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# MICROBIAL BIOSENSORS FOR ENVIRONMENTAL MONITORING

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***Microbial biosensors for environmental monitoring***

Microbial biosensors are analytical devices capable of sensing substances in the environment due to the specific biological reaction of the microorganism or its parts. Construction of a microbial biosensor requires knowledge of microbial response to the specific analyte. Linking this response with the quantitative data, using a transducer, is the crucial step in the construction of a biosensor. Regarding the transducer type, biosensors are divided into electrochemical, optical biosensors and microbial fuel cells. The use of the proper configuration depends on the selection of the biosensing element. With the use of transgenic *E. coli* strains, bioluminescence or fluorescence based biosensors were developed. Microbial fuel cells enable the use of the heterogeneous microbial populations, isolated from wastewater. Different microorganisms are used for different pollutants – pesticides, heavy metals, phenolic compounds, organic waste, etc. Biosensing enables measurement of their concentration and their toxic or genotoxic effects on the microbes. Increasing environmental awareness has contributed to the increase of interest for biomonitoring. Although technologies, such as bioinformatics and genetic engineering, allow us to design complex and efficient microbial biosensors for environmental pollutants, the transfer of the laboratory work to the field still remains a problem to solve.

**Key words:** microbiology / environmental protection / microbial biosensors / environmental pollutants / microbial fuel cells / bioluminescence / genetics / bioinformatics / genetic engineering

***Mikrobni biosenzorji za monitoring okolja***

Mikrobni biosenzorji so analitske naprave, ki nam omogočajo zaznavanje snovi v okolju zaradi specifične biološke reakcije, ki poteka v mikroorganizmu ali njegovem delu. Če želimo takšno napravo uporabiti za monitoring onesnažil v okolju, moramo dobro poznati odziv mikroorganizma na specifičen analit in ga preko pretvornika signala spremeniti v kvantitativno obliko. Poznamo različne konfiguracije mikrobnih biosenzorjev glede na pretvornik signala – elektrokemijske in optične biosenzorje ter mikrobne gorivne celice. Vsaka ima svoje prednosti in slabosti, katero uporabimo, je v veliki meri odvisno od izbora biosenzorskega organizma. Transgene celice *E. coli* nam omogočajo bioluminescenčno ali fluorescenčno zaznavo, v mikrobne gorivne celice pa lahko vključimo mešane mikrobne združbe. Z izborom organizma se prilagajamo tudi onesnažilu. Med najpogostejšimi onesnažili so pesticidi, težke kovine, fenoli, organski odpadki. Z biosenzorji ne spremljamo le njihovih koncentracij v okolju, pač pa beležimo tudi toksične in genotoksične vplive analitov na mikroorganizme. S povečevanjem skrbi za okolje narašča tudi pomen mikrobnih biosenzorjev. Razvoj tehnologij, kot sta bioinformatika in genetski inženiring, nam omogoča temeljitejše in uspešnejše načrtovanje uporabe mikrobnih biosenzorjev v okoljskih aplikacijah. Izziv za prihodnje pa ostaja prenos mikrobne biosenzorske tehnologije na teren.

**Ključne besede:** mikrobiologija / varstvo okolja / mikrobni biosenzorji / okoljska onesnažila / mikrobne gorivne celice / bioluminescenca / genetika / bioinformatika / genetski inženiring

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## 1 INTRODUCTION

A biosensor is a self-contained integrated device, capable of providing specific quantitative or semi-quantitative analytical information using a biosensing element connected with a transducer (IUPAC 1996, Thevenot *et al.*, 2001). Biosensor construction, a three-step process, involves combining two elements with different characteristics. First, a biological sensing element is chosen, then a transducer is selected, and finally the biological component (detection element) is fixed to the transducer (Xu and Ying, 2011). Enzymes, antibodies, cell receptors, microorganisms, animal and plant cells or tissue cultures can be used as biorecognition components of a biosensor. Microorganisms have a huge potential for detection of a wide spectrum of chemical substances and their mixtures, they are adjustable to different reaction conditions and compared to enzymes or antibodies do not require expensive preparation processes (Shin 2010, Xu and Ying, 2011). They can be genetically modified, too. This characteristic enables the use of microbial biosensors in the fields of environmental monitoring, food safety and medicine.

## 2 CHOICE AND APPLICATION OF A PROPER ORGANISM

Choice of a proper microorganism for the detection of pollutants and their effects in the environment and its incorporation with the competent transducer is a key step in the development of an environmental biosensor. Bacteria and yeast are the most commonly used (Xu and Ying, 2011). The chosen microorganism must be robust and capable of specific pollutant detection in small concentrations, to ensure price efficient detection. Recently whole-cell biosensors (Chan *et al.*, 2013; Niazi *et al.*, 2008; Mulchandani and Rajesh, 2011; Anu Prathap *et al.*, 2012) and microbial fuel cells (Di Lorenzo *et al.*, 2014; Shen *et al.*, 2013; Liu *et al.*, 2014; Ayyaru and Dharmaligman, 2013) draw special attention on the field of environmental monitoring. Genetic engineering became important, too. We can manipulate organisms to improve mechanisms of analyte detection or express them in new organisms (Mulhandani and Rajesh, 2011). DNA segments coding for detection mechanisms can be transferred into model organisms with optimized growing conditions, such as *Escherichia coli* and *Saccharomyces cerevisiae*. The organism and the detection configuration should be combined properly to achieve the best possible detection of the signal.

## 3 BIOSENSOR CONFIGURATION

There are three main types of microbial biosensors classified, based on different signal transducers: electrochemical, optical and microbial fuel cells (Xu and Ying, 2011).

Electrochemical transducers use the change of the electric current, potential and conductivity, caused by microbial-analyte contact. They can be further divided into amperometric, potentiometric and conductometric biosensors. Amperometric microbial biosensors operate at a fixed potential with respect to a reference electrode, and then the corresponding current is obtained due to the oxidation or reduction of electroactive species at the surface of the electrode (Xu and Ying, 2011). This configuration has been described by Yong *et al.* (2011), Anu Prathap *et al.* (2012) and Wang *et al.* (2013); on the other hand, the potentiometric transducer was constructed by Mulchandani and Rajesh (2011). Transducers of this kind use ion-selective electrodes to transmit the biological signal into an electric signal. They are less sensitive, produce higher relative error and a worse linear relationship between the exporting signal and the concentration of the detected analyte (Xu and Ying, 2011). As it is obvious from their name, conductometric biosensors measure changes in conductivity of the media, caused by the target analyte. Although the conductance measurements are extremely sensitive, the detection of solution conductance is considered to be nonspecific (Xu and Ying, 2011).

Optical biosensors can be defined as sensor devices that make use of optical principles, such as bioluminescence, fluorescence and colorimetry for transduction of a biochemical interaction into a suitable output signal (Xu and Ying, 2011). The use of genetic engineering enables an expression of fluorescence and bioluminescence in the target organism. Scientists report of luciferase (Niazi *et al.*, 2008; Shin, 2010; Chan *et al.*, 2013) and green fluorescent protein (GFP) applications (Wei *et al.*, 2013; Kim *et al.*, 2015). Microbial fuel cells (MFC) are bioelectrochemical devices that produce electrical energy through the action of specific microbes (known as anodophiles), capable of transferring the electrons generated from the oxidation of organic compounds (the fuel) to an anode electrode (Di Lorenzo *et al.*, 2014). In a typical two-chamber MFC (Fig. 1), the electrons are absorbed by the anode and are transported to the cathode through an external circuit. After crossing a proton exchange membrane, the protons enter the cathodic chamber where they combine with oxygen to form water (Du *et al.*, 2007). Electric current, produced by fuel oxidation, can serve as a transducer of a microbial response to the analyte. Due to their simple design and low cost, single-chamber MFC, where the cathode is exposed to air, are extensively used in envi-

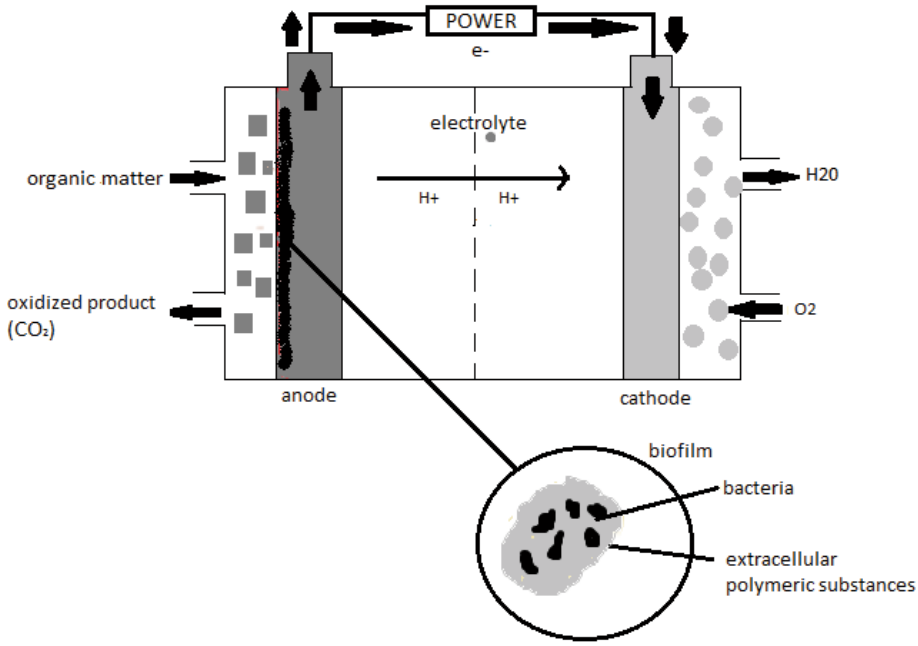


***Figure 1:*** *A scheme of a two-chamber microbial fuel cell*
***Slika 1:*** *Shema dvoprekatne mikrobne gorivne celice*

ronmental applications (Du *et al.*, 2007). They are useful for the detection of biochemical oxygen demand (BOD) (Liu *et al.*, 2013, Ayyaru and Dharmaligman, 2014), heavy metals and their toxicity (Shen *et al.*, 2013; Liu *et al.*, 2014; Di Lorenzo *et al.*, 2014). MFC enable the use of heterogeneous microbial populations, isolated from wastewater plants and other working MFC. This characteristic makes them very successful for the development of sensitive, specific and cost efficient biosensors.

## 4 ENVIRONMENTAL APPLICATIONS

Organisations, like WHO and FAO, have realised the negative effect of pollutants on human health (Bereza-Malcolm *et al.*, 2015). Together with their concern grows the need for monitoring of dangerous substances in the environment. Pollutant residues can eventually accumulate in our food and drinking water. Food quality control systems are established to prevent that kind of cases. A biosensor, used instead of conventional chemical monitoring methods, must be easy to use, cost-efficient, stable when stored and capable of the detection of small amounts of the analyte. It must have a low detection limit and a short response time (Kumar *et al.*, 2006). Transferring a working microbial biosensor under controlled laboratory conditions onto the field requires a lot of work and optimization. What follows is an overview of current trends in the field of environmental microbial monitoring.

### 4.1 PESTICIDES

Pesticides are chemical or biological substances meant for pests control. Considering the target organism, we can divide them into insecticides, herbicides, fungicides, bacteriocides, nematocides and others. The use of the first three listed above represent 95 % of world consumption (Aktar *et al.*, 2009). Insecticides are the most acute toxic group of pesticides. Their extensive use has a major environmental impact, resulting in water and ground accumulation. The negative effect of the pesticides rose awareness decades ago and led to the development of detection methods like gas and liquid chromatography for the monitoring of organophosphate pesticides. These methods have brought high selectivity and sensitivity, but are inappropriate for field detection, require expensive equipment and a skilled technician (Mulchandani and Rajesh, 2011). Furthermore, chemical analytical methods only provide the information of pesticide identity and quantity, but no information about their toxicity. Some biosensors are capable to detect pesticide toxicity and therefore they are suitable for their detection. Biosensors represent their alternative.

Kumar *et al.* (2006) report of an optical biosensor with bacteria *Flavobacterium* cells, adsorbed on the glass fiber. The cells express organophosphate hydrolase on their surface, an enzyme, capable of the hydrolysis of the organophosphate pesticides to the optically measurable colour products. Synthetic samples of the methyl

parathion have been tested with the biosensor and a gas chromatography analysis. Biosensoric analysis was based on the ratio between the hydrolysed methyl parathion and the amount of the colourful product – p-nitrophenol. The methods have comparable results, but the biosensoric analysis is more cost-efficient. The developed biosensor showed a lower detection limit compared to other similar devices. The same detection principle of the p-nitrophenol as a hydrolysing product of the methyl parathion, was applied by Kumar and D'Souza (2010). They immobilized bacteria *Sphingomonas* JK1 on the bottom side of the microplate and linked them with an optical plate reader, to form an optical biosensor. This system enables multiple sample detection on one plate. Biosensor can detect 4–80 μM concentrations of methyl parathion and can be reused up to 75 times. Kumar and D'Souza (2011) also report of a recombinant *E. coli*, periplasmically expressing enzymes for methyl parathion hydrolysis, as a biorecognition element. Microbial cells were immobilized on a screen printed electrode, using glutaraldehyde. The researchers observed the changes in the electric current, caused by different concentrations of the methyl parathion. Biosensor showed good selectivity – it did not react to the addition of glucose, sucrose and endosulfan; the response to the phenol and p-aminophenyl sulfate was insignificant and had good stability – it preserved 80 % of the enzymatic activity after being used in 32 reactions. Expression of the organophosphate hydrolase on the surface of the cells is efficient, its sensitivity can be improved by the application of the genetic engineering methods.

Recombinant biofilm forming bacteria *Moraxella*, containing the ICN protein from the *Pseudosomonas syringae* INA5, are capable of the detecting 1 μM methyl parathion and 0.2 μM paraoxon (Mulchandani and Rajesh, 2011). Overexpression of the linA2 gene, encoding the γ-hexachlorocyclohexane dehydrochlorinase (LinA2) in *E. coli* BL21, has been used by Anu Prathap *et al.* (2012) for the development of a sensitive, selective and fast electrochemical biosensor. LinA2 protein catalyses the dehydrochlorination of lindane into trichlorobenzene, forming HCl as a by-product and causing an increase in the conductivity of the cell microenvironment that can be detected with the pulse amperometry. Authors report about the detection limit of 2 ppt for lindane.

*E. coli* is among the most frequently used organisms in the field of microbial biosensors. A bioluminescent strain PGRFM, including luxCDABE operon and promotor region of the pgi gene, important for the metabolic answer to the oxidative stress, was applied by Niazi *et al.* (2008) for the construction of an optical biosensor. The sensor showed excellent response to the methyl viologen, pesticide that causes the induction of reactive oxygen species, with the detection limit of 0.6 ppm, when exposed to starving conditions. Another detection principle for the toxicity of ametryn, fenamiphos and endosulfan was reported by Yong *et al.* (2011). Amperometrically working ferricyanid was used as a redox probe to measure the overall toxicity of the chemicals on the *E. coli* respiration. Endosulfan was the most toxic, with the $IC_{50}$ = 5.7 mg/L.

## 4.2 HEAVY METALS

Heavy metals are extensively used in several industry branches such as mining, metallurgical, electronics, electroplating and metalfinishing (Wang *et al.*, 2013). The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (Järup, 2003). Standard detection techniques – spectrometry, ionic chromatography, potentiometric electrodes – are expensive, sometimes time consuming and require high skilled technicians. Development of simple methods, suitable for field application, is the priority in the field of heavy metal analysis. Biosensor detection is among them.

Yüce *et al.* (2010) reported on the inclusion of the cyanobacterium *Phormidium* as the biosensing element of an amperometrical biosensor. Heat treated dead cyanobacterial biomass was mixed with carbon dust and added to a steal rod to form an electrode, capable of the detection of Pb(II) in water solution. The Ag/AgCl reference electrode and platinum wire as counter electrode were also the part of the apparatus that measures the changes in the electric field, induced by heavy metal water solution. Results showed good stability and repeatability, a hypothetical limit of detection was set for 5 X $10^{-8}$ M.

Microbial fuel cells (MFC) became important as well. The presence of a pollutant in wastewater can inhibit the metabolic activity of the electrochemically active bacteria, leading to the reduced electron transfer and weak current production. Single-chamber air–cathode MFC, enriched with real domestic wastewater have been applied by Shen *et al.* (2013) for the detection of Cu (II). They were interested in the response of a biofilm, formed by microorganisms in wastewater, at different flow rates. Higher feed rate causes higher shear rate in the surrounding of the MFC, leading to the overproduction of the extracellular polymeric substances and reduced biosensor sensitivity.

The effect of metals on microbes can be also measured with an oxidative stress biosensor. Ooi *et al.* (2015) constructed a biosensor, using *E. coli* DH5α™ transformed with pRSET-roGFP2 plasmid that enables fluorescent de-

tection of arsenic induced oxidative stress. The biosensor is fast, efficient and enables detection down to 0.2 µg/l of arsenic. The same microorganism was used by Arias-Barierro *et al.* (2010) for the detection of $Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$ and arsenite. The described biosensor is even more sensitive and enables the detection down to $1*10^{-7}$ mg/l arsenite, 0.001 ppm copper and zinc ions, 0.01 ppm cadmium ion and 5 ppm lead ions.

A continuous flow of the analyte to the biosensor is the most recent improvement in the field of biomonitoring. Kim *et al.* (2015) incorporated *E. coli* DH5 in a microfluidic device, capable of feeding nutrients and various concentrations of heavy metals ions under continuous-feed mode, for the detection of $Pb^{2+}$ and $Cd^{2+}$. The detection mechanism is based on the negative control of the GFP reporter gene, mediated by CadC-type transcriptional repressors, which bind to $Pb^{2+}$ or $Cd^{2+}$ divalent ions and derepress the GFP reporter promoters. They observed 3–4 fold increase in the sensitivity of the biosensor and good specificity dynamics to detect $Pb^{2+}$ in $Cd^{2+}$, comparing to conventional batch-type detection modes.

An alternative approach in the construction of a biosensor for the detection of heavy metal pollution on the field enables synthetic biology. The environmental pressure – oscillation in the temperature, pH, access to the nutrients and toxicants in the environment affect a diverse set of regulatory elements, controlling the downstream signal cascade (Bereza-Malcolm *et al.*, 2015). Microbial biosensor can be constructed de novo, using regulatory elements for the production of new genetic circuits. The authors estimate that this biosensor application can solve the problem of weak specificity and the toxic nature of heavy metals to the microbial chassis in real world applications.

### 4.3 TOXICITY AND GENOTOXICITY

The overall effect of the pollutants on the environment cannot be determined without an estimation of their toxicity. For the measurements of toxicity of water and ground samples, we use commercially developed tests – Mictorox® and ToxAlert® with *Vibrio fischeri*, Cellsense® with *Escherichia coli* (Rodriguez-Mozaz *et al.*, 2004). They use fluorescent and amperometric detection. These systems no longer fulfil the need for monitoring of the toxicants in the environment, so the development of new methods is of great interest.

The secondary plant metabolites can show antimicrobial activity. Chan *et al.* (2013) developed two biosensors for the evaluation of aldehyde and phenolic terpenes and isothiocyanate on the microbes. The first biosensor combined the characteristics of commercial biosensors – they used *E. coli* HB101 with the luxCDABE gene from *V. fischeri*, the other biosensor consisted of *Acinetobacter baylyi* ADP1_recA_lux, transformed with the luxCDABE gene from *Photorhabdus luminescens*. These transgenic bacteria produce light in the presence of toxicants, damaging the DNA; the intensity of the light is directly correlated with the recA expression level. RecA is an essential DNA repair gene. Isothiocyanate and cinnamaldehyde are the most toxic substances for *E. coli* – they mechanically damage plasmalemma, weaken the cell metabolism and the production of the energy, but they do not activate recA *A. baylyi* – it is less plausible that they damage the microbial genome.

Many studies examine the toxicity of heavy metals. An amperometrical microbial biosensor ToxTell applies different microbial species as a biosensing element, giving the optimal results of the toxicity of the real samples (Wang *et al.*, 2013). The test organisms, *Psychrobacter* bacteria, isolated from the wastewater plant were immobilized on a polycarbonated screen printed electrode membrane. They investigated the toxicity of $Cu^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Cr^{6+}$, $Hg^{2+}$ and $Pb^{2+}$ to determine the $EC_{50}$ value. The highest $EC_{50}$ value was observed for Pb – 110 mg/l, the lowest for Hg – 0.8 mg/l. The toxicity of the metals increases with the decrease of particle size, as shown by Ivask *et al.* (2014). They investigated the toxicity of the silver nanoparticles, according to their size, to bacteria *E. coli* and *Pseudomonas fluorescens*, yeast *S. cerevisiae* and microalgae *Pseudokirchneriella subcapitata*. The latter showed the highest sensitivity. Liu *et al.* (2014) reported on the use of MFC as a real-time wastewater toxic shock biosensor. They monitored the response of the microbes, isolated from a wastewater plant, to the shocks of $Cr^{6+}$, $Fe^{3+}$, $NO_3^-$ and sodium acetate. The growth of a biofilm on the anodic electrode was observed after five days. The biofilm enables the support and protection for electrogenic bacteria and improves the biosensor specificity – it can differentiate the chromium, iron, nitrate and sodium acetate shock. Single-chamber air–cathode MFC was used by Di Lorenzo *et al.* (2014) for the detection of cadmium. At optimal pH and temperature, the addition of cadmium in feeding water caused immediate change in the outgoing current. The biosensor enabled the detection of cadmium in the range of 1 to 50 µg/l.

In an extensive study of the application of yeast in a hypersensitive biosensor, capable of automatic detection of a broad spectrum of genotoxic pollutants, Wei *et al.* (2013) used transformed and mutated *S. cerevisiae* BY4741 cells to establish their response on genotoxic chemicals (methyl sulfonyl methane (MMS), 4-nitroquinoline-oxide (4-NQO), phleomycin, hydrogen peroxide, tert butyl hydroperoxide methil viologen, chlorambucil

***Table 1:*** *Overview of the biosensors, according to the target analyte, used microorganism, transducer type and detection specificity*
***Preglednica 1:*** *Pregled biosenzorjev po tarčnih analitih, uporabljenem mikroorganizmu, vrsti prevodnika in specifičnosti detekcije*

| Analyte | Microorganism | Transducer type | Detection limit (LOD), $EC_{50}$ or $IC_{50}$ | Reference |
|---|---|---|---|---|
| Methyl parathion | *Flavobacterium* | Optical | LOD = 0.3 µM | Kumar *et al.* (2006) |
| Methyl parathion | *Sphingomonas* JK1 | Optical | Detection range: 4–80 µM | Kumar and D'Souza (2010) |
| Methil parathion | Recombinant *E. coli* | Electrochemical | LOD = 0.5 µM | Kumar and D'Souza (2011) |
| Methyl parathion, paraoxon | Recombinant *Moraxella* | Electrochemical | Methil parathion: LOD = 1 µM, paraoxon: LOD = 0.2 µM | Mulchandani and Rajesh (2011) |
| Lindane | Recombinant *E. coli* BL21 | Electrochemical | LOD = 2 ppt | Anu Prathap *et al.* (2012) |
| Methyl viologen | Recombinant *E. coli* | Optical | LOD = 0.6 ppm | Niazi *et al.* (2008) |
| DCP, ametryn, endosulfan, fenamiphos | *E. coli* | Electrochemical | $IC_{50}$ = 5.7–22 mg/L | Yong *et al.* (2011) |
| Lead | *Phormidium* | Electrochemical | LOD = $2.5*10^{-8}$ M | Yüce *et al.* (2010) |
| Copper | Heterogeneous microbial populations | MFC | LOD = 5 ppm | Shen *et al.* (2013) |
| Arsenite, selenite | Recombinant *E. coli* DH5α™ | Optical | Arsenite: LOD = 0.2 µg/l, selenite: LOD = 5.8 ng/l | Ooi *et al.* (2015) |
| Cadmium, copper, lead, zinc, arsenite | Recombinant *E. coli* DH5α™ | Optical | Pb: LOD = 5 ppm, Cd: LOD = 0.01 ppm, Cu, Zn: LOD = 0.001 ppm, arsenite: LOD = $1*10^{-7}$ mg/l | Arias-Barierro *et al.* (2010) |
| Zinc, cadmium | Recombinant *E. coli* DH5 | Optical | / | Kim *et al.* (2015) |
| Isothiocyanate, cinnamaldehyde | Recombinant *E. coli* HB101 | Optical | / | Chan *et al.* (2013) |
| Isothiocyanate, cinnamaldehyde | Recombinant *Acinetobacter baylyi* ADP1 | Optical | / | Chan *et al.* (2013) |
| Copper, cadmium, zinc, chromium, mercury, lead | *Psychrobacter* | Electrochemical | $EC_{50}$: 0.8–110.1 mg/l | Wang *et al.* (2013) |
| Silver nanoparticles | *E. coli*, *P. fluorescens*, *S. cerevisiae*, *P. subcapitata* | Optical | $EC_{50}$: 0.01–8.17 mg/l | Ivask *et al.* (2014) |
| Chromium, iron, nitrate, sodium acetate | Heterogeneous microbial populations | MFC | / | Liu *et al.* (2014) |
| Cadmium | Electroactive mixed bacteria | MFC | Detection range: 1–50 µg/l | Di Lorenzo *et al.* (2014) |
| MMS, 4-NQO, phleomycin, hydrogen peroxide, tert butyl hydroperoxide methyl viologen, chlorambucil and cisplatin | *S. cerevisiae* BY4741 | Optical | 4-NQO: LOD = 0.12 ng/ml<br>MMS: LOD = 0.36 µg/ml | Wei *et al.* (2013 |
| Zinc, copper, 3,5-DCP, benzene, toluene, bronopol | Recombinant *E. coli* HB101 | Optical | $EC_{50;}$ 0.09–21.0 mg/l | Horsburgh *et al.* (2002) |
| Catechol | *Lactobacillus* | Electrochemical | Detection range: 0.5–5.0 mM | Sagiroglu *et al.* (2011) |

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| | | | | |
|---|---|---|---|---|
| Salicylate | Recombinant *E. coli DH5α* | Optical | LOD = 0.1 μM | Shin (2010) |
| BOD | *P. putida* SG10 | Electrochemical | Detection range: 0.5–10 mg/l | Chee (2013) |
| BOD | Electroactive mixed bacteria | MFC | Detection range: 100–750 ppm | Ayyaru and Dharmaligman (2014) |
| BOD | Heterogeneous microbial populations | MFC | Detection range: 3–164 ppm | Di Lorenzo *et al.* (2014) |
| BOD | Heterogeneous microbial populations | Electrochemical | Detection range: 20–450 mg/l | Vaiopoulou *et al.* (2005) |

and cisplatin). Transcripts for RNR3 and HUG1 genes that work as a sensor due to their overexpression induced with DNA damage were linked with the yEGFP reporter gene, enabling the fluorescent detection. They report of improved detection at mutants with five or seven genes deleted. The highest sensitivity was observed with quintuple and septuple mutants. The septuple mutant of the HUG1 sensor gene showed the greatest sensitivity (relative sensitivity: 0.12 ng/ml for 4-NQO and 0.36 μg/ml for MMS). Real-time monitoring is a key in the cases of large spills of toxicants to ensure an immediate response and reduce the negative effects on the environment (Di Lorenzo *et al.*, 2014). On-line microbial biosensor can be used for automatized detection of toxicity. The *E. coli* HB101 cell suspension transformed with the pUCD607 plasmid with a lux CDABE insert was applied by Horsburgh *et al.* (2002) for the detection of the toxicity of environmental samples from a metal plating plant, a paper mill and a distillery. They constructed a pump system, enabling continuous flow of the cells, mixed with the samples to a light detection unit. This biosensor is sensitive on a broad spectrum of chemicals (zinc, copper, 3,5-DCP, benzene, toluene, bronopol), the $EC_{50}$ values measured with a biosensor for zinc and bronopol were significantly more reliable than $EC_{50}$ measured by batch mode in a cuvette. The biosensor of this kind enables quick and cheap making of environmental samples fingerprint, without the use of chemicals.

## 4.4 PHENOLIC COMPOUNDS

Phenolic compounds that appear in the environment originate from the paper and pulp industry and from the production of drugs, dyes, and antioxidants (Rodriguez-Mozaz *et al.*, 2004). Lyophilised cells of the bacteria *Lactobacillus*, that were immobilised on a teflon-membrane oxygen electrode, work as a practical biosensor, suitable for the detection of catechol in wastewater and dairy products (Sagiroglu *et al.*, 2011). The sensor measures the difference in the concentration of dissolved oxygen depending on the concentration of catechol and shows good sensitivity, substrate specificity, repeatability and cost-efficiency. Aromatic compounds raise special awareness due to their toxicity and environmental resistance. The microbial activation mechanism, triggering the NahR regulatory protein synthesis in the presence of salicylate, was used by Shin (2010) for the construction of a biosensor. The *E. coli* DH5α was transformed with a pNRSAL plasmid containing the nahR gene and luciferase reporter gene, for the bioluminescent detection of salicylate. The response of the mutants, introduced by side directed mutagenesis at the residues 169 and 248 of the nahR gene was compared to the response of the wild type organism. The substitution of the amino acids leads into drastic changes in the microbial response to salicylate, including the 50-fold increase of sensitivity.

## 4.5 BIOCHEMICAL OXYGEN DEMAND

Biochemical oxygen demand (BOD or $BOD_5$) can be measured by a dedicated BOD test that applies aerobic microorganisms that consume the organic compounds in water systems for biochemical decomposition (Chee, 2013). BOD represents the oxygen used for neutralisation of organic compounds in 5 days, at 20 °C. Its conventional determination is time consuming and requests an expert to achieve repeatable results (Ayyaru and Dharmaligman, 2013). The use of biosensor enables us to avoid long-lasting incubation. They are mainly appropriate for the detection of BOD in samples with high concentration of easy-degradable organic compounds. Chee (2013) used five microorganisms (*P. putida* SG10, *P. fluorescens* IAM12022, *P. putida* IAM1236, *B. subtilis* IAM12118, *T.cutaneum* IFO10466) that were immobilized on a porous cellulose-nitrate membrane of an oxygen electrode for the detection of BOD in river samples. All of the organisms were exposed to artificial wastewater and standard solutions of glucose and glutaminic acid. The most sensitive one (*P. putida* SG10 with the detection limit of 0.5 mg/l) was applied for the characterization of

river samples. This biosensoric method is comparable with the determination of $BOD_5$ by the standard method.

MFC are also suitable for the detection of BOD. Ayyaru and Dharmaligman (2014) report of a single chamber MFC, enriched with electrochemically active bacteria, isolated from the University of Anna water treatment plant as a suitable biosensor for the characterization of the unstable BOD. They monitored the electric current, produced by MFC at continuous feeding of the properly diluted samples of artificial wastewater. The anodic electrode senses the BOD as a current, produced by electrogenic bacteria, when in contact with organic compounds. A similar principle was used by Di Lorenzo *et al.* (2014) for the evaluation of a single-chamber air–cathode MFC with multilayer 3D printing. MFC was enriched with heterogeneous microbial populations from another working MFC. They monitored the amperometrical response of the sensor on increasing concentration of acetate in water. The biosensor enabled a fast linear detection 3–164 ppm of chemical oxygen demand (due to the acetate, used in the study, it is similar to $BOD_5$)

The CO2 concentration in gas phase, a by-product of microbial respiration activity during the catalysis of organic compounds, can be measured for the determination of current BOD values in wastewater samples. This principle was used by Vaiopoulou *et al.* (2005) for the development of a microbial biosensor, consisting of a conical fluidized bed reactor and cylindrical oxygen saturation chamber. The cell biomass from the activated sludge was used as an immobilized biosensing component. The biosensor was firstly calibrated in a laboratory with artificial wastewater with the addition of glucose and acetic acid and later used for the detection of BOD in a wastewater treatment plant Xianthi. The biosensor is adjustable for a broad range of wastewater.It enables the use of microbial populations from existing wastewater treatment plant and shows high activity of the immobilized cells, due to the continuous oxygen feeding.

## 5 SUMMARY

Microorganisms are appropriate biosensing elements for the construction of environmental pollutants biosensors. They are used for the detection of heavy metals, pesticides, phenolic compounds, BOD and toxicity or genotoxicity. MFC and whole cell biosensors are the most frequently used biosensor types. The development of genetic engineering enables organism manipulation and improved action of the sensory system. The majority of biosensors stated above, show excellent performance in laboratory conditions, but are not yet all optimized for field applications.

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