Chapter 3

Whole cell biosensors

César A. Hernández, Johann F. Osma

CMUA. Department of Electrical and Electronics Engineering. Universidad de los Andes, Colombia.

ca.hernandez11@uniandes.edu.co

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1. Introduction

The term biosensors can be used in a very broad sense for a large set of devices that use biologically active components, conjugated with appropriate transducers, which bear the detection of sought substances (Thévenot, Toth, Durs & Wilson, 2001). A recurrent example when referring to biosensors is the coal-mines canary, often used to depict the early use of living species to provide environmental information of potential hazards. Such example has been expedient to spot the advantages of higher organisms on the detection of menaces, reports (Van der Schalie, Gardner, Bantle, De Rosa, Finch, Reif et al., 1999; Rabinowitz, Gordon, Chudnov, Wilcox, Odofin, Liu et al., 2006; Gubernot, Boyer & Moses, 2008; Rabinowitz, Scotch & Conti, 2009) render an extensive guide on case-study and case of application on animals that can be used as biological markers for early warning systems, in Table 1, a compiled list of examples using animals as sentinels is provided. Physiological and behavioral responses as exhibited by aquatic organisms are a benchmark of this postulate (Van der Schalie, 1977; Morgan & Young, 1984; Kramer, 2009): Architectures using fishes (Morgan & Eagleson, 1983), mussels (Butterworth, Gunatilaka & Gonsebatt, 2001) and invertebrates (Lechelt, Blohm, Kirschneit, Pfeiffer, & Gresens, 2000) are described as reliable markers for commercially available systems (Fish Toximeter- Detection of Toxic Substances in the Water - Bbe Moldaenke, 2013; Musselmonitor (Mosselmonitor): a Biological Early Warning System, 2013).

Sentinel Incidents					
Species	**Toxicant**	**Country**	**Date**	**Related events**	**References†**
Canaries	Carbon monoxide	England	1870s		(Burrell & Seibert; 1916; Schwabe, 1984)
Cattle	Smog	England			(Veterinarian, 1874a, 1874b)
Cattle	Fluoride	England	1910s		
Horses	Lead	United States			
Cattle	TCE	Scotland			(Haring & Meyer, 1915; Stockman, 1916; Holm, Wheat, Rhode & Firch, 1953; Medtronics Associates, 1970)
Cats	Mercury	Japan	1950s		(Kurland, Faro & Siedler, 1960)
Birds	DDT	United States			
Cattle	Smog	England			
Chickens	PCBs	Japan	1960s	Silent Spring published (1962)	(Carson, 1962; Kuratsune, Yoshimura, Matsuzaka &. Yamaguchi, 1972)
Sheep	OP agents	United States			(Van Kampen, James, Rasmussen, Huffaker & Fawcett, 1969)

Sentinel Incidents					
Species	**Toxicant**	**Country**	**Date**	**Related events**	**References†**
Horses and other animals	Dioxin	United States	1970s	NCR Symposium on Pathobiology of Environmental Pollutans: Animal Models and Wildlife Monitors (1979)	(Case & Coffman, 1973; Carter, Kimbrough, Liddle, Cline, Zack, Barthel et al., 1975; Northeastern Research for Wildlife Diseases, Registry of Comparative Pathology & Institute of Laboratory Animal Resources (U.S.), 1979)
Dairy cattle	PBBs	United States			(Jackson & Halbert, 1974; Welborn, Allen, Byker, DeGrow, Hertel, Noordhoek et al.,1975)
Sheep	Zinc	Peru	1980s	Task Force on Environmental Cancer and Heart and Lung Disease established (1981) NAS risk assessment paradigm (1983) NCI report, Use of Small Fish Species in Carcinogenicity Testing (1984)	National Research Council, 1983)
Aligators	DDT, dicofol	United States	1990s	NRC report, Animals as Sentinels of Environmental Health Hazards (1991) Public Law 103-43 enacted (1993) NIEHS establishes ad hoc ICCVAM (1993) ICCVAM report, Validation and Regulatory Acceptance of Toxicological Methods (1997)	(National Research Council, 1991; U.S. Congress 1993; Jr & Gross, 1994)
Fish	*Pfiesteria* toxins	United States			(Interagency Coordinating Committee on the validation of alternative Methods, 1997)

Abbreviations: TCE, tetrachloroethylene; PCBs, polychlorinated biphenyls; OP, organophosphate; NCR, National Research Council; PBBs, polybrominated biphenyls; NAS, National Academy of Sciences; NCI, National Cancer Institute; NIEHS, National Institute of Environmental Sciences; ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods

† All references as cited by (Van der Schalie et al., 1999)

Table 1. Timeline of examples of animals as sentinels of environmental toxicants and noteworthy events, extracted from (Van der Schalie et al., 1999)

The thermodynamic reaction path provides an advantageous guideline to allocate two well defined strategies, namely: Stimuli-Response-Based (SRB) and Biotransformation-Based (BtB) strategies, which respond to the equation posed by Willard Gibbs for describing the free energy of a reaction (Gibbs, 1873). Additionally, the proposed strategies acknowledge the signal reading as direct (SRB) and indirect (BtB) transduction methods. Some advantages and disadvantages of using both strategies are listed in Table 2.

	SRB Strategy	BtB Strategy
Advantages	• Direct assessment of electrical measures • Faster response • Less complex measurement mechanism • No need for additional reagents/elements for measuring • Lower probability for occurrence of side-effect reactions • Swifter usage for the assembled device	• Yield a ready amplified signal • No need for immobilization of cells • Wide variety of response signals • Some cases don't need to use measurement equipment
Disadvantages	• Low level signals • High noise to signal ratio (NSR) • Need for an output signal external amplifier for portable applications • Need for immobilization and precise biomass control • Need for measuring devices to read the output signal	• Indirect measurement • The output signal is mediated by complementary reactions • Response dependent on the reaction and metabolic pathway time • Usual need for additional reagents/elements for measuring • Higher probability for occurrence of side-effect reactions

Table 2. Advantages and disadvantages of the SRB and BtB strategies

Some of the more relevant issues of tissue-based biosensors are described in (Arnold & Rechnitz, 1980; Rechnitz & Ho, 1990; Wijesuriya & Rechnitz, 1993; Safronova, Khichenko & Shtark, 1995; Rudolph & Reasor, 2001), a classification of mammalian cells is presented in Table 3 according to their function and mechanism of biosensing, they are set as an example of the multiple nature of tissue used for biosensing purposes, multiple mechanisms are used in order to harvest information derived from tissues, namely transduction methods, ranging from measurements that rely on electric, physiological, metabolic, optical and genetic changes (Arnold, 1986; Hansen, Wittekindt & Sherry, 2009; Acha, Andrews, Huang, Sardar & Hornsby, 2010; Belkin & Gu, 2010).

A ready-witted approach to biosensing, has been made by the isolation and utilization of biocatalysts to attain a variety of reactions that allow the addressing of different substrates (Milner & Maguire, 2012), which pose particular relevance for health, environment and industrial purposes. Among the used biocatalysts in biosensors, enzymes, which expedite and manage chemical reactions, to ensure the bearing and survival of whole-cells, have drawn the attention of academic and industrial communities mainly due to their high specificity, portability, miniaturization capacity, ease of in-situ utilization and fast response (Wilson & Hu, 2000; Newman & Setford, 2006).

The question that arises is then: Why is it appealing to deepen into whole-cell biosensors? Consider the whole-cell as a natural factory of biocatalysts: usually, the method for acquiring isolated biocatalysts, like the aforesaid enzymes, requires a process of separation and purification from the raw whole-cell strain or tissue, which is time and resource consuming. Furthermore, the ensuing enzyme sometimes requires cofactors and coenzymes, to carry out a complete reaction or to recognize a substrate; such are paired by adding them to the purified enzyme or by combining a supplementary enzyme, which implies a recycling process to attain the required cofactor, then eliciting the need for further separation and purification steps.

Function/ mechanism at cellular levels	Cell types	Primary signals derived from cells	Device/method used for secondary transduction of signals	Application areas	Strategy	References
Excitable/ electrogenic	Neuron, cardiac cells, neuronal network	pH, flow of ions	LAPS, microelectrodes	Drug discovery and testing (dose response) Toxicology Pharmacology	SRB	(Pancrazio, Gray, Shubin, Kulagina, Cuttinom, Shaffer et al., 2003; Liu, Cai, Xu, Xiao, Yang & Wang, 2007; Parviz & Gross, 2007)
Electrical responses	Epithelial cells, cardiomyocytes, neurons	Electric current, flow of ions	ECIS, Impedance, IDES, electrophysiology and electric potential, BERA	Bio-assays Drug discovery and testing (dose response) Toxicology	SRB	(Giaever & Keese, 1993; Gilchrist, Giovangrandi, Whittington & Kovacs, 2005; Asphahani & Zhang, 2007; Kloss, Fischer, Rothermel, Simon & Robitzki, 2008; Slaughter & Hobson, 2009)
Cellular receptors	Epithelial cells, hepatocytes, stem cells, mast cells, mononuclear cells, T- or B-cells	pH, alteration of molecules within cells	Cell-signaling molecules, LAPS, optical methods, DPSCA	Pathogen and toxin testing Combinatorial chemistry Bio-assays Toxicology Environmental monitoring Biosecurity	SRB/ BtB	(Kamei, Haruyama, Mie, Yanagida, Aizawa & Kobatake, 2003; Rider, Petrovick, Nargi, Harper, Schwoebel, Mathews et al. 2003; Trask, Baker, Williams, Nickischer, Kandasamy, Laethem et al., 2006; Curtis, Naal, Batt, Tabb & Holowka, 2008)
Cellular metabolism	Epithelial cells, hepatocytes, stem cells	pH, ion channel, molecular flux	pH-sensitive ISFETs, LAPS, ion-sensors	Drug discovery and testing (dose response) Toxicology Bio-assays	SRB/ BtB	(Xu, Ye, Qin, Xu, Li, Li et al., 2005; Ceriotti, Kob, Drechsler, Ponti, Thedinga, Colpo et al., 2007; Liu et al., 2007)
Cytotoxicity	Epithelial cells, endothelial cells, macrophages, myeloma, mononuclear cells, T- or B-cells	Changes in membrane integrity, cellular morphology	Optical methods, potentiometric methods	Pathogen and toxin testing Bio-assays Toxicology Environmental monitoring Biosecurity	BtB	(Banerjee, Lenz, Robinson, Rickus & Bhunia, 2008; Lee, Kumar, Sukumaran, Hogg, Clark & Dordick, 2008; Tong, Shi, Xiao, Liao, Zheng, Shen et al., 2009)
Genomic responses	Epithelial cells, endothelial cells, macrophages, myeloma cells, T- or B-cells	Changes in gene expression	Reporter gene assay, optical methods, cytometry	Bio-assays Drug discovery and testing (dose response) Toxicology Environmental monitoring	BtB	(Haruyama, 2006; Trask et al., 2006; East, Mauchline & Poole, 2008)

BERA, bioelectric recognition assay; DPSCA, double potential step chronoamperometry; ECIS, electric cell-substrate impedance sensing; IDES, interdigitated electrode structures; ISFET, ion-sensitive field effect transistor; LAPS, light-addressable potentiometric sensor

Table 3. Classification of cell-based biosensors based on the function and mechanism of action of the biosensing, modified from (Banerjee & Bhunia, 2009; Fleming, 2010)

Whole-cells contain a complete metabolic aggregate of enzymes, cofactors and coenzymes, constituting a well suited mechanism to assure chemical reactions that are fundamental for their function, in addition they self-regulate the recycling process for such substances; analog processes can be found in tissues, but the requirements related to maintenance and cost for culturing microorganisms are below from those of tissue cultures.

By following the route of different metabolic paths, where one or more enzymes are involved, whole-cells can yield a series of reactions that can be readily detected: One advantage on the usage of whole-cells is that very complex reactions can be attained by harnessing the presence of multiple enzymes in one single step. The resulting processes derived from the considered enzymatic activities, such as physiological responses – to mention motility, growth, respiration, digestion, among others – reveal prospects in which the advantageous properties of whole-cells are profited.

The selection of a relevant whole-cell for a biosensing application, primarily, would obey to the characteristics rendered by the selected strain on an observed environment, to set an example: Strains that grow on harsh environments yield to metabolic activities that require specific compounds to be performed and are copiously available on the targeted environment. The previous example is not a restriction, innate selectivity of whole-cells is not limited to strains that are harvested under extreme conditions, strains cultured in a friendlier environment can respond to very specific stimuli, such condition allows the screening of different strains as prospect candidates for a desired biosensing application.

In addition, whole-cells endure the modification and inclusion of regulatory mechanisms, such can be detached from other organisms or being synthetically tailored in a laboratory (Siegfried, 2011; Thomas, 2013). It is possible to alter genetic configuration of whole cells thus changing the enzymatic expression of a given strain, conveying the possibility to react upon different substrates or to include responses that can be easily monitored, the induced response is mediated for what is known as a bioreporter gene (Daunert, Barrett, Feliciano, Shetty, Shrestha & Smith-Spencer, 2000; Leveau & Lindow, 2002; Belkin, 2003; Jansson, 2003; Sørensen, Burmølle & Hansen, 2006; Yagi, 2007; Salis, Tamsir & Voigt, 2009; van der Meer & Belkin, 2010).

Numerous reviews have been made to present the advances, methods and characteristics of whole-cell biosensors (D'Souza, 2001; Farré, Pasini, Carmen Alonso, Castillo & Barceló, 2001; Harms, Wells & van der Meer, 2006; Tecon & van der Meer, 2008; Reshetilov, Iliasov & Reshetilova, 2010; van der Meer, 2011; Su, Jia, Hou & Lei, 2011; Shimomura-shimizu & Karube, 2010); the following pages present a general approach to the fundamentals and applications of whole-cell biosensors, the first section refers to the principles on whole-cell sensing, it deepens into the concept of enzymatic catalysis induced in living cells and divides the sensing strategies in two: Stimuli-Response-Based whole-cell biosensors and Biotransformation-Based whole-cell biosensors. A similar division was proposed in (Aston & Turner, 1984), referred as direct and indirect systems, analogue to SRB and BtB strategies respectively, such division is not explained on the basis of thermodynamic properties, but the use of a secondary transduction method. This section is directed to the non-biology-related readers, and might be only a quick reference for biologists.

The consecutive section deals with the relationship in between whole-cell transduction and chemical transduction, benefiting from the proposed classification of the latter section, it serves as a bridge between disciplines, it is here explained the parameters to be aware of in both interactions. It settles the differences from electrochemical effects in whole-cells (amperometric,

potentiometric, conductometric and impedance -sensors) and the physiological effects, either due to respirometry, external stimuli or bioreporters associated results.

The last section presents the current advances and the market-available options using whole cell biosensors, the chapter is closed with the discussion of the future treads and challenges that they offer to be developed.

2. General principles on whole-cell biosensors

Whole-cells possess the ability to conduct important changes on different substrates through very well defined succession of reactions, such transformations are profited as energy or as essential elements for vital processes of the cell. A single strain can interact with different substrates at the same time, each of which is guided on every instance by a very specific series of chemical reactions, which compose a metabolic pathway.

Enzymes are the mediators that promote the occurrence of such chemical reactions; they are set to complete a chain of activities that secure that the whole-cells accomplish metabolic and physiological responses for assuring survival. An enzyme-catalyzed reaction would be a naturally occurring event which presents a modified energetic route.

The enzyme production is concurrently regulated by the genetic code of the whole-cell, which is in ensemble a unique imprint for every different strain. The integrity of the genetic information is kept in what can be compared to a "storage unit" i.e. the DNA, the information set itself is referred as the genome. While the genome can vary from subject to subject, the basic function structure remains the same, which ultimately will indicate the identity of a given strain. The sequential arrangement of the resulting aminoacid constitutes the enzymatic structure as a complete programming code (Siegfried, 2011).

The very specific 3D structures of enzymes, formed by the folding and coiling of aminoacids, form fragments that act as pockets disposed for the coupling of the substrate, such are known as active sites, they are endowed with unique geometric shape and an atomic disposition complementary to those of the substrate.

A general approach for substrate-enzyme binding mechanisms can be envisioned through the "lock-and-key theory" (Koshland, 1995): In 1894 Emil Fischer proposed that enzymes were rigid structures, they would act as a lock with a specific shape; the substrate, on the other hand, resembles a key: If a properly shaped active site is available then it will serve as an adequate key hole, thus, it would "unlock" the consequent reaction (Figure 1).

In 1958 David Koshland offered an alternative to the Fischer's postulate: He stated that enzymes are flexible rather than rigid, hence the active site is keenly changing its shape to adapt to the substrate (Figure 1), the reaction would only take place if there are certain number of chemical bonds constructed between the active site and the substrate and are aligned to the catalytic groups, substrates can bind through e.g. van der Waals forces, ionic-, hydrogen- and covalent bonds; these interactions are generally weak, but with many of these interactions taking place at the same time, it constructs a solid binding. Koshland's postulate is recognized as the “induced-fit theory” (Koshland, 1995).

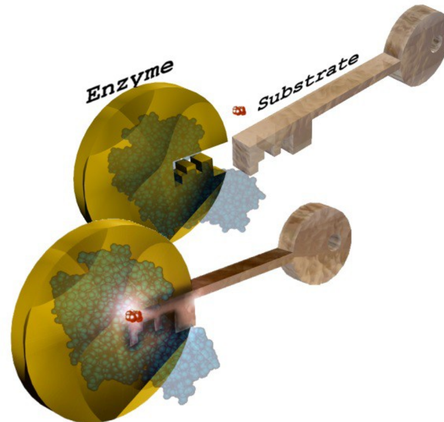

Figure 1. Representation of the lock and key theory: Only equally shaped substrates will bind to specific enzyme, circle represents the enzyme while the key represents the substrate. A comparison with the induced-fit is made, in blue, a 3D representation of an enzyme with flexible structure, the shape adapts to the substrate, depicted in red, though the active site. When the substrate is bonded the catalytic reaction takes place

The distinctive binding mechanism not only encloses the necessary conditions to understand selectivity, the core of the enzymatic process relies on the chemical reaction rate acceleration. As it is inferred from the induced-fit theory both mechanisms are dependent on the progression of molecular interactions with the substrate: While the initial enzyme-substrate interaction is feeble, the increasing amount of bindings formed between the active site and the substrate, which is only possible when an appropriate substrate is present, would prompt structural changes on it until the substrate is firmly attached to the enzyme, analogous mechanism can arrange multiple substrates, coenzymes and cofactors. The correct alignment might induce any of four acceleration mechanisms, to wit: Approximation of the reactants, covalent catalysis, general acid-base catalysis or the introduction of distortion or strain in the substrate (Jencks, 1987).

A reaction can be understood in terms of the thermodynamic properties of the system, relying on the first and second laws of thermodynamics, these are conservation of energy and increasing entropy: Willard Gibbs proposed in 1873 a model, analogous to the potential energy in classical mechanics, considering that a given system would have a thermodynamic potential to generate work under constant conditions of volume and temperature in a closed system, namely Gibbs free energy (denoted as G), originally denominated as available energy (Gibbs, 1873; Newman & Thomas-Alyea, 2012). The maximum amount of free energy derived in any chemical reaction is defined from the difference between the free energy of the products and the free energy of the reactants (ΔG), when the reaction consumes energy i.e. the free energy of the product is greater than the free energy of the reactants, it is said to be an endergonic reaction, that is, it requires an external source of energy e.g. heat. When the reaction releases energy it is said to be exergonic; it is thus considered a thermodynamically favorable reaction, meaning that it can spontaneously occur. Then, when an exergonic (spontaneous) reaction takes place, the entropy (S) will increase if no energy is provided to the system ($\Delta S>0$). On the other hand, if no

change in entropy is considered, the release of energy can only be attributed to the internal energy of the system, property known as enthalpy (H), as it is usually measured as heat, the difference between the initial and the resulting reaction state (ΔH) is known as the heat of a reaction. Accordingly, when the system's resulting process releases energy, it is known as exothermic (ΔH<0), on the contrary case it is called endothermic (ΔH>0); it is important to stress that not every exergonic reaction is necessarily exothermic i.e. the energy is not always released as heat, thus a reaction with ΔG<0 may have ΔH<0, ΔH=0 or ΔH>0. These characteristics were properly explained in Gibbs fundamental equation as follows:

$$\Delta G = \Delta H - T\,\Delta S \tag{1}$$

Gibbs equation represents a state function, which means that it solely depends on the equilibrium state of the system, indistinctly of how the system got to that state. However, catalytic mechanisms move in a tighter boundary of the chemical reaction process, they will not influence the initial nor the final state of the reaction, but will influence in the path leading from one to the other: The enzymatic process diminishes the required free energy for the reaction to take place (Figure 2). The occurrence of spontaneous chemical reactions is restricted by the influence of an energetic barrier, appointed as activation energy; in order for any reaction to take place, there must be enough energy provided to the system to outperform it into a transition state – in a non catalyzed reaction, this can be done e.g. by heating up the system –, this critical instant represents a state where the reactants and the products are simultaneously existent, due to the concurrence of bonds both from the reactant state and the product state, such molecular form is highly unstable, hence related to a large amount of free energy. The enzyme-catalyzed reaction will present an alternative path for the reaction to take place (Jencks, 1987; Copeland, 2004).

By understanding the catalytic process as an energy state transformation, it results easier to elucidate an expected outcome by analytical means without much regard on the details on a specific metabolic pathway. Lets consider again the Gibbs fundamental free energy equation under constant temperature conditions, as given in Equation 1. Consider as well an exergonic reaction, thus no energy is provided to the system, nor by external means neither as a result of a previous enzymatic reaction on the metabolic pathway: For the simplest case we can suppose that no energy was released from the system, the only possible outcome would be an increase on the entropy of the system. When the substrate is composed from molecules constructed by different chemical elements, a dissociation of the elements can be expected, in some cases at least one of the molecules can be readily detected through a known chemical-sensing method. If such is the case, it can be said that the sensing strategy would not be targeted to the substrate, but to a byproduct or a related reaction, this case will be referred as a Biotransformation-Based whole-cell sensing strategy (BtB Strategy) – A special situation will be considered when the result of such biotransformation yield a readily detection signal, such as bioluminescence, often addressed as bioreporter (Leveau & Lindow, 2002; Jansson, 2003; van der Meer & Belkin, 2010).

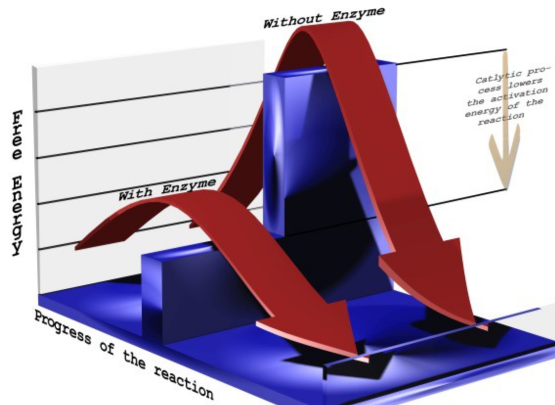

Figure 2. Free energy diagram through the progress of a reaction comparing a catalyzed reaction against a non-catalyzed reaction. The enzymatic catalyst would lower the activation energy, increasing the rate where a normally "spontaneous" reaction would take place

An additional case can be elucidated when no entropy change is considered, hence, the only effect on the resulting free energy equation would be induced by a change of enthalpy, if the initial conditions agree on those previously proposed, the reaction would be exothermic, the outcome is then a release of energy which can be measured by suitable methods. This approach will be labeled as a Stimuli-Response-Based whole-cell strategy (SRB Strategy).

The actual scenarios of the enzyme-catalyzed reaction are much more complicated than the above considered, the metabolic pathway sets a complex network of reactions that can be interrelated with various pathways, some of them active all-through the cell vital routines, other related to the specific social behavior of different strains, in cases for both intra- and inter-species responses (Waters & Bassler, 2005; Shank & Kolter, 2009). Such scenarios combine different characteristics on the entropic and enthalpic properties of the reaction, and the endergonic-exergonic nature of the reaction, nonetheless such combinations would lead to a similar analysis, favoring either the expression of byproducts, according to the BtB strategy, or by energetic measurable changes approached by the SRB strategy.

3. Whole-cell sensing methods, transduction

In the previous section it was introduced two biosensing strategies based on the specific contributory thermodynamic effects of each term, i.e. Stimuli-response-based strategy (SRB strategy), grounded on the concept of enthalpy (energy related response), and Biotransformation-based strategy (BtB strategy), linked to entropy related intake (molecular related response). Transduction is referred, in this section, as the process in which the presence of a given substrate triggers a suitable reaction to an information translated measurable unit.

The concept in this section is closer to that posed by the analytical procedure of the physical context, for both SRB and BtB strategies; the scope approached refers to the interfaced-measurable signal, anyway, few insights on the physics and thermodynamic background of some concepts are given, which are usually neglected in literature.

3.1. Stimuli-Response-Based strategy (SRB strategy)

The SRB strategy is assumed under the energy release/intake principle, the main scenario, presented at the introduction, would be the case of an exothermic reaction. Given this first glimpse, such strategy might be conceived of being based on the whole-cell heat production as a responsive event when interacting with a substrate, yet, even though the concept encloses direct reference to the thermal properties of the reaction, the release/intake of energy is not necessarily of thermal nature, effects on the electric domain are also to be considered. The reactions driven by the whole-cell enzymatic activity, for the consideration of an SRB strategy, rely upon the ionic effects, as are ionic transport phenomena (Borkholder, 1998; Ikeda & Kano, 2001), which provoke alterations over an adequate material, namely electrode, readily measurable on the electric domain.

The most general reaction in which biological systems are involved is known as reduction-oxidation (redox) reaction. As it deploys, reactants undergo an electron transfer process, which produces fractional quantities of Gibbs free energy (Marcus, 1956a; Marcus, 1956b), that are released in the form of heat; the breaking, formation and reconfiguration of atomic bonds contribute likewise to the production of heat, both effects reflect the metabolic activity of a given strain when interacting with specific substrates. Thermal changes respond directly to these chemical reactions (Newman & Thomas-Alyea, 2012), the energy released in such reactions is easily profited by the usage of temperature detection methods, applicable to biological interactions through microcalorimetric processes, early described by Max Rubner (Rubner, 1911), assessing the measure of heat flow of a biological process, which develops proportionally as chemical interactions take place (Braissant, Wirz, Göpfert & Daniels, 2010).

Akin interaction occurs on the boundaries of a whole-cell-electrode interface; the electrode serves as a suitable electron donor/acceptor for the redox reaction deployed on the presence of the substrate, the released ionic clusters move from a higher concentration region to a lower concentration region, a diffusion transport effect due to the concentration gradient (Newman & Thomas-Alyea, 2012); the cell potential was first explained by Nernst, who related such potential to the Gibbs free energy state of the reactants. (Nernst & Barr, 1926). For every chemical reaction, there is a free energy dependent change (Ulstrup & Jortner, 1975); the alterations induced on the electrode modify its electric structure and generate readily measurable electrical units, typically, such reaction achieves either a difference in current (amperometric), a potential or charge accumulation (potentiometric), alters the conductive properties between surfaces (conductometric), produces changes on the impedance (impedimetric) or potentiometric changes on a gate electrode (field-effect) (Ikeda & Kano, 2001; Thévenot et al., 2001; Grieshaber, MacKenzie, Vörös & Reimhult, 2008). An additional interaction for the considered reaction is posed by microbial fuel cells (MFC) biosensors (Aston & Turner, 1984; Stein, Keesman, Hamelers & van Straten, 2011), the resulting ions that are needed by the product of the whole-cell enzymatic reactions or that are detached from them can be exchanged through the whole-cell membrane, the ionic concentration gradient produces an electromotive force (EMF) measurable as an electric energy difference. However, by reason of the non-conductive nature of the membrane, not every cell posses the ability to directly transfer the ion cluster towards the electrode; a membrane is commonly used that would act as a mediator to selectively transfer electrons/protons to the electrode, yet, some electrochemically active strains (Chang, Moon & Bretschger, 2006), as *Aeromonas hydrophilia* (Pham, Jung, Phung, Lee, Chang, Kim et al., 2003), *Clostridium butyricom* (Park, Kim, Kim, Kim, Kim, Kim et al., 2001), *Desulfoblus propionicus* (Holmes, Bond & Lovley, 2004), *Enterococcus gallinarum* (Kim, Hyun, Chang, Kim, Park, Kim et

al., 2005), *Geobacter sulfurreducens* (Bond & Lovley, 2003), *Rhodofoferax ferrireducens* (Chaudhuri & Lovley, 2003) and *Shewanella putrefaciens* (Kim, Park, Hyun, Chang, Kim & Kim, 2002), would be able to provide a mediator less MFC.

As the reaction is driven by the transformation of the substrate, the resultant electric gradient is proportional to the substrate's concentration; in consequence, the output signal is a quantitative indicator dependent on the amount of the specific substrate interacting with the whole-cell surface (Aston & Turner, 1984). Under the present construct, the detection proposed for the use of the SRB strategy is only mediated by the utilization of an electrode, directly interfaced with the whole-cell or mediated through an electron/proton exchange membrane.

For any of the exposed cases, the SRB strategy is considered when the interaction with a given substrate generates a measurable change over an electrode interfacing the whole-cell, these methods usually require the immobilization of the whole-cell on the surface of the electrodes, techniques for cell entrapment as polyvinyl alcohol (PVA) immobilization (Rouillon,, Tocabens & Carpentier, 1999), hydrogel immobilization (Gäberlein, Spener & Zaborosch, 2000), immobilization crosslinking method (Babu, Patra, Karanth, Kumar & Thakur, 2007), physical confinement (Hernandez, Gaviria, Segura & Osma, 2013), among others.

There are two characteristics shared by the methods arranged under this strategy, the prevalence of the contribution of the enthalpy of the system on the overall reaction and the direct usage of electrodes to assess a proportional electrical measure. The consideration to use an SRB strategy with a suitable whole-cell strain is advantageous when a one reaction one response measures is required as a non mediated direct response, which is translated in faster acquisition of the measure, less complex systems for data transduction and lesser probability of side effect-reactions to add noise to the measure.

3.2. Biotransformation based strategy (BtB strategy)

BtB strategy is considered, under the present scope, as a set of byproduct mediated-sensing methods. Many reaction results can be contemplated strictly within the chemical realm of the dissociation and consumption of different compounds. The transformation occurred within the whole-cell can be readily detected by different methods established for precise substances and not by the energy produced directly within the whole-cell. Some examples of the used electrodes are listed in Table 4.

The conformation of chemical bonds, from the perspective of the molecular interaction, derived from specific metabolic pathways, serves as a further option for targeting a substrate. The processes delivered by the whole-cell might be only possible if complemented with a different substance, e.g. aerobic organisms depend on the consumption of oxygen to complete the processes within their complete metabolic network, the amount of oxygen on a contained environment can be used as a measure unit of the whole-cell activity. Further applications related to the produced substances due to whole-cell respiration products and effects, such as the detection on pressure change and CO_2 (Lei, Chen & Mulchandani, 2006), other products due to the separation of compounds that can be easily assessed can be profited.

Transducer	Species detected
Amperometric electrodes	O_2, H_2O, NADH, I_2
Ion-selective electrodes	H^+,NH_4^+, NH_3, CO_2, I^-, CN^-
Field-effect transistors	H^+, H_2, NH_3
Photomultiplier (in conjunction with fiber optics)	Light emission or chemiluminescence
Photodiode (in conjunction with a light-emitting diode)	Light absorption
Piezoelectric crystal	Mass adsorbed

Table 4. Some transducers used under the scope of the BtB strategy (indirect electrodes), table extracted from (Aston & Turner, 1984)

The transduction for a BtB strategy would require further steps beyond the strain-specific reactions; the biological task is limited to the production of intermediate agents to unleash further chemical reactions that could be translated e.g. into electrical signals, chemical analogous to the methods presented for the transduction on SRB strategy. The recognition and quantification of the produced byproduct must comply with simple procedures.

The targeting of the production and intake of substances for reporting the presence of a given substrate might be addressed as the utilization of bioreporters (Daunert et al., 2000), identified by means of the specific reporter gene responsible to manage the production of the substance on a specific metabolic pathway. The term bioreporter, is usually referred in regards to very specific products that can be detected e.g. by means of optical instruments based on fluorescence and phosphorescence (Pringsheim, 1949).

Bioreporter genes can be introduced within the cell genome by different methods leveraged from genetic engineering (Salis et al., 2009; van der Meer & Belkin, 2010), they are attached to precise metabolic pathways, due to the linear nature of the deployment of reactions within the metabolic pathways, the bioreporter would only be active when the correspondent pathway has reached the stage in which the specific reporter gene is reached, thus reporting on the presence of the sought substrate.

The released molecules become the target of chemical sensing methods, bridging the translation for the quantification of the substrate, in the same way the electrical response on the SRB method is proportional to the amount of substrate detected, this molecules would be released/consumed as there is more/less concentration of the substrate.

4. Current advances in whole-cell biosensors

The selection of a suitable strategy for the development of a biosensor responds to different properties rendered by the selection of the used strain. One of the main challenges is to recognize the specific substrate which can be targeted by different strains, or which strain best adapts to a specific target. In Table 5, an extensive review on 163 cases is made, compiling 116 different whole-cell strains, differentiating the reported genetically modified ones, and a record of about 100 different targets that have been claimed to be recognized for such strains.

Microorganism	Target	Detection method	Strategy	Reference
A. aceti (IFO 3284)	Ethanol	Amperometric	SRB	(Ikeda, Kato, Maeda, Tatsumi, Kano & Matsushita, 1997)
A. adeninivorans LS3	BOD	Amperometric	SRB	(Riedel, Lehmann, Tag, Renneberg & Kunze, 1998; Chan, Lehmann, Tag, Lung, Riedel, Gruendig et al.,1999; Chan, Lehmann, Chan, Chan, Chan, Gruendig et al., 2000; Tag, Kwong, Lehmann, Chan, Renneberg, Riedel et al., 2000; Tag, Lehmann, Chan, Renneberg, Riedel & Kunze, 2000)
A. ferrooxidans	Fe^{2+} $S_2O_3^{2-}$ $Cr_2O_7^{2-}$	mperometric	SRB	(Zlatev, Magnin, Ozil, & Stoytcheva, 2006a; Zlatev, Magnin, Ozil, & Stoytcheva, 2006b; Zlatev, Magnin, Ozil, & Stoytcheva, 2006c)
A. globiformis	Choline	Amperometric	SRB	(Stoytcheva, Zlatev, Valdez, Magnin & Velkova, 2006)
Arthrobacter. sp. JS 443	p-Nitrophenol	Amperometric	SRB	(Lei, Mulchandani, Chen, Wang & Mulchandani,2003; Lei, Mulchandani, Chen, Wang & Mulchandani, 2004)
A. nicotianae (acyl-CoA oxidase)	Short chain fatty acids in milk (butyric acid)	Oxygen electrode (Polyvinyl alcohol)	BtB	(Ukeda, Wagner, Bilitewski & Schmid, 1992; Ukeda, Wagner, Weis, Miller, Klostermeyer & Schmid, 1992; Schmidt, Standfuß-Gabisch & Bilitewski, 1996)
A. niger	Ethanol	Amperometric	SRB	(Subrahmanyam, Shanmugam, Subramanian, Murugesan, Madhav & Jeyakumar, 2001)
	Glucose	Oxygen electrode (entrapment Glucose) in dialysis membrane)	BtB	(Katrlík, Švorc, Rosenberg & Miertuš, 1996)
A. peroxydans	Hydrogen peroxide	Amperometric	SRB	(Sumathi, Rajasekar & Narasimham, 2000)
A. phenologenes (Tyrosine-phenol lyase)	Tyrosine	NH3 gas sensing electrode (direct immobilisation on sensor membrane)	BtB	(Di Paolantonia & Rechnitz, 1982)
Activated sludge	BOD	Amperometric	SRB	(Kumlanghan, Kanatharana, Asawatreratanakul, Mattiasson & Thavarungkul, 2008)
Activated sludge (mixed microbial consortium)	BOD	Oxygen electrode/flow injection system (entrapped in dialysis membrane)	BtB	(Liu, Björnsson & Mattiasson, 2000)
Anaerobic sludge	BOD	MFC	SRB	(Di Lorenzo, Curtis, Head & Scott, 2009)
B. ammoniagenes	Urea	Conductometric	SRB	(Jha, Kanungo, Nath & D'Souza, 2009)

Microorganism	Target	Detection method	Strategy	Reference
B. stearothermophilus var. calidolactis	β-lactams	Potentiometric	SRB	(Ferrini, Mannoni, Carpico & Pellegrini, 2008)
B. subtilis	Peptides (aspartame)	Oxygen electrode (filter paper strip and dialysis membrane)	BtB	(Renneberg, Riedel & Scheller, 1985)
	Enalapril maleate (angiotensin)	Oxygen electrode		(Fleschin, Bala, Bunaciu, Panait & Aboul-Enein, 1998)
	BOD	Amperometric	SRB	(Riedel, Renneberg, Kühn & Scheller, 1988)
B. subtilis (heat killed)	BOD	Amperometric	SRB	(Tan & Qian, 1997)
B. subtilis and *B. licheniformis 7B*	BOD	Amperometric	SRB	(Tan, Li, Neoh & Lee, 1992; Tan, Li & Neoh, 1993; Li, Tan & Lee, 1994)
Bacillus sp.	Urea	NH_4^+ ion selective electrode	BtB	(Verma & Singh, 2003)
Bacteria consortium	Glucose	MFC	SRB	(Kumlanghan, Liu, Thavarungkul, Kanatharana & Mattiasson, 2007)
Bioluminescent recombinant *E. colir::luxAB* strain	Tributyltin	Luminescence	BtB	(Thouand, Horry, Durand, Picart, Bendriaa, Daniel et al., 2003)
BOD-Multiple organisms	BOD	Amperometric	SRB	(Tan & Wu, 1999)
Brevibacterium sp.	Acrylamide; acrylic acid	Oxygen electrode (free cells)	BtB	(Ignatov, Rogatcheva, Kozulin & Khorkina, 1997)
C. parapsilosis	BOD	Amperometric	SRB	(König, Reul, Harmeling, Spener, Knoll & Zaborosch, 2000)
C. sp.	Cu^{2+}	Voltammetric	SRB	(Alpat, Cadirci, Yasa & Telefoncu, 2008)
C. tropicalis	Ethanol	Amperometric	SRB	(Akyilmaz & Dinçkaya, 2005)
Candida vini	Alcohol	Oxygen electrode (porous acetyl cellulose filter)	BtB	(Mascini, Memoli & Olana, 1989)
Chorella vulgaris (algae)	Atrazine	Amperometric	SRB	(Shitanda, Takamatsu, Watanabe & Itagaki, 2009)
	Cd^{2+}	Conductometric		(Guedri & Durrieu, 2008)
	Cd^{2+} and Zn^{2+} Paraoxon-methyl			(Chouteau, Dzyadevych, Durrieu & Chovelon, 2005)
	Phosphate	Oxygen electrode (polycarbonate membrane)	BtB	(Matsunaga, Suzuki & Tomoda, 1984)
Chloroplast /thylakoid membranes	Herbicides[c] (diuron and atrazine)	Pt-electrode in microelectrochem ical cell(photo cross linkable PVA bearing styrylpyridium group	BtB	(Rouillon, Sole, Carpentier & Marty, 1995)

Microorganism	Target	Detection method	Strategy	Reference
CO_2 utilizing autotropic bacteria (*Pseudomonas)*	CO_2	Oxygen electrode (bound on cellulose nitrate membrane)	BtB	(Suzuki, Tamiya & Karube, 1987)
Comamonas testosterone TI	Non-ionic surfactants	Amperometric	SRB	(Taranova, Fesay, Ivashchenko, Reshetilov, Winther-Nielsen & Emneus, 2004)
E. coli	Vitamin B-12	Oxygen electrode (trapped in porous acetyl cellulose membrane)	BtB	(Karube, Wang Tamiya, & Kawarai, 1987)
	Phenol	Amperometric	SBR	(Neufeld, Biran, Popovtzer, Erez, Ron & Rishpon, 2006)
	Nalidixic acid			(Ben-Yoav, Biran, Pedahzur, Belkin, Buchinger, Reifferscheid et al., 2009)
E. coli bearing *fab A'::lux* fusions	Pollutants/toxicity	Luminescence	BtB	(Bechor, Smulski, Van Dyk, LaRossa & Belkin, 2002)
E. coli DH5α (pPR-arsR-ABS, expressing *egfp)*	Arsenite	Fluorescence	BtB	(Wells, Gösch, Rigler, Harms, Lasser & van der Meer, 2005)
E. coli DPD1718 containing *recA'::lux fusion*	Genotoxicants	Luminescence	BtB	(Polyak, Bassis, Novodvorets, Belkin & Marks, 2000)
E. coli HB101 pUCD607 containing *luxCDABE* cassette	Water pollutants/toxicity	Luminescence	BtB	(Horsburgh, Mardlin, Turner, Henkler, Strachan, Glover et al., 2002)
E. coli HMS174 harboring *mer-lux* plasmid *pRB27*	Bioavailable mercury	Luminescence	BtB	(Rasmussen, Turner & Barkay, 1997)
E. coli HMS174 harboring *mer-lux* plasmid *pRB27 or pRB28*	Hg^{2+}	Luminescence	BtB	(Rasmussen, Sørensen, Turner & Barkay, 2000)
E. coli HMS174 harboring *mer-lux* plasmid *pRB27 or pRB28*	Bioavailable mercury	Luminescence	BtB	(Barkay, Gillman & Turner, 1997)
E. coli HMS174 harboring *mer-lux* plasmid *pRB28, pOS14orpOS15*	Bioavailable mercury	Luminescence	BtB	(Selifonova, Burlage & Barkay, 1993)
E. coli K12	Mono-and /disaccharides	Amperometric	SRB	(Held, Schuhmann, Jahreis & Schmidt, 2002)
E. coli MC1061 harboring *mer-lux* plasmid *pTOO11*	Urinary mercury (II)	Luminescence	BtB	(Roda, Pasini, Mirasoli, Guardigli, Russo, Musiani et al., 2001)
E. coli MC4100 harboring *pAHL-GFP*	N-Acyl homoserine lactones in soil	Fluorescence	BtB	(Burmølle, Hansen, Oregaard & Sørensen, 2003)
E. coli WP2	Tryptophan	LAPS	SBR	(Seki Kawakubo, Iga & Nomura, 2003)

Microorganism	Target	Detection method	Strategy	Reference
E. coli, Pantoea agglomerans and *Pseudomonas syringae*	Water availability	Fluorescence	BtB	(Axtell & Beattie, 2002)
F. solani	Acetic acid	Amperometric	SRB	(Subrahmanyam, Kodandapani, Shanmugam, Moovarkumuthalvan, Jeyakumar & Subramanian, 2001)
Flavobacteium sp.	Organophosphates	pH electrode	SRB	(Gäberlein et al., 2000)
G. oxydans	Ethanol	Amperometric	SRB	(Tkáč, Voštiar, Gemeiner & Šturďık, 2002; Tkac, Vostiar, Gorton, Gemeiner & Sturdik, 2003)
	Total sugars			(Tkáč, Gemeiner, Švitel, Benikovský, Šturďık, Vala et al., 2000)
	Glucose			(Odaci, Timur & Telefoncu, 2009)
				(Tuncagil, Odaci, Varis, Timur & Toppare, 2009; Tuncagil, Odaci, Yildiz, Timur & Toppare, 2009)
	Ethanol			(Valach, Katrlík, Šturdík & Gemeiner, 2009)
	1,3-Propanediol			(Katrlík, Vostiar, Sefcovicová, Tkác, Mastihuba, Valach et al., 2007)
G. oxydans (D-glucose dehydrogenase), *S.cerevisiae* (invertase), *K. marxianus* (galactosidase)	Glucose, sucrose, lactose	Oxygen electrode (gelatine)	BtB	(Svitel, Curilla & Tkác, 1998)
G. oxydans or P. methanolica	Ethanol	Amperometric	SRB	(Reshetilov, Trotsenko, Morozova, Iliasov & Ashin, 2001)
G. suboxydans	Ethanol	Amperometric	SRB	(Kitagawa, Ameyama, Nakashima, Tamiya & Karube, 1987)
G. sulfurreducens	Acetate	MFC	SRB	(Tront, Fortner, Plötze, Hughes & Puzrin, 2008)
GEMb E. coli (organophosphorous hydrolase)	Organophosphate nerve agents (paraxon, methyl parathion, diazinon)	Potentiometric (adsorption on electrode surface)	SRB	(Mulchandani, Mulchandani, Kaneva & Chen, 1998)
		Fiber-optic (agarose)	BtB	(Mulchandani, Kaneva & Chen, 1998)
H. polymorpha	L-lactate	Amperometric	SRB	(Smutok, Dmytruk, Gonchar, Sibirny & Schuhmann, 2007)
K. oxytoca AS1	BOD	Amperometric	SRB	(Ohki, Shinohara, Ito, Naka, Maeda, Sato et al., 1994)
LAS degrading bacteria isolated from activated sludge	Aniomic surfactants (linear alky benzene sulfonates- LAS)	Oxygen electrode, (reactor type sensor, ca-alginate)	BtB	(Nomura, Ikebukuro, Yokoyama, Takeuchi, Arikawa, Ohno et al., 1994)

Microorganism	Target	Detection method	Strategy	Reference
Moraxella sp.	β-d-Glucuronidase	Amperometric	SRB	(Togo, Wutor, Limson & Pletschke, 2007)
	p-Nitrophenol			(Mulchandani, Lei, Chen, Wang & Mulchandani, 2002; Mulchandani, Hangarter, Lei, Chen & Mulchandani, 2005)
	Paraoxon			(Mulchandani, Chen & Mulchandani, 2006)
Microbial consortium	BOD	Amperometric	SRB	(Rastogi, Kumar, Mehra, Makhijani, Manoharan, Gangal et al., 2003; Liu, Olsson & Mattiasson,2004a; Liu, Olsson & Mattiasson, 2004b; Dhall, Kumar, Joshi, Saxsena, Manoharan, Makhijani et al., 2008)
Nitrobacter vulgaris DSM10236	Nitrite	Oxygen electrode (adsorption on Whatman paper)	BtB	(Reshetilov, Iliasov, Knackmuss & Boronin, 2000)
P. aeruginosa	Cephalosporins	Potentiometric	SRB	(Kumar, Kundu, Pakshirajan & Dasu, 2008)
P. aeruginosa + K. sp.	Methane	Amperometric	SRB	(Wen, Zheng, Zhao, Shuang, Dong & Choi, 2008)
P. aeruginosa FRD1 carrying plasmid *pMOE15* with *recA::luxCDABE*	UV	Luminescence	BtB	(Elasri & Miller, 1999)
P. aeruginosa JI104	Trichloroethylene	Chloride ion selective electrode	BtB	(Han, Kim, Sasaki, Yano, Ikebukuro, Kitayama et al., 2001; Han, Sasaki, Yano, Ikebukuro, Kitayama, Nagamune et al., 2002)
P. alcaligenes	Caffeine	Amperometric	SRB	(Babu et al., 2007)
P. angusta	Ethanol	Amperometric	SRB	(Voronova, Iliasov & Reshetilov, 2008)
P. fluorescens	BOD	Amperometric	SRB	(Yoshida, Yano, Morita, McNiven, Nakamura & Karube, 2000; Yoshida, Hoashi, Morita, McNiven, Nakamura & Karube, 2001)
	Glucose Galactose Mannose Xylose			(Kirgoz, Timur, Odaci, Pérez, Alegret & Merkoçi, 2007; Odaci, Kiralp Kayahan, Timur & Toppare, 2008; Yeni, Odaci & Timur, 2008; Tuncagil, Odaci, Varis, Timur & Toppare, 2009)
				(Odaci, Timur & Telefoncu, 2008)
P. fluorescens 10586r pUCD607	Toxicity of chlorophenol	Luminescence	BtB	(Tiensing, Strachan & Paton, 2002)
P. fluorescens A506 (pTolLHB) and *E. cloacae JL1157 (pTolLHB)*	Bioavailable toluene and related compounds	Fluorescence	BtB	(Casavant, Thompson, Beattie, Phillips & Halverson, 2003)

Microorganism	Target	Detection method	Strategy	Reference
P. fluorescens DF57 with a *Tn5::luxAB* promoter probe transposon	Bioavailable copper	Luminescence	BtB	(Tom-Petersen, Hosbond & Nybroe, 2001)
P. fluorescens pUCD607	Pollution-induced stress	Luminescence	BtB	(Porteous, Killham & Meharg, 2000)
P. fluorescents NCIMB 11764	Cyanide	Amperometric	SRB	(Lee & Karube, 1996)
P. putida	BOD	Oxygen electrode (adsorption on porous nitro cellulose membrane)	BtB	(Chee, Nomura & Karube, 1999)
	Phenolic compounds	Oxygen electrode (reactor with cells adsorbed on PEI glass)		(Nandakumar & Mattiasson, 1999b)
	3-Chloro-benzoate	Oxygen electrode (PVA)		(Riedel, Naumov, Boronin, Golovleva, Stein & Scheller, 1991)
	Phenolic compounds	Amperometric	SRB	(Timur, Pazarlioğlu, Pilloton & Telefoncu, 2003; Timur, Della Seta, Pazarlioğlu, Pilloton & Telefoncu, 2004)
	Phenol			(Kirgöz, Odaci, Timur, Merkoçi, Pazarlioğlu, Telefoncu et al., 2006)
	Galactose Glucose			(Timur, Anik, Odaci & Gorton, 2007)
	Catechol			(Timur, Haghighi, Tkac, Pazarlioğlu, Telefoncu & Gorton, 2007)
	2,4-Dichloro phenoxy acetic acid			(Odaci, Sezgintürk, Timur, Pazarlioğlu, Pilloton, Dinçkaya et al., 2009)
P. putida carrying *NAH7* plasmid and a chromosomally inserted gene fusion between the *sal* promoter and the *luxAB* genes	Bioavailable naphthalene	Luminescence	BtB	(Werlen, Jaspers & van der Meer, 2004)

Microorganism	Target	Detection method	Strategy	Reference
P. putida F1	Benzene Toluene Ethylbenzene	Amperometric	SRB	(Rasinger, Marrazza, Briganti, Scozzafava, Mascini & Turner, 2005)
P. putida JS444	Paraoxon Parathion Methyl parathion Fenitrothion EPN	Amperometric	SRB	(Lei, Mulchandani, Chen & Mulchandani, 2005; Lei, Mulchandani, Chen, Wang & Mulchandani, 2005) (Lei, Mulchandani, Chen & Mulchandani, 2006; Lei, Mulchandani, Chen & Mulchandani, 2007)
P. putida SG10	BOD	Amperometric	SRB	(Chee, Nomura, Ikebukuro & Karube, 2005)
Pseudomonas. sp.	Microbiologically influenced corrosion p-Nitrophenol	Amperometric	SRB	(Dubey & Upadhyay, 2001; Banik, Prakash & Upadhyay, 2008)
	BOD			(Li & Chu, 1991)
P. syringae	BOD	Amperometric	SRB	(Kara, Keskinler & Erhan, 2009)
P. vulgaris (Phenylalanine deaminase)	Phenylalanine	Amperometric oxygen electrode	BtB	(Liu, Cui & Deng 1996)
P.fischeri and *P. putida BS566::luxCDABE*	Toxicity of waste water treatment plant treating phenolics-containing waster	Luminescence	BtB	(Philp, Balmand, Hajto, Bailey, Wiles, Whiteley et al., 2003)
Potato *(S. tuberosum)* slices (polyphenol oxidase inhibition)	Mono and polyphenols (atrazine)	Oxygen electrode (tissue slice sandwitched between membranes)	BtB	(Mazzei, Botrè, Lorenti, Simonetti, Porcelli, Scibona et al., 1995)
Pseudomonas and *Archromobacter*	Anionic surfactants	Amperometric	SRB	(Taranova, Semenchuk, Manolov, Iliasov & Reshetilov, 2002)
Psychrophilic *D. radiodurans*	Sugars (glucose)	Oxygen electrode (agarose)	BtB	(Nandakumar & Mattiasson, 1999a)
R. erthropolis	2,4-Dinitrophenol	Amperometric	SRB	(Emelyanova & Reshetilov, 2002)
Ralstonia eutropha AE2515	Ni^{2+} and Co^{2+}	Luminescence	BtB	(Tibazarwa, Corbisier, Mench, Bossus, Solda, Mergeay et al., 2001)
Recombinant *E. coli*	Penicillin	Flat pH electrode	BRB	(Galindo, Bautista, García & Quintero, 1990; Chao & Lee, 2000)
	Cadmium	Amperometric		(Biran, Babai, Levcov, Rishpon & Ron, 2000)
Recombinant *E. coli* containing DL-2-haloacid dehalogenase encoding gene and *luxCDABE* genes	Halogenated organic acids	Luminescence	BtB	(Tauber, Rosen & Belkin, 2001)

Microorganism	Target	Detection method	Strategy	Reference
Recombinant *E. coli* containing *recA'::lux* fusion	UV	Luminescence	BtB	(Rosen, Davidov, LaRossa & Belkin, 2000)
Recombinant *Moraxella*	Organophosphates	Amperometric	SRB	(Mulchandani, Chen, Mulchandani, Wang & Chen, 2001)
Recombinant *P. putida JS 444*	Organophosphates	Amperometric	SRB	(Lei, Mulchandani, Chen & Mulchandani, 2005)
Recombinant *Pseudomonas syringae* carrying *gfp* gene	Bioavailable iron	Fluorescence	BtB	(Joyner & Lindow, 2000)
Recombinant *S. cerevisiae*	Cu^{2+}	Amperometric	SRB	(Lehmann, Riedel, Adler & Kunze, 2000)
Rhodococcus erythropolis DSM Nr. 772 and *Issatchenkia orientalis DSM Nr. 3433*	BOD	Amperometric	SRB	(Heim, Schnieder, Binz, Vogel & Bilitewski 1999)
Rhodococcus sp. DSM 6344	Chlorinated and brominated hydrocarbons (1-chlorobutane and ethylenebromide)	Ion selective electrodes (alginate)	BtB	(Peter, Hutter, Stöllnberger& Hampel, 1996)
Rhodococcus sp.; Trichosporon beigelii	Chlorophenols	Oxygen electrode (PVA)	BtB	(Riedel, Hensel, Rothe, Neumann & Scheller, 1993; Riedel, Beyersdorf-Radeck, Neumann & Schaller, 1995)
S. cerevisiae	Sucrose	Amperometric	SRB	(Rotariu , Bala & Magearu, 2000)
	Cyanide	Oxygen electrode (PVA)	BtB	(Ikebukuro, Honda, Nakanishi, Nomura, Masuda, Yokoyama et al., 1996; Nakanishi, Ikebukuro & Karube, 1996; Ikebukuro, Miyata, Cho, Nomura, Chang, Yamauchi et al., 1996)
	BOD	Amperometric	SRB	(Nakamura, Suzuki, Ishikuro, Kinoshita, Koizumi, Okuma et al., 2007)
	Vitamin B1	Amperometric	SRB	(Akyilmaz, Yaşa & Dinçkaya, 2006)
	L-lysine	Amperometric	SRB	(Akyilmaz, Erdoğan, Oztürk & Yaşa, 2007)
	Sucrose	Oxygen	BtB	(Rotariu, Bala & Magearu, 2002)
S. cerevisiae (I) (II)	Cu^{2+}	Amperometric	SRB	(Tag, Riedel, Bauer, Hanke, Baronian & Kunze, 2007)
S. ellipsoideus	Ethanol	Amperometric	SRB	(Rotariu & Bala, 2003)
S. ellipsoideus	Ethanol	Oxygen	BtB	(Rotariu, Bala & Magearu, 2004)
S. typhimurium	2-Amino-3-methylimidazo[4,5-f]quinoline	Amperometric	SRB	(Ben-Yoav, Elad, Shlomovits, Belkin & Shacham-Diamand, 2009)

Microorganism	Target	Detection method	Strategy	Reference
S. uvarum	Vitamin B-6	Oxygen electrode (adsorption on cellulose nitrate membrane)	BtB	(Endo, Kamata, Hoshi, Hayashi & Watanabe, 1995)
Salt tolerant mycelial yeast *A. adeninivorans LS3*	BOD	Oxygen electrode (PVA)	BtB	(Tag, Lehmann, Chan, Renneberg, Riedel & Kunze, 1998)
Serratia marcescens LSY4	BOD	Amperometric	SRB	(Kim & Kwon, 1999)
Sinorhizobium meliloh containing a *gfp* gene fused to the *melA* promoter	Galactosides	Fluorescence	BtB	(Bringhurst, Cardon & Gage, 2001)
Sphingomonas yanoikuyae B1 or *Ps. fluorescens WW4*	Polycyclic aromatic hydrocarbons (Naphthalene)	Oxygen electrode (polyurethane based hydrogel)	BtB	(König, Zaborosch, Muscat, Vorlop & Spener, 1996; König, Zaborosch & Spener, 1997)
Streptococcus faecium (Pyruvate dehydrogenase complex)	Pyruvate	CO2 gas sensing electrode (direct immobilisation on sensor membrane)	BtB	(Di Paolantonia & Rechnitz, 1983)
Synechococcus PCC 7942 reporter strain	Bioavailable phosphorus	Luminescence	BtB	(Schreiter, Gillor, Post, Belkin, Schmid & Bachmann, 2001)
Synechococcus sp. PCC 7942	Pollutants such as diuron and mercuric chloride	Photoelectrochem ical (photo cross linkable PVA bearing styrylpyridium group)	BtB	(Rouillon et al., 1999)
T. bacteria	BOD	Amperometric	SRB	(Karube, Yokoyama, Sode & Tamiya, 1989)
T. candida	BOD	Amperometric	SRB	(Sangeetha, Sugandhi, Murugesan, Murali Madhav, Berchmans, Rajasekar et al., 1996)
T. cutaneum	BOD	Oxygen electrode array (Miniature electrode photo cross-linkable resin) (Entrapment)	BtB	(Marty, Olive & Asano, 1997; Yang, Sasaki, Karube & Suzuki, 1997)
T. cutaneum and *B. subtilis*	BOD	Amperometric	SRB	(Jia, Tang, Chen, Qi & Dong, 2003)
T. ferrooxidans	Cyanide	Amperometric	SRB	(Okochi, Mima, Miyata, Shinozaki, Haraguchi, Fujisawa et al., 2004)

Microorganism	Target	Detection method	Strategy	Reference
Trichosporum cutaneum	BOD	Miniature oxygen electrode (UV cross-linking resin ENT-3400)	BtB	(Yang, Suzuki, Sasaki & Karube, 1996)
Yeast	BOD	Amperometric	SRB	(Chen, Cao, Liu, & Kong, 2002)
Yeast cells	Bioavailable organic carbon in oxic sediments	Oxygen electrode (PVA)	BtB	(Neudörfer & Meyer-Reil, 1997)
Yeast SPT1 and SPT2	BOD	Amperometric	SRB	(Trosok, Driscoll & Luong, 2001)

Table 5. Different whole-cell strains for use in biosensing, it is listed the target, detection method and attributed strategy. Table modified from (D'Souza 2001; Lei, Chen et al., 2006; Su et al., 2011)

From the reviewed literature, approximately 51% of the strains respond to an SRB strategy, 45% to a BtB strategy and a 4% to both, 19 different methods are listed, 7 of them attributed to a SRB strategy, namely amperometric, conductometric, pH electrode, LAPS, MFC, potentiometric and voltametric, and the remaining 12 to the BtB strategy, comprising different chemical-compound-selective electrodes, and fluorescent and luminescent bioreporters.

The given classification is based on the nature of the different electrodes, if any, and aims to serve as a base for the correct identification of a specific strategy according to the application goals of the designing process of a biosensor. According to the U.S. Environmental Protection Agency (EPA), whole-cell biosensors display a promising alternative to the usage in early warning screening due to their fast reaction to toxins (EPA, 2005); in the same report, some technologies are disclosed under the current commercial application, although more accordingly with the definition of biological test, such systems are ToxScreen-II(currently III) (EPA, 2006; CheckLight Highlighting Water Safety-TOX-SCREEN 3, 2013), BioTox™ (EPA, 2006), DeltaTox® (EPA, 2003a), ToxTrak™ (Environmental technology verification program, 2006; ToxTrakTM Toxicity Reagent Set, 25-49 tests- Overview | Hach, 2013), POLYTOX™ (EPA, 2003b; InterLab Supply-Products-Biological Oxygen Demand (BOD) and Toxicity Testing Technology, 2013) and microMAX-TOX, the latter a promising device announced by the italian company Systea S.p.a. (SYSTEA S.p.A., 2013) which would cover the expected properties of a whole-cell based biosensor for online and continuous monitoring. Other commercially available whole-cell based biosensors are related in Table 6.

It is clearly observed the current preference to the utilization of the BtB strategy for commercial use, although the efforts posed by researchers to develop biosensors on the margins of the SRB strategy. The trending posed by the current developments under the scope of the SRB strategy will lead a new generation of biosensors based on the possibilities of different whole-cells strains to modify the electric structures of a given electrode, furthermore, the advantages regarding the needless utilization of reagents and the faster response will definitively play an important role on the favoring of inclusion of the development of SRB strategy biosensors.

The future developments would include the utilization of Archaea as an auspicious prospect on the development of highly effective SRB strategy biosensors, the affinity of such domain-type whole-cells with different substrates and the possibility it offers for strong electrode reactions is an interesting field to be explored.

Technology name	Biological system	Strategy	Reference
Aquasentinel	Algae	BtB	(Aqua Sentinel, 2013)
Fluotox	Algae (*Scendedesmus subspicatus*)	BtB	(Fluotox, 2013)
Lumitox	Algae (*Pyrocystis lunula*)	BtB	(Stiffey & Nicolaids, 1995)
Amtox	Bacteria	BtB	(Upton & Pickin, 1996)
Baroxymeter	Bacteria	BtB	(Baroxymeter, 2013)
BioTox Flash Test	Bacteria (*Vibrio fischerei*)	BtB	(Aboatox Environmental analysis, 2013)
Cellsense	Bacteria, *algae*	SRB	(Farré et al., 2001)
GreenScreen EM	Yeast (*Saccharomyces cerevisiae*)	BtB	(Keenan, Knight, Billinton, Cahill, Dalrymple, Hawkyard et al., 2007)
LUMIStox	Bacteria (*Vibrio fischerei*)	BtB	(Hach-Lange UK-LUMIStox, 2013)
MetPlate	Bacteria (*Escherichia coli*)	BtB	(MetPLATETM, 2013)
Sinorhizobium mellioti Toxicity Test	Bacteria (*Sinorhizobium mellioti*)	BtB	(van der Schalie, James & Gargan, 2006)

Table 6. Current commercially available whole-cell based biosensor, table modified from extract (Walther & Wurster, 2007)

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