensor loaded by a buffer solution with the cells before (green line) and after (pink line) the addition of antibodies. One can see that addition of antibodies leads to significant change in values of depth of resonant peaks. The control experiments including nonspecific interaction "microbial cells–antibodies" showed the absence of changes in peaks parameters. This sensor showed the possibility of recording the interaction of microbial cells with specific antibodies in suspensions with a conductivity of 5–50 μS/cm and with a minimum cell concentration of 10^4–10^3 cells/ml. A

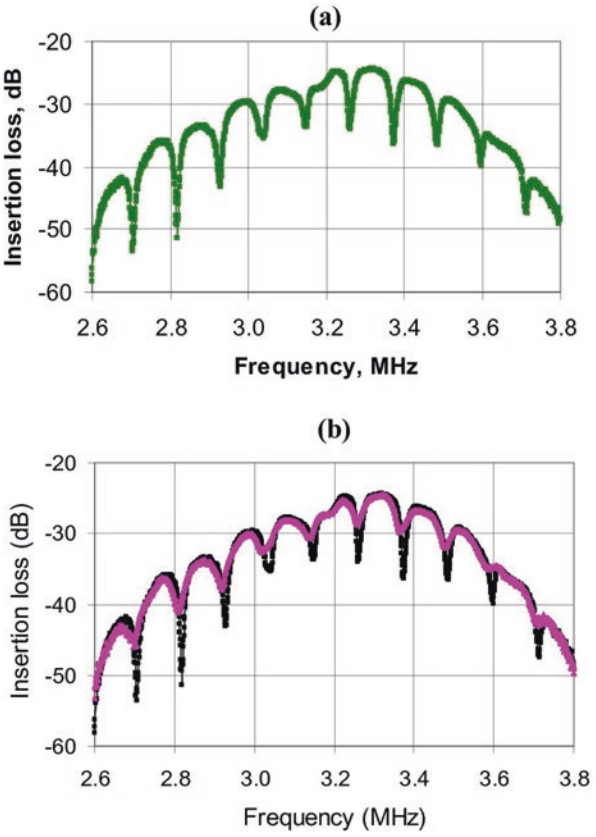

Fig. 11.8 The frequency dependence of the insertion loss of the sensor loaded by the buffer solution with the conductivity of 20 μS/cm without (**a**) and (**b**) with the cells *A. lipoferum* Sp59b before (black line) and after the addition of the specific Abs (pink line)

distinctive feature of the sensor is the lack of contact of the investigated suspension with a thin (200 μm) acoustic waveguide. An additional advantage of the sensor is the presence of a removable liquid container, which allows to reuse it and facilitates the process of cleaning the container from the sample.

11.6 Conclusion

The capabilities of electroacoustic biological sensors based on resonators with a longitudinal and lateral electric field, based on delay lines with a propagating piezoactive acoustic wave, and based on a delay line with a slot mode are demonstrated. These sensors can conduct cell detection and identification of bacteria using immobilized microorganisms or directly in cell suspension. The sensitivity range of microbial cell detection is 10^3–10^8 cells/ml and for suspension with the conductivity of 5–50 μS/cm. At that the analysis time varies from 5 min to several hours. Obtaining reliable information is carried out by registering and processing changes in the analytical signal of the sensor when specific agents are introduced into the cell suspension. The presented possibilities of electroacoustic biological sensors for the detection of bacteria are focused on the clinical use of *onsite* as a personalized diagnostic device. The possibility of rapid detection of microflora will allow timely diagnosis of the disease and timely medical assistance. An important feature of acoustic biological sensors is the detection of any biochemical and/or biophysical signal associated with a particular microorganism. Further studies in the development of acoustic biological sensors are aimed at adapting the method for detection of microbial cells of different taxonomic groups, optimizing the measurement procedure and developing criteria for specific binding to the selective agent in real conditions.

In general, acoustic biological sensors form a wide class of detection systems and are very promising for use in microbiology, medicine, and veterinary medicine for solving the problems of detection and identification of bacteria.

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