CellPress

Review

Yeast Biosensors for Detection of Environmental Pollutants: Current State and Limitations

Sergio Jarque,[1,*] Michal Bittner,[1] Ludek Blaha,[1] and Klara Hilscherova[1]

Yeast biosensors have become suitable tools for the screening and detection of environmental pollutants because of their various advantages compared to other sensing technologies. On the other hand, many limitations remain with regard to their optimal performance and applicability in several contexts, such as low-concentration samples and on-site testing. This review summarizes the current state of yeast biosensors, with special focus on screening and assessment of environmental contaminants, discusses both pros and cons, and suggests steps towards their further development and effective use in the environmental assessment.

Yeast Biosensing Principle and Applicability Domains

Numerous chemical compounds that may represent a threat in terms of both environmental risk and human health are released into the environment, contaminating vital resources including water, air, and soil. Natural populations of many species have been reported to be affected by environmental pollutants, not only in industrial areas [1–3] but also in remote ecosystems once considered as pristine [4,5]. Given the widespread use of chemicals in products and industrial processes, exposure of both humans and wild biota may result in physiological alterations and adverse effects including developmental toxicity, reduced fertility, and neurological disorders [6–8]. In addition, diverse human activities such as farming, industry, untreated waste waters, and recreation abnormally increase the concentrations of degradable organic compounds in waters, which are then metabolized by aquatic microorganisms, resulting in a dramatic depletion of oxygen levels and posing a threat for other living organisms in the ecosystem. The rapid detection of both contaminants and oxygen shortage is required to prevent health and environmental risks.

Scientists have developed various biological tools for the rapid screening of a large number of chemicals in the environment to promptly detect those that could constitute a hazard (Box 1) [9–11]. Among the available tools, *in vitro* models such as genetically modified yeast have become particularly suitable for use in biosensors because of several advantages, including time- and cost-effectiveness, sensitivity, reproducibility, and scalability to high-throughput formats. Unlike other *in vitro* sensors not based on whole-cell systems, yeast biosensors allow specific detection of compounds in their bioavailable forms and, given the less-demanding growth conditions of yeasts, they are potentially adaptable to portable devices for in-field testing [12].

Yeast biosensors are constructed by coupling yeast cells sensitive to environmental compounds with electronic transducers. Yeast can be naturally sensitive to pollutants, for example,

Trends

Yeast biosensors are *in vitro* tools used routinely for environmental monitoring.

Current yeast biosensors are still far from optimal performance.

Recent improvements in yeast biosensing include increase of sensitivity, adaptation to high-throughput formats, and immobilization of cells into/onto supporting matrices that simplify procedures and enhance cell durability.

Further improvements such as portability and combination with online applications are necessary to make yeast biosensors accessible to in-field monitoring.

[1]Masaryk University, Faculty of Science, RECETOX, Kamenice 5, 62500 Brno, Czech Republic

*Correspondence: jarque@recetox.muni.cz (S. Jarque).

 http://dx.doi.org/10.1016/j.tibtech.2016.01.007

Box 1. Strategies Used for Detection of Environmental Pollutants

To help to contextualize the use of yeast biosensors for environmental monitoring purposes, Table I summarizes the advantages and disadvantages of other approaches commonly used for the detection of environmental pollutants. Given that yeasts are unicellular eukaryotic organisms, yeast-based methods combine the advantages of prokaryotic assays with being more representative of higher organisms.

Table I. Methods Used for Detection of Environmental Pollutants

Detection Method	Advantages	Disadvantages
Chemical analysis	Identification of specific compounds Low limits of detection No ethical issues	No information on biological activity Sample pre-cleaning usually needed Cost of analytical devices
Enzyme-based	Information on specific enzyme–substrate interaction Low limits of detection Results in minutes or hours Scalable to high-throughput formats No ethical issues	Low relevance for the cell or the whole-organism
Bacteria-based	Easy and inexpensive Results in hours or days Scalable to high-throughput formats Adaptable to portable devices	Low relevance for eukaryotic organisms Possible ethical issues of using genetic modifications Requires special equipment for sterile work
Yeast-based	Eukaryotic organism Easy and inexpensive Transfection with fully functioning vertebrate genes possible Results in hours or days Scalable to high-throughput formats Adaptable to portable devices	Unicellular organism Possible ethical issues of using genetic modifications Requires special equipment for sterile work
Algae-based	Easy and inexpensive Results in hours or days Scalable to high-throughput formats	Specific light requirements Nutrients in complex samples may mask effects of toxicants
Vertebrate cell *in vitro*	Specific signaling pathways can be studied separately without interference of whole-organism responses Numerous cell types available Results in hours or days Lower ethical issues compared to *in vivo* assays 3D cell cultures can mimic the responses of tissue explants	Not possible to replicate precise cellular conditions of a vertebrate organism Possible ethical issues of using genetic modifications Requires special equipment for sterile work Laborious and expensive compared to bacteria and yeast
Tissue explants	More comprehensive and complex functional information on pollutant effects than unicellular assays Possibility to use waste tissues from butchers	Ethical issues (if animals raised only for testing purposes) Laborious Duration of experiments Deterioration of tissues after a relatively short time Do not reflect systemic factors
Animals *in vivo*	Invertebrate and vertebrate models are available Reflect systemic factors The most accurate sensing system for quantifying animal-threat agents The most accurate sensing system for translation of results to human	Ethical issues Time and costs of raising and maintaining animals Duration of experiments Not suitable for use in biosensors High variability between animal species

Glossary

Amperometry: detection of analytes based on differences in the electric current after applying a potential.
Biodegradable organic compounds: organic contaminants relevant for waters, where their biodegradation by bacteria consumes oxygen. The amount of these substances is usually quantified as BOD.
Colorimetry: detection of analytes based on a chemical reaction that induces a change of color in the medium.
Cytotoxins: substances that have deleterious effects on cell function and viability.
Endocrine-disrupting compounds (EDCs): exogenous substances or mixtures that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, its progeny, or (sub) populations.
Fluorimetry: detection of analytes based on light emission. The light emission is triggered by light previously absorbed.
Genotoxins: chemical or other agent that directly or indirectly damages cellular DNA, resulting in mutations and consequent adverse outcomes (e.g., diseases including cancer).
Ligand: compound that interacts with a recognition biomolecule forming a complex that triggers a biological response.
Limit of detection (LoD): the lowest amount of substance that a given assay can significantly distinguish from controls (no presence of substance).
Luminometry: detection of analytes based on light emission. Unlike fluorescence, the light emission is induced by a chemical reaction and not by light.
Reporter gene: gene whose expression is easily detectable after linkage to the regulatory sequences of another gene of interest. It allows the assessment of gene expression related to that gene of interest. Reporter gene expression is typically measured as change of color, luminescence, or fluorescence signal.
Toxic metals: metals that can exert harmful effects at the levels that are relevant for polluted environment. These metals can be from the groups called biologically essential (e.g., Cu, Zn) or non-essential (e.g., Cd, Pb).

biodegradable organic compounds (see Glossary) and toxins, because of the intrinsic presence of molecular targets in the cell. In those cases, cells may need to be transfected with a **reporter** gene as a detection system to measure the chemical activity. However, their sensitivity is generally acquired by introducing specific foreign biorecognizing molecules able to recognize analytes of interest, such as **endocrine-disrupting compounds** (EDCs) and **toxic metals**. This process requires the transfection of cDNA encoding a specific receptor together with interacting partners and target promoters of a cotransfected reporter gene whose expression is then regulated by the analyte–receptor interaction. During the sensing process, chemicals enter yeast cells and interact with biorecognition molecules, including nuclear receptors, DNA, and enzymes, triggering a specific response that can be detected either by an electric current (**amperometry**) or by reporter gene activity (color, fluorescence, or luminescence), depending on the yeast biosensor design (Figure 1). The response signal is proportional to the initial biorecognition event, and it is thus possible to determine the effective concentration of the **ligand** of interest. This has been used (i) to characterize the actions of individual chemicals towards different biological targets for assessing hazards, and this in turn allows the calibration of sensors for individual prototypical compounds and estimation of their relative toxic potencies (equivalency factors), and (ii) to assess toxic activities in contaminated environmental samples. While the first hazard assessment approach has only partially employed yeast biosensors (owing to lower relevance of results to humans compared to other *in vitro* and *in vivo* models), many studies have used yeast biosensors as a tool for

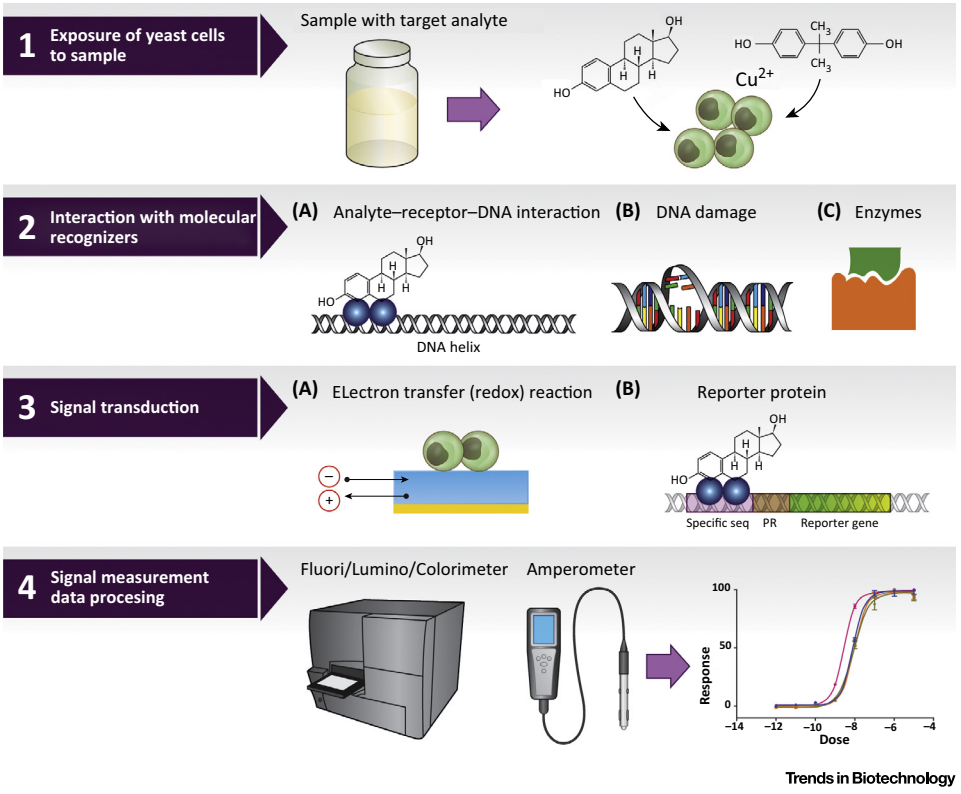

Figure 1. Yeast Biosensing Detection Principle. (1) Preparation of chemicals or environmental samples (including, e.g., extraction or pre-concentration of the samples) and exposure of yeast cells (exposure time varies depending on the yeast assay). (2) The compounds trigger a biological response in the yeast cells such as induced activity of a reporter gene, DNA damage, or metabolic reaction. (3) The signal associated with the biological response is (4) measured, amplified, and transformed into quantifiable values using an electronic transducer. The values are analyzed to evaluate effects of chemicals or environmental sample. Abbreviation: PR, promoter.

environmental monitoring in air, soil, aquatic, and biological samples. For example, yeast strains transfected with the aryl hydrocarbon receptor (AhR) have been used to estimate toxic potential in aerosols, combustion particles, and effluents from kraft mills and wastewater treatment plants [13–15]. Yeast biosensors have also been broadly used to assess anti-/estrogenic and anti-/androgenic potencies associated with waste, surface, bottled, and tap waters, as well as with suspended matter and sediments [16–19]. Similar approaches have been applied for other EDCs such as glucocorticoids or retinoids, and to evaluate metal concentrations and related toxicity in water samples [10,20]. Another application of yeast biosensors includes assessment of biochemical oxygen demand (BOD), an endpoint reflecting one of the key quality parameters in water monitoring studies.

When moving from research towards practical monitoring, standardization, and validation of any method (including yeast biosensors) is essential for its regulatory acceptance. With this respect, two yeast assays are undergoing the validation process within International Organization for Standardization Committee Draft (ISO/CD) as a standard biological method for the determination of the estrogenic potential of water and waste water (ISO/CD 19040-1).

Available Yeast Biosensors

The yeast biosensors developed so far have focused on several groups of environmental pollutants with well-characterized mechanisms of action and simple molecular targets (Table 1). In general, there are two different approaches: (i) biosensors for contaminants sharing specific biological or toxic potentials in a mixture (e.g., groups of biodegradable organics, groups of EDCs such as estrogens or androgens, metals, **cytotoxins**, **genotoxins** etc.), and (ii) biosensors highly specific for individual compounds (e.g., metals such as copper or priority EDCs such as bisphenol A). The biosensing principle is similar in all cases, and only the specificity of the target determines the range of detected compounds.

Biodegradable organics, generally quantified as BOD, comprise a large group of important aquatic contaminants. Although traditional and standardized analytical methods for BOD measurements exist, they are time-consuming, generally taking 5 days to obtain reliable values (BOD_5), and show relatively high interlaboratory variability due to fluctuations in the microbial diversity of the inoculum. By contrast, current electrochemical yeast biosensors reliably measure cellular respiration in only a few minutes [21]. Moreover, yeast cells are more resistant and tolerant than bacteria to aquatic chemical stress, and yeast biosensors thus remain functional for longer periods. It is also beneficial to use co-cultures of several yeast species with similar growth parameters and different substrate specificities to broaden the range of oxidized substrates covered [22]. Nevertheless, the number of yeast and fungal species used for BOD biosensing applications is smaller than the number of bacterial species that have been exploited [23].

Yeast biosensors targeting EDCs are particularly prominent because of the intrinsic ability of yeast cells to correctly express transfected vertebrate nuclear receptors, which allows relatively easy preparation of suitable transgenic yeast strains. Several key receptors involved in endocrine signaling have been utilized in yeast biosensors, and even transgenic strains cotransfected with two different receptors have been developed [24]. Among them, estrogen and androgen receptors have by far attracted the most attention given the still-growing knowledge of environmental occurrence and relevance of estrogenic and androgenic compounds. Several transgenic strains specific for single EDCs of particular interest such as tributyltin or bisphenol A have been developed. However, environmental samples often contain complex mixtures of contaminants, and the specific endocrine-disrupting potency of the sample is therefore better reflected by biosensors responding to a broader range of endocrine ligands sharing the same mode of action.

Table 1. Yeast Biosensors for Detection of Environmental Pollutants[a]

Pollutant Group	Detected Compound(s)	Receptor/ Gene	Detection	LoD (Range μM)	Ligand	Refs
Metals	Copper	CUP1	Amperometry Colorimetry Fluorescence Luminescence	0.11–500 1 0.5 0.5	Copper	[39,40] [20] [64] [65]
	Cadmium	AtPCS1	Fluorescence	0.2	Cadmium	[66]
	Heavy metals	cAMP-PKA	Fluorescence	12–45		[25]
Endocrine disruptors	Thyroids	TR	Luminescence Colorimetry	3.7×10^{-3} 0.5×10^{-3} to 0.075	T_3	[62] [62,67,68]
	Retinoids	RXR	Colorimetry Luminescence	0.01 0.06	9cRA TBT	[69,70] [71]
		RXR/RAR	Colorimetry	0.01	9cRA	[71]
	Tributyltin	RXR-ER	Luminescence	0.06	TBT	[69]
	Androgens	hAR	Colorimetry Luminescence Luminescence Fluorescence	0.2×10^{-3} 0.05×10^{-3} 0.5×10^{-3} 3×10^{-3}	DHT T DHT DHT	[73] [74] [26] [72]
	Estrogens	hER hER hERα hERβ hERα	Colorimetry Fluorescence Luminescence Luminescence Amperometry	0.01×10^{-3} 0.03×10^{-3} 0.03×10^{-3} to 0.04×10^{-3} 0.03×10^{-3} 1×10^{-3}	E2	[75–77] [78] [26,30,79] [26] [80]
	Progestagens	PR	Fluorescence Colorimetry	0.1×10^{-3} to 1 1	PG	[78,81] [78]
	Dioxin-like	AhR	Colorimetry Fluorescence	0.3×10^{-3} 5×10^{-3}	TCDD β-NF	[82] [78]
	Glucocorticoids	GR	Fluorescence	0.5	Budesonide	[83]
	Metabolic disruptors	PPARγ PPARγ antagonism	Amperometry Colorimetry	0.01 0.1	GI262570 Rosiglitazone	[84] [85]
	Bisphenol A	BPA-R	Luminescence	0.11	BPA	[86]
	Serotonin	HTR1A	Fluorescence	10	5-HT	[87]
Genotoxins	NA	*RAD54*	Fluorescence	50–90	MMS	[88–90]
		RNR2	Fluorescence	3–50		[89–91]
		RNR3	Fluorescence	10		[92]
		PLM2	Fluorescence	50		[90]
		DIN7	Fluorescence	50		[90]
		HUG1P	Fluorescence	3–45		[92,93]
Cytotoxins	Inhibitors of respiratory system	Cell respiratory system	Amperometry	0.8–1.2	KCN	[94]
	ROS inducers	*SOD1*	Fluorescence	0.1	Menadione	[27]
	General cytotoxins	NA	Luminescence	NA	NA	[26]
Biodegradable organics	NA	NA	Amperometry	39–206	BOD	[41,42,95,96]

[a]Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); 9cRA, 9-*cis*-retinoic acid; β-NF, β-naphthoflavone; AhR, aryl hydrocarbon receptor; AtPCS1, phytochelatin synthase from *Arabidopsis thaliana*; BOD, biochemical oxygen demand, BPA, bisphenol A, BPA-R, bisphenol A receptor; DHT, dihydrotestosterone; E2, estradiol; ER, estrogen receptor; GR, glucocorticoid receptor; hAR, human androgen receptor; hER, human estrogen receptor; HTR1A, 5-hydroxytryptamine receptor 1A; KCN, potassium cyanide; LoD, limit of detection; MMS, methylmethane sulfonate; NA, not applicable; PG, progesterone; PPAR, peroxisome proliferator-activated receptor; PR, progesterone receptor; T_3, triiodothyronine; TR, thyroid receptor; RAR, retinoid A receptor; RXR, retinoid X receptor; ROS, reactive oxygen species; TBT, tributyltin; T, testosterone; TCDD, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin.

Toxic heavy metals, one of the most hazardous groups of contaminants, can be detected by both non-specific and specific biosensors. The sensing element in non-specific biosensors is based on the cAMP/PKA (cAMP-dependent protein kinase) signaling pathway which mediates gene expression in response to generic toxic substances in yeast. These biosensors have been shown to recognize As^{3+}, Fe^{2+}, Pb^{2+}, and Cd^{2+}, but they seem to be nonselective to metals, responding to other generally toxic compounds [25]. In addition, the sensitivity of these biosensors is about two orders of magnitude lower than that of specific biosensors, and they are thus suggested for monitoring general toxicity rather than toxicity specifically associated with metals. Among the different metal species, copper has received the most attention because of the native presence of copper-inducible promoters (*CUP1*) in yeast cells, which has facilitated the development of specific strains for biosensing.

Another group of yeast sensors detects chemicals altering DNA structure. Unlike bacteria, yeast cells have similar DNA repair mechanisms to higher eukaryotes, sharing some genes or vertebrate homologs such as *RAD54*, *RNR2*, *RNR3*, *PLM2*, *DIN7*, *HUG1P.* This has been used to develop transgenic yeast strains responsive to DNA damage by coupling genes involved in DNA repair to reporter genes, mainly fluorescence-based systems. Given that some biological functions are phylogenetically conserved in higher organisms, responses in yeasts are considered representative indicators of genotoxicity. However, it remains unclear whether results obtained from yeast may be indicative of potential carcinogenicity in vertebrates.

Cytotoxicity testing using yeast biosensors is possible in different ways. Wild-type yeast cells can be used to detect changes in the oxygen respiratory activity in response to chemical exposure by amperometric methods. Similarly, cytotoxicity can be detected as an inhibition of luminescence in yeasts constitutively expressing luciferase enzyme [26]. Another indirect approach uses oxidative defense proteins, such as Cu,Zn-superoxide dismutase (*SOD1*), which are induced in response to the toxic effects of, for example, metal-related oxidative stress. GFP was fused to a chromosomal copy of *SOD1* to create a new yeast biosensor originally designed to assess material biocompatibility [27], although the potential applications are general and include testing of chemicals or environmental samples.

Despite the relevance of the aforementioned contaminants, the number of compounds covered by current yeast biosensors is relatively limited compared to the diversity of hazardous chemicals released into the environment. One of the main reasons for this is the high complexity of some biological systems that complicates their translation into simple *in vitro* models. In addition, molecular targets for many compounds are still not known, and this precludes the development of efficient yeast biosensors. It should also be highlighted that the positive response of the yeast biosensor indicates only the potency to interfere through a specific mode of action, and does not directly imply the effect (considering the complexity of regulatory and compensatory mechanisms feedbacks *in vivo*).

Detection Systems and Their Sensitivity

Yeast biosensors can be classified as amperometric, **colorimetric**, **luminometric**, or **fluorometric** according to the detection system used (Table 1; Figure 2), which partially pre-determines the sensitivity and thus the **limit of detection** (LoD). Amperometric biosensors detect analytes by measuring changes in electric current using specific electrodes after applying a potential. This strategy is used, for instance, to monitor water quality by measuring BOD values. Colorimetric, luminometric, or fluorometric biosensors most commonly use reporter genes associated to specific sequences to generate a measurable signal to quantify analyte–molecular target interactions [28]. Such biosensors are typically obtained by introducing two foreign elements into the yeast cell: a biorecognition molecule, notably vertebrate receptors able to recognize an analyte of interest (e.g., hormones), and a reporter gene under the transcriptional control of that biomolecule.

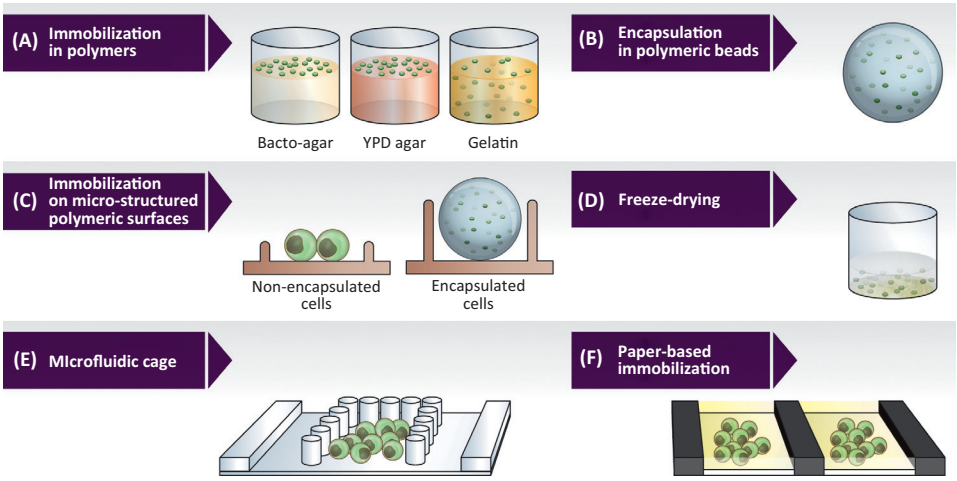

Figure 2. Examples of Immobilization Strategies for Yeast Biosensors. Times in parentheses below indicate typical duration of fully sensitive and viable cells. (A) Immobilization onto/into biocompatible polymeric matrices (90 days) [47]. (B) Encapsulation of cells into polymeric beads, for example, alginate-based beads, isolating cells from less-optimal environmental conditions (1 month) [44,45]. (C) Immobilization of yeast cells or beads onto micro-structured polymeric surfaces to increase bioavailability of analytes. Durability of cells and beads is estimated to be similar in A and B, respectively. (D) Lyophilization at low pressures and low temperatures to eliminate water content in cells and to achieve a biological 'standby' state (at least 10 months when stored at −18 °C) [55]. (E) Microfluidic cages used to trap yeast cells or beads while a solution flows through them. The microfluidic platform is mounted on a fluorescence microscope where the signal from cells is captured (several days) [38]. (F) Paper-integrated yeasts remain viable for periods longer than 1 year when stored at −20 °C. Yeast cells are immobilized into a polymeric channel where they will be exposed to the samples. To avoid cross-contamination, channels are separated by hydrophobic barriers. Abbreviation: YPD, yeast extract/peptone/dextrose.

Direct comparability of various detection systems and their sensitivities is difficult given the limited number of studies that use the same conditions. Moreover, sensitivity for each detection system may vary depending on the nature of the compound to be detected. For example, while amperometric yeast biosensors for the detection of metals show similar sensitivities to fluorometric and luminometric biosensors (e.g., in the range 0.1–1 μM for copper, Table 1), amperometric detection of EDCs is scarce and usually less sensitive than fluorescence and luminescence. The situation regarding the comparability of sensitivities of color-based versus fluorometric and luminometric detection systems is similar, ranging from comparable LoDs for some compounds to one order of magnitude less-sensitive colorimetric detection for other ligands (Table 1). However, fluorometric and luminometric systems often use simplified protocols in which yeast cell lysis and/or addition of external substrates are not required. As a consequence, current efforts to develop new yeast biosensors are more oriented to fluorescence and luminescence than to color-based systems.

Yeast biosensors show LoDs in the μM range for metals, cyto-/genotoxic compounds and BOD, and nM for EDCs (Table 1). Therefore, while levels of sensitivity for the first three groups of compounds are comparable to those obtained with other *in vitro* cell-based sensors (i.e., bacteria and vertebrate cells) [29], yeast biosensors for the detection of EDCs are still at least one order of magnitude less sensitive than their mammalian cell-based counterparts [30,31]. Thus, some EDC-contaminated samples which are detected by mammalian assays could go undetected by the yeast biosensor. This disadvantage can be minimized by extraction and pre-concentration of the samples tested; however, this is time- and labor-demanding. Moreover, pre-concentration of complex samples may increase non-specific cytotoxicity, making the assessment of EDCs technically impossible. The limited sensitivity of yeast sensors is especially relevant for particular compound classes such as alkylphenols [32]. Potential strategies to

enhance sensitivity should be focused on both key elements in yeast biosensors, in other words the bioreceptor and the reporter gene. In this respect, the study of the mechanistic details of reporter genes has arisen as a crucial task to improve sensitivity [33]. For instance, a new version of the luciferase reporter gene has been developed by removing the peroxisomal targeting codons, resulting in higher levels of light emission and, consequently, avoiding the need for cell lysis before response detection [34]. Computational analysis may help to detect the key amino acid residues involved in the enzymatic reactions associated with reporter genes, facilitating further modification and optimization of their structures using protein engineering techniques [35]. Molecular techniques allow up- and downstream sequences that regulate gene transcription to be modified to enhance reporter expression and the detected signal. However, this may also result in increased background noise. Finally, the validation and establishment of new reporter genes could contribute to both increased sensitivity and broadened applicability of yeast biosensors. For example, a new secretory luciferase from Japanese ostracod has successfully been implemented recently [36]. Unlike other luciferases, this secretory luciferase is highly stable at neutral pH and does not require cofactors for the enzymatic reaction. Because cell lysis is not required for measuring the luminescence signal, assays can be adapted to automated high-throughput platforms employed by other luciferase-based assays.

Scalability to High-Throughput Formats

Environmental assessment often includes large numbers of samples, thus demanding the development of efficient high-throughput approaches. Accordingly, yeast assays originally designed to be performed as single tests in tubes have been adapted to different multi-well microplate formats, increasing biosensor throughput and reducing the volumes required for the assays.

Reporter gene-based yeast assays are now typically performed in 96-well microplates, although higher-density designs, for example, 384- and 1536-well microplates, have been used as well [37]. Apart from microplates, even larger throughputs may be achieved with miniaturized microfluidic platforms [38]. The high throughput can also be enhanced to detect multiple toxic potencies on a single plate by combining, for example, two yeast populations with different reporter genes that elicit responses at different wavelengths [12]. In addition, the use of automated platforms and robots may further increase the time- and cost-efficiencies of yeast biosensors. To increase the ready-to-use nature of the methodology, approaches for immobilization of yeast have been explored, and most of the methods described are compatible with miniaturized and multi-analyte detection formats (see the following section).

High-throughput formats have also been gradually implemented for amperometric biosensors. First, copper-sensitive yeast strains were adapted to stirred measuring chambers to determine Cu^{2+} content [39]. Further, amperometric yeast biosensors using flow injection analysis have been developed that allow semi-continuous assessment of metals. Current versions of these biosensors can measure about 50 samples using the same sensor and remain stable for up to 2 months [40]. Similar methods have been also applied to biosensors for BOD assessment [41,42], where yeast cells were immobilized on high-density transducer surfaces and coupled to flow injection systems. As a result, assessment of BOD is now possible in only a few minutes and is compatible with semi-continuous measurement.

On-Site and On-Line Applicability

On-site applicability of yeast biosensors requires ready-to-use protocols able to preserve the viability and sensitivity of yeast after long periods of storage such that they can be used without previous reconstitution from frozen stocks and precultivation, a process that must be done under sterile conditions and requires 3–4 additional days. For this purpose, cells are immobilized or encapsulated in different matrices to create favorable microenvironments in which cells can survive

in 'standby' state (Figure 2) [43]. Although this latent state typically interrupts cell metabolism, cells are more easily reconstituted than those mixed with glycerol and stored at −80 °C, allowing fast transition to the fully-active state. Therefore, immobilized yeast biosensors reconstituted in growth medium are ready for use after short reconstitution times. Encapsulation may also be a good strategy to isolate cells from undesired nonspecific toxic compounds present in the media. The cells can then respond to compounds of interest without interference from the complex sample matrix, which would otherwise require additional sample pre-treatment [44].

Several polymers such as calcium alginate, polyvinyl alcohol, pectin, gelatin, agar, and silica gel have been used for cell immobilization, assuring cell viabilities from 28 days to 4 months (Figure 2) [44–48]. Yeast cells can be directly immobilized onto specific micropatterns of polymer surfaces, which enhance the efficiency of ligand–cell interactions [49], or can be encapsulated in polymer beads. The two strategies can be also combined to increase both the viability and stability of the cells and the bioavailability of compounds and samples. Some of these polymers have been successfully combined with commercial and prototype portable luminometers [12,47]. However, the use of polymers in yeast biosensors also has important limitations. First, the sensitivity of immobilized cells is typically at least one order of magnitude lower than that of their non-immobilized counterparts because of lower bioavailability, in other words limited diffusion of the analytes through polymers and gels [50]. Second, matrices tested so far degenerate after relatively short time-periods, mainly due to water evaporation, a process closely related to storage temperature, which results in alterations or partial cracking of the supporting matrix. Both limitations can be overcome by encapsulating cells in synthetic materials such as ceramic-like or artificial polyelectrolyte matrices with high stability and porosity as well as adaptability to different biosensor designs [51,52]. In experiments with bacteria, such advanced matrices maintained the viability and luminescence responses for several months [53]. Recently, microfluidic cages have been also used to immobilize bacterial biosensors; however, these platforms maintained functionality of cells for only short periods of time [38]. This approach could be potentially applied to yeast biosensors because microfluidic platforms have been demonstrated to be applicable to yeast cells for various purposes, including biosensing [54].

So far, the most efficient approach for the long-term storage of viable yeast cells is freeze-drying, which ensures the activity of cells for at least 10 months with no significant effects on biosensor sensitivity when stored at −18 °C [55]. However, a limitation for rapid on-site applications could be the extra few hours needed for recovery from the freeze-dried state to a completely active yeast biosensor. Similarly, yeast biosensors designed for pharmaceutical analysis and integrated into paper-based carriers were shown to be viable for more than 1 year when stored at −20 °C [56]. Importantly, the durability of both freeze-dried and paper-immobilized yeast cells was significantly shortened to 4 and 6 months, respectively, in biosensors stored at 4 °C, demonstrating that temperature is a key factor in maintaining the viability of yeast biosensors during long storage periods. The most likely explanation for this decrease in cell survival is partial rehydration of yeast cells associated with temperatures above zero. Moreover, given that yeast integrated into paper have been previously embedded into a polymeric layer, positive temperatures may facilitate the degradation of polymers, leading to subsequent loss of activity. Indeed, alginate beads stored at −80 °C have been shown to harbor viable and active cells for longer periods compared to beads stored at 4 °C [45]. This particular study, however, showed that the optimum storage temperatures may vary depending on the immobilization technique and the initial freezing process.

Immobilized yeast biosensors have recently been combined with flow injection platforms where cells are semi-continuously or continuously exposed to waters for quality monitoring [40,42,57]. Such injection systems use peristaltic pumps and press valves to control water flows. Yeast biosensors are located in isolated measuring chambers where cells are exposed to water samples, and the response signal is automatically measured. In these applications, waste

treatment systems have been included (e.g., high-temperature treatment) to avoid the release of viable transgenic cells into the environment [58]. Although yeast-based flow injection analysis platforms designed so far are basically prototypes, their effectiveness has been demonstrated by measuring metals and estrogenic compounds in industrial effluents [40], and BOD in bioreactors [42]. Recent studies with bacterial sensors showed that this approach is also applicable for assessing DNA damage, oxidative stress, and cytotoxicity [59].

It is important to remark that, although on-site testing approaches are designed to test environmental samples in field, yeast cells are not placed into direct contact with natural water bodies, thus excluding the possibility of escape of microorganisms and contamination of the environment. However, potential risks related to unintentional release of transgenic yeast into the environment should also be considered and minimized accordingly. In all cases, future development and use of yeast biosensors in field should meet current regulations on genetically modified organisms.

Concluding Remarks and Future Steps

Yeast biosensors are suitable screening tools for the detection of chemical compounds, but their applicability for routine environmental monitoring may be in some cases limited by drawbacks such as lower sensitivities than their mammalian cell-based counterparts. Nevertheless, significant advances such as protocol optimization, improvement of detection systems, and the development of new specific biosensors have been achieved recently. As a result, yeast biosensors are now an alternative to other analytical tools, and are being standardized as methods for environmental monitoring purposes. However, the number of compounds currently covered by yeast biosensors is limited to biodegradable organics (determination of BOD), EDCs, metals, cytotoxins, and genotoxins, which may constitute only a small fraction of the total toxic potency of a complex contaminated environmental sample. Because monitoring of all potential toxic compounds is impossible, development of rational prioritization schemes is a major challenge for future screening programs. Further needs include the development of yeast biosensors for new classes of prioritized chemicals and improvement of sensitivity to detect low levels of the molecules that are of concern in contaminated environmental matrices.

Important advances have been achieved in terms of high-throughput and on-site applicability, and yeast biosensors are now adaptable to various miniaturized platforms compatible with immobilization strategies. Although high-density portable detection devices are not yet available, medium-throughput prototype models have been successfully developed [12]. Current efforts further move towards the improvement of such devices and the development of high-throughput alternatives to obtain 'ready-to-use' biosensors that would allow on-site testing of large number of samples in a short time. In-field applicability may be further increased by using yeast based-sensors that do not require extraction and concentration of samples [60–62], and by the use of strains tolerant to more adverse environmental conditions such as high salinity [57]. Ultimately, portable devices could be combined with incipient online applications for continuous water monitoring [57,59,63].

Outstanding Questions

How can we improve the sensitivity of yeast biosensors to avoid pre-concentration treatments to detect chemicals in less-concentrated environmental samples?

How can we improve current yeast biosensors to obtain 'ready-to-use' versions more accessible to less-equipped laboratories and adaptable to high-throughput portable devices for in-field applications?

Which strategies should be implemented to identify chemicals of special concern and thus to be prioritized in screening programs?

Acknowledgments

This work was supported by the Ministry of Education, Youth, and Sports of the Czech Republic (projects LO1214 and LM2011028).

References

1. Sanchez, W. *et al.* (2011) Adverse effects in wild fish living downstream from pharmaceutical manufacture discharges. *Environ. Int.* 37, 1342–1348
2. Hallmann, C.A. *et al.* (2014) Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* 511, 341–343
3. Asker, N. *et al.* (2015) A gene to organism approach – assessing the impact of environmental pollution in eelpout (*Zoarces viviparus*) females and larvae. *Environ. Toxicol. Chem.* 34, 1511–1523
4. Jarque, S. *et al.* (2014) Analysis of hepatic deiodinase 2 mRNA levels in natural fish lake populations exposed to different levels of putative thyroid disrupters. *Environ. Pollut.* 187, 210–213
5. Jarque, S. *et al.* (2015) Background fish feminization effects in European remote sites. *Sci. Rep.* 5, 11292
6. Nair, R. *et al.* (2014) Methyl parathion inhibits the nuclear maturation, decreases the cytoplasmic quality in oocytes and alters the developmental potential of embryos of Swiss albino mice. *Toxicol. Appl. Pharmacol.* 279, 338–350

7. Préau, L. *et al.* (2015) Thyroid hormone signaling during early neurogenesis and its significance as a vulnerable window for endocrine disruption. *Biochim. Biophys. Acta* 1849, 112
8. Lelieveld, J. *et al.* (2015) The contribution of outdoor air pollution sources to premature mortality on a global scale. *Nature* 525, 367–371
9. Mehinto, A.C. *et al.* (2015) Interlaboratory comparison of in vitro bioassays for screening of endocrine active chemicals in recycled water. *Water Res.* 83, 303–309
10. Escher, B.I. *et al.* (2014) Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environ. Sci. Technol.* 48, 1940–1956
11. van der Meer, J.R. and Belkin, S. (2010) Where microbiology meets microengineering: design and applications of reporter bacteria. *Nat. Rev. Microbiol.* 8, 511–522
12. Roda, A. *et al.* (2011) A portable bioluminescence engineered cell-based biosensor for on-site applications. *Biosens. Bioelectron.* 26, 3647–3653
13. Olivares, A. *et al.* (2013) Developmental effects of aerosols and coal burning particles in zebrafish embryos. *Environ. Pollut.* 178, 72–79
14. Chamorro, S. *et al.* (2013) Analysis of aryl hydrocarbon receptor ligands in kraft mill effluents by a combination of yeast bioassays and CG-MS chemical determinations. *J. Environ. Sci. Health. A Tox. Hazard. Subst. Environ. Eng.* 48, 145–151
15. Allinson, M. *et al.* (2011) In vitro assessment of retinoic acid and aryl hydrocarbon receptor activity of treated effluent from 39 wastewater-treatment plants in Victoria, Australia. *Arch. Environ. Contam. Toxicol.* 61, 539–546
16. Jálová, V. *et al.* (2013) Estrogen-, androgen- and aryl hydrocarbon receptor mediated activities in passive and composite samples from municipal waste and surface waters. *Environ. Int.* 59, 372–383
17. Zhao, J-L. *et al.* (2011) Estrogenic activity profiles and risks in surface waters and sediments of the Pearl River system in South China assessed by chemical analysis and in vitro bioassay. *J. Environ. Monit.* 13, 813–821
18. Osman, A.G.M. *et al.* (2015) Screening of multiple hormonal activities in water and sediment from the river Nile, Egypt, using in vitro bioassay and gonadal histology. *Environ. Monit. Assess.* 187, 317
19. Nie, M. *et al.* (2015) Occurrence, distribution and risk assessment of estrogens in surface water, suspended particulate matter, and sediments of the Yangtze Estuary. *Chemosphere* 127, 109–116
20. Vopálenská, I. *et al.* (2015) New biosensor for detection of copper ions in water based on immobilized genetically modified yeast cells. *Biosens. Bioelectron.* 72, 160–167
21. Jouanneau, S. *et al.* (2014) Methods for assessing biochemical oxygen demand (BOD): a review. *Water Res.* 49, 62–82
22. Yudina, N.Y. *et al.* (2015) A yeast co-culture-based biosensor for determination of waste water contamination levels. *Enzyme Microb. Technol.* 78, 46–53
23. Lagarde, F. and Jaffrezic-Renault, N. (2011) Cell-based electrochemical biosensors for water quality assessment. *Anal. Bioanal. Chem.* 400, 947–964
24. Kawanishi, M. *et al.* (2008) Construction of a reporter yeast strain to detect estrogen receptor signaling through aryl hydrocarbon receptor activation. *Environ. Sci. Technol.* 42, 6897–6902
25. Radhika, V. *et al.* (2005) Engineered *Saccharomyces cerevisiae* strain BioS-1, for the detection of water-borne toxic metal contaminants. *Biotechnol. Bioeng.* 90, 29–35
26. Leskinen, P. *et al.* (2005) Bioluminescent yeast assays for detecting estrogenic and androgenic activity in different matrices. *Chemosphere* 61, 259–266
27. Yang, H-C. *et al.* (2005) In vitro assessment of biocompatibility of biomaterials by using fluorescent yeast biosensor. *Curr. Appl. Phys.* 5, 444–448
28. Close, D.M. *et al.* (2009) Reporter proteins in whole-cell optical bioreporter detection systems, biosensor integrations, and biosensing applications. *Sensors* 9, 9147–9174
29. Bohrn, U. *et al.* (2013) A critical comparison of cell-based sensor systems for the detection of Cr(VI) in aquatic environment. *Sensors Actuators B Chem.* 182, 58–65
30. Schultis, T. and Metzger, J.W. (2004) Determination of estrogenic activity by LYES-assay (yeast estrogen screen-assay assisted by enzymatic digestion with lyticase). *Chemosphere* 57, 1649–1655
31. Sanseverino, J. *et al.* (2009) Screening of potentially hormonally active chemicals using bioluminescent yeast bioreporters. *Toxicol. Sci.* 107, 122–134
32. Brix, R. *et al.* (2010) Evaluation of the suitability of recombinant yeast-based estrogenicity assays as a pre-screening tool in environmental samples. *Environ. Int.* 36, 361–367
33. Ding, B. *et al.* (2015) Mechanistic insight into marine bioluminescence: photochemistry of the chemiexcited *Cypridina* (sea firefly) lumophore. *J. Chem. Theory Comput.* 11, 591–599
34. Leskinen, P. *et al.* (2003) One-step measurement of firefly luciferase activity in yeast. *Yeast* 20, 1109–1113
35. da Silva, L.P. and Esteves da Silva, J.C.G. (2011) Computational studies of the luciferase light-emitting product: oxyluciferin. *J. Chem. Theory Comput.* 7, 809–817
36. Tochigi, Y. *et al.* (2010) Sensitive and convenient yeast reporter assay for high-throughput analysis by using a secretory luciferase from *Cypridina noctiluca*. *Anal. Chem.* 82, 5768–5776
37. Rajasärkkä, J. and Virta, M. (2011) Miniaturization of a panel of high-throughput yeast-cell-based nuclear receptor assays in 384- and 1536-well microplates. *Comb. Chem. High Throughput Screen.* 14, 47–54
38. Buffi, N. *et al.* (2011) Development of a microfluidics biosensor for agarose-bead immobilized *Escherichia coli* bioreporter cells for arsenite detection in aqueous samples. *Lab Chip* 11, 2369–2377
39. Lehmann, M. *et al.* (2000) Amperometric measurement of copper ions with a deputy substrate using a novel *Saccharomyces cerevisiae* sensor. *Biosens. Bioelectron.* 15, 211–219
40. Tag, K. *et al.* (2007) Amperometric detection of Cu^{2+} by yeast biosensors using flow injection analysis (FIA). *Sensors Actuators B Chem.* 122, 403–409
41. Renneberg, T. *et al.* (2004) A salt-tolerant yeast-based microbial sensor for 24 hour community wastewater monitoring in coastal regions. *Microchim. Acta* 148, 235–240
42. Seo, K.S. *et al.* (2009) A flow injection analysis system with encapsulated high-density *Saccharomyces cerevisiae* cells for rapid determination of biochemical oxygen demand. *Appl. Microbiol. Biotechnol.* 83, 217–223
43. Borovikova, D. *et al.* (2014) Immobilisation increases yeast cells' resistance to dehydration-rehydration treatment. *J. Biotechnol.* 184, 169–171
44. Ponamoreva, O.N. *et al.* (2015) Yeast-based self-organized hybrid bio-silica sol-gels for the design of biosensors. *Biosens. Bioelectron.* 67, 321–326
45. Fine, T. *et al.* (2006) Luminescent yeast cells entrapped in hydrogels for estrogenic endocrine disrupting chemical biodetection. *Biosens. Bioelectron.* 21, 2263–2269
46. Liu, K. *et al.* (2012) Generation of disk-like hydrogel beads for cell encapsulation and manipulation using a droplet-based microfluidic device. *Microfluid. Nanofluidics* 13, 761–767
47. Bittner, M. *et al.* (2015) Polymer-immobilized ready-to-use recombinant yeast assays for the detection of endocrine disruptive compounds. *Chemosphere* 132, 56–62
48. Cha, C. *et al.* (2012) Tuning structural durability of yeast-encapsulating alginate gel beads with interpenetrating networks for sustained bioethanol production. *Biotechnol. Bioeng.* 109, 63–73
49. Krol, S. *et al.* (2005) Encapsulated living cells on microstructured surfaces. *Langmuir* 21, 705–709
50. Chou, J. *et al.* (2012) Modeling analyte transport and capture in porous bead sensors. *Anal. Chem.* 84, 2569–2575
51. Rapoport, A. *et al.* (2011) Immobilisation of yeast cells on the surface of hydroxyapatite ceramics. *Process Biochem.* 46, 665–670
52. Böttcher, H. *et al.* (2004) Biocers: ceramics with incorporated microorganisms for biocatalytic, biosorptive and functional materials development. *J. Mater. Chem.* 14, 2176
53. Meunier, C.F. *et al.* (2010) Encapsulation of cells within silica matrixes: Towards a new advance in the conception of living hybrid materials. *J. Colloid Interface Sci.* 342, 211–224

54. García-Alonso, J. *et al.* (2010) Rapid and direct magnetization of GFP-reporter yeast for micro-screening systems. *Biosens. Bioelectron.* 25, 1816–1819
55. Jarque, S. *et al.* (2016) Freeze-drying as suitable method to achieve ready-to-use yeast biosensors for androgenic and estrogenic compounds. *Chemosphere* 148, 204–210
56. Weaver, A.A. *et al.* (2015) Incorporating yeast biosensors into paper-based analytical tools for pharmaceutical analysis. *Anal. Bioanal. Chem.* 407, 615–619
57. Pham, H.T.M. *et al.* (2013) Application of modified *Arxula adeninivorans* yeast cells in an online biosensor for the detection of estrogenic compounds in wastewater samples. *Sensors Actuators B Chem.* 185, 628–637
58. Pham, H.T.M. *et al.* (2012) EstraMonitor – a monitor for amperometric detection of estrogenic activity with *Arxula adeninivorans* yeast cells as the biocomponent. *Sensors Actuators B Chem.* 161, 137–145
59. Elad, T. *et al.* (2011) Online monitoring of water toxicity by use of bioluminescent reporter bacterial biochips. *Environ. Sci. Technol.* 45, 8536–8544
60. Balsiger, H.A. *et al.* (2010) A four-hour yeast bioassay for the direct measure of estrogenic activity in wastewater without sample extraction, concentration, or sterilization. *Sci. Total Environ.* 408, 1422–1429
61. Colosi, J.C. and Kney, A.D. (2011) A yeast estrogen screen without extraction provides fast, reliable measures of estrogenic activity. *Environ. Toxicol. Chem.* 30, 2261–2269
62. Li, J. *et al.* (2014) A yeast bioassay for direct measurement of thyroid hormone disrupting effects in water without sample extraction, concentration, or sterilization. *Chemosphere* 100, 139–145
63. Yagur-Kroll, S. *et al.* (2015) A miniature porous aluminum oxide-based flow-cell for online water quality monitoring using bacterial sensor cells. *Biosens. Bioelectron.* 64, 625–632
64. Shetty, R.S. *et al.* (2004) Fluorescence-based sensing system for copper using genetically engineered living yeast cells. *Biotechnol. Bioeng.* 88, 664–670
65. Roda, A. *et al.* (2011) Analytical strategies for improving the robustness and reproducibility of bioluminescent microbial bioreporters. *Anal. Bioanal. Chem.* 401, 201–211
66. Matsuura, H. *et al.* (2013) Development of surface-engineered yeast cells displaying phytochelatin synthase and their application to cadmium biosensors by the combined use of pyrene-excimer fluorescence. *Biotechnol. Prog.* 29, 1197–1202
67. Li, J. *et al.* (2008) A two-hybrid yeast assay to quantify the effects of xenobiotics on thyroid hormone-mediated gene expression. *Environ. Toxicol. Chem.* 27, 159–167
68. Shiizaki, K. *et al.* (2010) Establishment of yeast reporter assay systems to detect ligands of thyroid hormone receptors alpha and beta. *Toxicol. In Vitro* 24, 638–644
69. Kabiersch, G. *et al.* (2013) Bioluminescent yeast assay for detection of organotin compounds. *Anal. Chem.* 85, 5740–5745
70. Li, J. *et al.* (2008) A two-hybrid yeast assay to quantify the effects of xenobiotics on retinoid X receptor-mediated gene expression. *Toxicol. Lett.* 176, 198–206
71. Shiizaki, K. *et al.* (2014) Development of yeast reporter assay for screening specific ligands of retinoic acid and retinoid X receptor subtypes. *J. Pharmacol. Toxicol. Methods* 69, 245–252
72. Bovee, T.F.H. *et al.* (2007) A new highly specific and robust yeast androgen bioassay for the detection of agonists and antagonists. *Anal. Bioanal. Chem.* 389, 1549–1558
73. Gerlach, T. *et al.* (2014) Development and assessment of a novel *Arxula adeninivorans* androgen screen (A-YAS) assay and its application in analysis of cattle urine. *Sci. Total Environ.* 490, 1073–1081
74. Michelini, E. *et al.* (2008) A sensitive recombinant cell-based bioluminescent assay for detection of androgen-like compounds. *Nat. Protoc.* 3, 1895–1902
75. Routledge, E.J. and Sumpter, J.P. (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem.* 15, 241–248
76. De Boever, P. *et al.* (2001) Optimization of a yeast estrogen screen and its applicability to study the release of estrogenic isoflavones from a soygerm powder. *Environ. Health Perspect.* 109, 691–697
77. Kaiser, C. *et al.* (2010) Evaluation and validation of a novel *Arxula adeninivorans* estrogen screen (nAES) assay and its application in analysis of wastewater, seawater, brackish water and urine. *Sci. Total Environ.* 408, 6017–6126
78. Noguerol, T-N. *et al.* (2006) Detection of hormone receptor ligands in yeast by fluorogenic methods. *Talanta* 69, 351–358
79. Sanseverino, J. *et al.* (2005) Use of Saccharomyces cerevisiae BLYES expressing bacterial bioluminescence for rapid, sensitive detection of estrogenic compounds. *Appl. Environ. Microbiol.* 71, 4455–4460
80. Schwartz-Mittelman, A. *et al.* (2005) Electrochemical detection of xenoestrogenic and antiestrogenic compounds using a yeast two-hybrid-17-β-estradiol system. *Bioelectrochemistry* 65, 149–156
81. Chatterjee, S. *et al.* (2008) Screening of some anti-progestin endocrine disruptors using a recombinant yeast based in vitro bioassay. *Toxicol. In Vitro* 22, 788–798
82. Miller, C.A. (1999) A human aryl hydrocarbon receptor signaling pathway constructed in yeast displays additive responses to ligand mixtures. *Toxicol. Appl. Pharmacol.* 160, 297–303
83. Bovee, T.F.H. *et al.* (2011) Recombinant cell bioassays for the detection of (gluco)corticosteroids and endocrine-disrupting potencies of several environmental PCB contaminants. *Anal. Bioanal. Chem.* 401, 873–882
84. Chen, Q. *et al.* (2004) A yeast two-hybrid technology-based system for the discovery of PPAR?? agonist and antagonist. *Anal. Biochem.* 335, 253–259
85. Mueller, M. and Jungbauer, A. (2009) Peroxisome proliferator-activated receptor γ is constitutively activated in yeast. *Anal. Biochem.* 385, 365–367
86. Rajasärkkä, J. and Virta, M. (2013) Characterization of a bisphenol A specific yeast bioreporter utilizing the bisphenol A-targeted receptor. *Anal. Chem.* 85, 10067–10074
87. Nakamura, Y. *et al.* (2015) Applications of yeast-based signaling sensor for characterization of antagonist and analysis of site-directed mutants of the human serotonin 1A receptor. *Biotechnol. Bioeng.* 112, 1906–1915
88. Billinton, N. *et al.* (1998) Development of a green fluorescent protein reporter for a yeast genotoxicity biosensor. *Biosens. Bioelectron.* 13, 831–838
89. Afanassiev, V. *et al.* (2000) Application of yeast cells transformed with GFP expression constructs containing the RAD54 or RNR2 promoter as a test for the genotoxic potential of chemical substances. *Mutat. Res. Toxicol. Environ. Mutagen.* 464, 297–308
90. Bui, V.N. *et al.* (2015) Genotoxicity of chemical compounds identification and assessment by yeast cells transformed with GFP reporter constructs regulated by the PLM2 or DIN7 promoter. *Int. J. Toxicol.* 34, 31–43
91. Lu, Y. *et al.* (2015) Dual fluorescent protein-based bioassay system for the detection of genotoxic chemical substances in *Saccharomyces cerevisiae*. *Toxicol. Mech. Methods* 25, 698–707
92. Wei, T. *et al.* (2013) Construction and evaluation of two biosensors based on yeast transcriptional response to genotoxic chemicals. *Biosens. Bioelectron.* 44, 138–145
93. Benton, M.G. *et al.* (2007) The utilization of a *Saccharomyces cerevisiae* HUG1P-GFP promoter–reporter construct for the selective detection of DNA damage. *Mutat. Res.* 633, 21–34
94. Goldblum, D.K. *et al.* (1990) Oxygen transport in biofilm electrodes for screening of toxic chemicals. *AIChE J.* 36, 19–28
95. Nakamura, H. *et al.* (2007) A new BOD estimation method employing a double-mediator system by ferricyanide and menadione using the eukaryote *Saccharomyces cerevisiae*. *Talanta* 72, 210–216
96. Chan, C. *et al.* (2000) Designing an amperometric thick-film microbial BOD sensor. *Biosens. Bioelectron.* 15, 343–353