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MINI-REVIEW

Application of genetically engineered microbial whole-cell biosensors for combined chemosensing

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Abstract The progress of genetically engineered microbial whole-cell biosensors for chemosensing and monitoring has been developed in the last 20 years. Those biosensors respond to target chemicals and produce output signals, which offer a simple and alternative way of assessment approaches. As actual pollution caused by human activities usually contains a combination of different chemical substances, how to employ those biosensors to accurately detect real contaminant samples and evaluate biological effects of the combined chemicals has become a realistic object of environmental researches. In this review, we outlined different types of the recent method of genetically engineered microbial whole-cell biosensors for combined chemical evaluation, epitomized their detection performance, threshold, specificity, and application progress that have been achieved up to now. We also discussed the applicability and limitations of this biosensor technology and analyzed the optimum conditions for their environmental assessment in a combined way.

Keywords Engineered microbial whole-cell biosensor · Bioavailability · Combined chemicals

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Introduction

The environment is a complex mixture of chemicals and molecules, which have different biological effects and influences. What chemicals exist and to what extent they affect the living world are key issues in environmental conservation and remediation. As a helpful supplement to traditional methods of chemical identification, biosensors, especially genetically engineered microbial whole-cell biosensors (EMWCBs), have been developed and are considered to be an effective method for the environmental assessment.

EMWCB technology is a sensing methods that uses living microbial cells (prokaryote or eukaryote) to inform us about the composition, security, and quality of chemicals to which living organisms are exposed. These biosensors, especially the "lights on" biosensor (depicted in Fig. 1a), are integrated and designed to produce electric current, potential, heat, conductivity, chromagen, luminescence, or fluorescence signals controlled by promoters that are activated via transcriptional regulators that interact with chemicals or environmental stresses. Once these output signals are detected and calibrated, the presence and relative quantity of the activator can be interpreted. Because of their relatively high substrate specificity, EMWCBs better reflect the effect of different chemicals with little to no interference from other substances. EMWCBs respond rapidly and efficiently to the environmental chemicals, nutrients, etc., which then can be further assessed by other conventional analytical techniques.

Unlike other biological or toxicological tests (LD_{50}, LC_{50}, etc.) performed by higher animals and plants, EMWCBs, which are constructed by genetically engineered microbial cells, can be considerably more time- and cost-effective. EMWCBs can not only qualitatively evaluate specific toxins under a sublethal dose but also semiquantitatively assess their chronic effects instead of the acute impact of

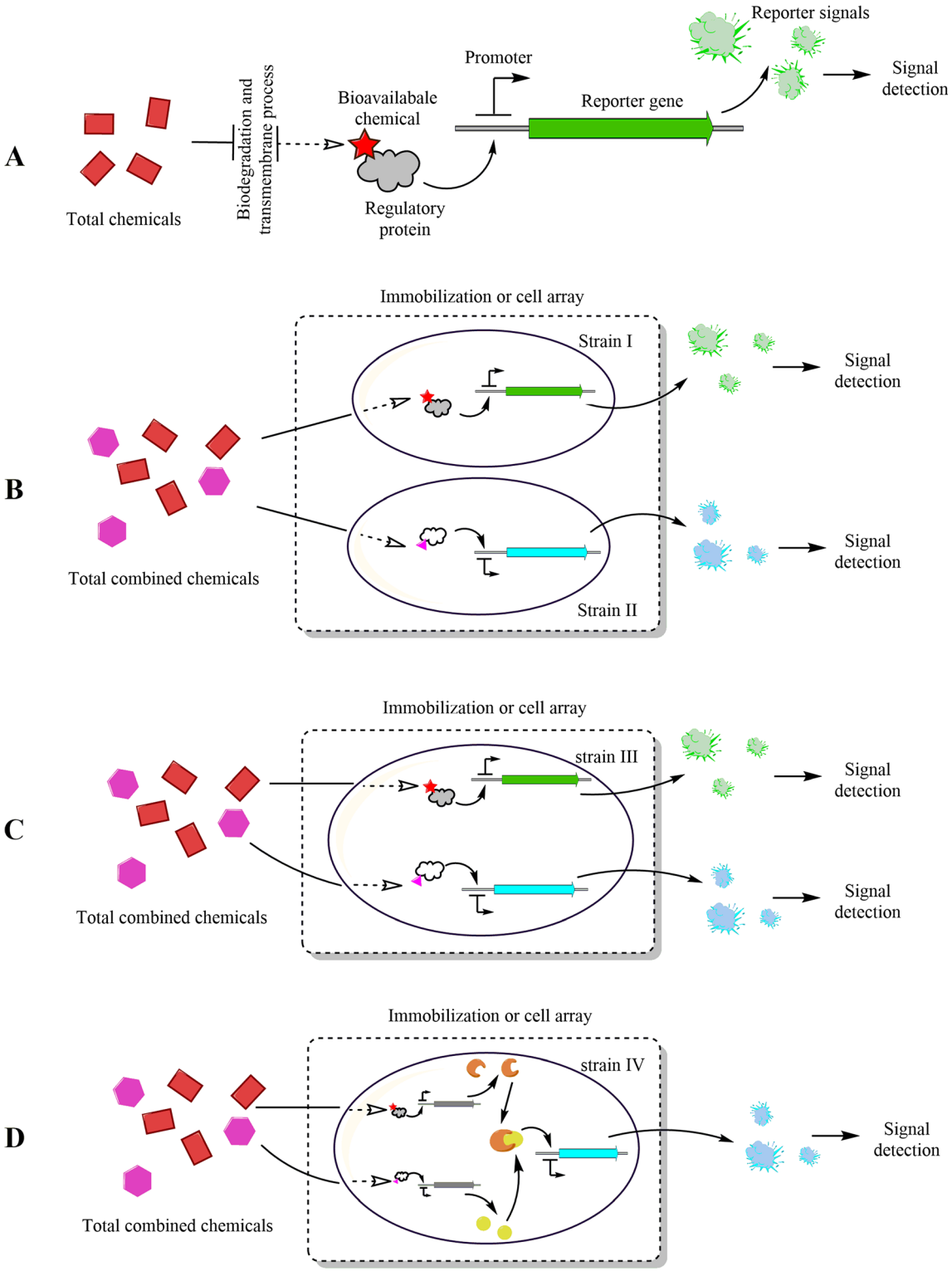

Fig. 1 General regulation, construction, and organization of EMWCBs. **a** General principle of the EMWCB construction, giving "lights on" biosensor as an example; **b** group of monofunctional EMWCBs for the combined chemical assessment; **c** multifunctional EMWCBs for the combined chemicals assessment; **d** the "AND gates" biosensor for multiple chemosensing

"global" contamination. With high specificities, EMWCBs can be used to monitor changes or online processes, inform us about the bioavailable fraction of certain chemicals. This information is interpreted as the maximum amount of a contaminant that is available for uptake by an organism within a specified period of time (Semple et al. 2007;

Riding et al. 2013), thus provides more biologically relevant information than traditional analytical or toxicological evaluations.

Studies investigating the use of EMWCBs for chemosensing have been previously reported, and an increasing number of strains have been constructed (Wei et al. 2014). Although they have been well tested in the laboratory, these biosensors and research into their applications are still confined to unitary function verification and simple assessment in simulated environmental systems. In this review, we will not repeat the minor details of the construction of EMWCBs (Belkin 2003; Magrisso et al. 2008; Tecon and van der Meer 2008; Xu et al. 2013), rather we will focus on the extent to which various chemicals can be assessed by EMWCBs. Moreover, readers will be introduced to where and how EMWCBs are used, in respect to their monitoring capacities and practical applicability in complex environmental systems.

Groups of the combined chemicals being assessed

Recognition between sensor cells and their target chemicals is based upon metabolic regulatory cassette (a transcriptional regulator connected to a promoter/operator), which are artificially fused with a promoterless reporter gene encoding an easily measurable protein (Harms et al. 2006) (Fig. 1a). This genetically engineered expression system determines the sensor's specificity and sensitivity. In this way, chemicals, especially combined organics, inorganics, and their mixtures, can be assessed by EMWCBs. Unlike most previous reviews, this section emphasizes recent approaches of EMWCBs in the combined assessment of chemicals instead of single substance evaluation. Note that the relationship between signal intensity and chemical bioavailability in real environmental samples is complex. The EMWCB tests do not result in absolute quantification but in semiquantitative assessments. According to earlier studies (He et al. 2010; Wei et al. 2014), although less than 10~20 % of the total pollutants (mercury and phenanthrene) were detected by sensor cells, the relative quantification results showed similar trends also applied for instrumental and analytical approaches. The results of several studies cited in the text also showed concordance with concentrations tested by traditional chemical analysis. Even so, it is too early to say that levels of chemicals detected by biosensors are relevant to the real environmental concentrations. In addition, the reporter's growth rate, physiology, etc. may interfere with the conclusions (Harms et al. 2006). As stated by Xu et al. (2013), different chemicals have different effects on different species. Results that are obtained using EMWCBs should be carefully explained, especially for the combined chemical evaluation.

The evaluation of combined organic chemicals

With rapid worldwide urbanization and industrialization, a large amount of organic waste has been generated and released by human activities. These organic chemicals, which have been classified as a major group of contaminants by the US Environmental Protection Agency (USEPA), are environmentally detrimental because of their potential carcinogenicity, teratogenicity, and other adverse effects on ecosystems. Due to their special chemical properties and complex migration and transformation patterns in the environment, the evaluation of the bioavailability of these organic pollutants is a problem, especially when they coexist with each other. Therefore, biosensors were exploited, and series of organic toxins have been measured under complex conditions. For example, construction of genetically engineered *Escherichia coli* bioreporter strains for the detection of 1,3-dinitrobenzene (1,3-DNB), 2,4,6-trinitrotoluene (2,4,6-TNT), and 2,4-dinitrotoluene (2,4-DNT) have been reported (Yagur-Kroll et al. 2014). Both *gfp* and *luxCDABE* fused *yqjF* and *ybiJ* reporters were immobilized in agar and induced by the three ligands. A clear bioluminescent signal was detected when they were placed on top of damp garden soil containing 2,4-DNT. Another successful case is *Acinetobacter baylyi* bioreporter ADPWH_alk, which was constructed by Zhang et al. (2012, 2013a, b). The sensor strain was used as detector for active sensing of alkanes and crude oil in seawater and sediment. This strain has the ability to detect a broad range of alkenes with carbon chain lengths from C7 to C36 and was employed to evaluate the seawater pollution by crude oil spill resulting from the Dalian oil tank explosion in 2010 in China. Similar to the those method, other biosensors have been developed for the detection of simple aromatic hydrocarbons (xylene, ethylbenzene, toluene, and benzene) (Selifonova and Eaton 1996; Applegate et al. 1997, 1998; Willardson et al. 1998; Stiner and Halverson 2002), middle-chain alkanes (Sticher et al. 1997), polychlorinated biphenyls (PCBs) (Feliciano et al. 2006), phenolic compounds (Shingler and Moore 1994; Hay et al. 2000; Abd-El-Haleem et al. 2002; Leedjarv et al. 2006), and polycyclic aromatic hydrocarbons (PAHs) (phenanthrene and naphthalene) (King et al. 1990; Tecon et al. 2006, 2009). A summarized description of those biosensors is listed in Table 1. Readers can also refer to the review by Tecon and van der Meer (2008) for more detailed information.

In addition to toxic pollutants, other organic matters, such as nutrients, secondary metabolites, and bioactive substances, have also been assessed by EMWCBs. Jaeger et al. (1999) used the reporter gene *inaZ* to construct biosensors 299R-sucr (*scrY::inaZ*) and 299R-tryp (*aatl::inaZ*) that respond to sucrose and tryptophan. Those two biosensors were used in the rhizosphere and showed significant differences in spatial distribution between tryptophan and sucrose in the space surrounding plant roots. Similarly, by constructing *lux* fused

Table 1 Applications of EMWCBs for environmental chemosensing and bioavailability assessment

Host strain	Reporter construct	Analyte (performance)	Application	Formation	Reference
E. coli XL1-Blue	*zraP-gfp*; *cusC-RFP*	Zn (>200 μM); Cu (>200 μM)	Spiked water samples	Free cells	(Ravikumar et al. 2012)
E. coli MC1061	*arsR-lux*	As(V) (80–141 μg/L), As(III) (8–18 μg/L)	Sediment (water suspension)	Fiber-optic immobilized and Free cells	(Ivask et al. 2007)
	cueR-luc, *copA-luc*	Cu (NR)			
	merR-luc	Hg (6.7 mg/L)			
	arsR-luc	As (6.1 μg/L)			
Ralstonia. metallidurans AE1433	*cupS-lux*	Pb (0.4 ppm), Zn (NR), Cd (NR), Co (NR)	Soil (water suspension)	Free cells	(Magrisso et al. 2009)
R. metallidurans AE2515	*cnrYXH-lux*	Ni (0.1 μM), Co (9 μM)	Soil (medium suspension)	Free cells	(Tibazarwa et al. 2001; Everhart et al. 2006)
Burkholderia. sartisoli RP007	*phn-luxAB*	Naphthalene (0.17 μM), phenanthrene	Seawater	Free cells	(Tecon et al. 2010)
E. coli DH5α	*alkS*, *PalkB-luxAB*	C6–C10 alkane, octane (24.5 nM)	Soil (DMSO or water extract), groundwater, seawater	Free cells	(Sticher et al. 1997; Bundy et al. 2001; Bhattacharyya et al. 2005; Tecon et al. 2010)
	capR-lacZ	Phenolic compounds sensing, phenol (0.1 μM)	Hospital water and soil (water extract)	Free cells	(Shin et al. 2005)
	tbuT-luxAB	BTEX, toluene (0.24 μM)	Seawater	Free cells	(Tecon et al. 2010)
E. coli	*yqjF-lux*, *ybiJ-lux*	DNT and TNT (5 mg/L)	Soil (water extract)	Free cells	(Yagur-Kroll et al. 2014)
	aaeXAB-lux	Phenolics sensing, ferulic acids (9.8 mg/L), vanillic acids (4.9 mg/L)	Hydrolysate	Free cells	(Monnappa et al. 2013)
Pseudomonas putida TVA8	*tod-lux*	BTEX (0.03–50 mg/L)	Soil, groundwater, and wastewater	Free cells	(Dawson et al. 2008; Diplock et al. 2009)
Methylobacterium extorquens	P_{cmuA}-*syfp2*	Methyl halide (10 pM)	In situ plant	Free cells	(Farhan Ul Haque et al. 2013)
Pseudomonas.fluorescens OS8	*dmpR-Po-lux*	Phenolic compounds sensing, phenol (0.08 mg/L), 2-methylphenol (0.03 mg/L)	Groundwater and dump leachate	Free cells	(Leedjarv et al. 2006)
Alcanivorax borkumensis SK2	*AlkSAb-PalkB1-luxAB*	Long-chain alkanes (5nM)	Seawater	Free cells	(Kumari et al. 2011)
Saccharomyces cerevisiae	*GPD-luxAB*; *ADHI-luxAB*	Dihydrotestosterone (2.5 nM), destosterone (0.25 nM)	Surface and drinking water	Free cells	(Eldridge et al. 2007; Di Dea Bergamasco et al. 2011)
E. coli; *P. putida*	*merR-gfp*; *nahR-lux*	Phenanthrene (~60 mg/kg), Hg^{2+} (~240 μg/kg)	Spiked soil samples	Immobilized cells	(Wei et al. 2014)
Anabaena torulosa		Cu (1.195–1.410 μg/L), Pb (0.027–0.25 μg/L), Cd (0.01–0.5 μg/L), 2,4-D (0.025–0.235 μg/L), Chlorpyrifos (0.025–0.117 μg/L)	Spiked water sample	Immobilized and Free cells	(Shing et al. 2013)
Acinetobacter baylyi	*ADPl-recA-lux*	MMC, BaP, Cr (VI), and Pb(II) (chemosensing)	Soil	Free cells	(Song et al. 2014)
E. coli	*nahR::lacZ*	salicylate and naphthalene (chemosensing)	Gas and aqueous samples	Immobilized and Free cells	(Cho et al. 2014)
S. cerevisiae	*golf-gfp*	2,4-DNT and 2,4,6-TNT (chemosensing)	Gas and aqueous samples	Free cells	(Radhika et al. 2007; Dhanasekaran et al. 2009)
P. putida	*xylR-lacZ*, *xylR-lux*	BTEX (chemosensing)	In situ soil	Immobilized and Free cells	(de las Heras and de Lorenzo 2011)

Table 1 (continued)

Host strain	Reporter construct	Analyte (performance)	Application	Formation	Reference
E. coli	P_{arsR}-*gfp*, P_{cusC}-*gfp*, P_{merT}-*gfp*, P_{luxI}-*gfp*, P_{zraP}-*gfp*, P_{zntA}-*gfp*	As, Hg, Cu, 3OC6HSL, Zn, Pb, Cd (chemosensing)	Spiked water sample	Free cells	(Wang et al. 2013)
	zntA-Pz$_{ntR}$-*gfp*	Zn, Pb, Cd	Soil	Free cells	(Hurdebise et al. 2015)

androgen and estrogen receptors, Eldridge and Sanseverino used genetically engineered *Saccharomyces cerevisiae* strains (BLYES and BLYAS) to detect the estrogenic endocrine-disrupting chemicals in raw and treated water samples (Sanseverino et al. 2005; Eldridge et al. 2007). Compared with the results of chemical analyses, the high-throughput, rapid screening of the compounds verified the biosensor method for the near-real-time detection of environmental hormone-disrupting chemicals.

The evaluation of combined inorganic chemicals

Except for natural causes, human activities, such as mining, smelting metalliferous ores, electroplating, and incomplete fossil fuel combustion, can produce a range of highly toxic inorganic chemicals. Heavy metal contaminants, for instance, are hazardous for almost all living organisms because of their massive environmental prevalence and strong biological toxicity. By linking a regulatory protein that recognizes specific metal ions to the expression of a downstream reporter gene, these circuits can be switched on and off. For instance, the regulatory protein MerR binds to an operator site close to the P_{merT} promoter. As long as mercurate is present, Hg(II) interacts with MerR, reducing its affinity for the target site and enhancing the expression of any downstream reporter genes, such as fluorescent proteins, luciferase, or β-galactosidase. Once variations in the level of signal protein or enzyme activity are quantified, the Hg(II) concentration can be evaluated. Although regulatory proteins have a high level of specificity for their targets, due to the characteristics of microbial metal resistance systems, interference from different metal ions should not be discounted, as real heavy metal contaminated samples always contain more than one metallic pollutant. Contrary to those studies that have merely focused on the demonstration of the proof-of-concept that bioavailability and toxicity could be assessed by biosensors (Ivask et al. 2002, 2009; Bondarenko et al. 2008; Brandt et al. 2008; Hynninen and Virta 2010), many groups have noticed the need for combined inorganic matter assessment in real environmental samples instead of those artificially amended ones. For example, Ravikumar et al. (2012) designed and constructed genetically engineered biosensors for the evaluation of zinc and copper levels after bioremediation. After genetically fusing the *zraP* and *cusC* promoters (components of regulatory circuit *CusSR* and *ZraSR*, respectively) to double-labeled signal protein genes, the multifunctional heavy metal detecting bacteria were able to efficiently sense low levels of heavy metals (16 μM zinc and 26 μM copper, respectively). The authors also assembled a bio-adsorption system and allowed the sensor cells to specifically assess the heavy metal toxins when at a combined concentration of 100 mg/L. Those findings suggest that, even when present in combination, zinc and

copper contaminants can be sensitively and effectively monitored by the multifunctional bacterial system.

It should be noted that the specificity is one of the key issues that determines the applicability of EMWCBs for combined chemosensing. The recognition of certain chemicals from an environmental mixture is controlled by the regulatory gene cassette within the microbial cells. Theoretically, as long as each sensor system specifically detect one chemical with sufficient sensitivity, together, they can detect combined chemicals (Fig. 1b). Related studies have been carried out by creating mutants in those key elements to enhance the biosensors' sensitivity and specificity (Gupta et al. 2012; Zhang et al. 2012; Lambreva et al. 2013). This refactoring, however, has a limit, as an infinite increase in the specificity of these proteins is unattainable. So far, the detection of a particular class of pollutants using EMWCBs is the easiest and the most practical choice. A concise overview of other applications for combined heavy metal assessment is presented in Table 1.

Evaluation of organic and inorganic chemical mixtures

EMWCBs have also been used to evaluate mixtures of organic and inorganic chemicals. Standing et al. (2003) used *Pseudomonas fluorescens* marked by *lux* to construct a triple reporter gene system that could report organic carbon, nitrogen, and inorganic phosphorus in soil. Combined with chemical analysis, this EMWCB system provides a quantitative description of the C, N, and P flow and a hyper spatial survey of root nutrient dynamics. This system also lays the foundation for in situ and real-time monitoring of nutrients. We also conducted related studies and used bacterial reporters to evaluate combined pollutants, especially mercury and phenanthrene in red soil (Wei et al. 2014). Bioavailable phenanthrene (~60 mg kg $^{-1}$) and Hg^{2+} (~240 μg kg $^{-1}$) were able to be detected and were assessed by relative fluorescent units and relative luminescence units. Detection of a single pollutant revealed a good correlation between signal strength and pollutant concentrations, whereas interference and bioavailability repression were observed in experiments where more than one pollutant was present. Other examples of sensors to detect inorganic and organic compounds in a complex mixture include a cyanobacterial sensor strain that used a fluorescence based reported to detect pesticides (chlorpyrifos and dichlorophenoxyacetic acid (2,4-D)) and heavy metals (Cd, Pb, Cu) (Shing et al. 2013). Immobilization was also conducted, and the reproducibility and long-term storage of the sensor cells were enhanced after coating with poly 2-hydroxyethyl methacrylate. Because of the obvious interference by mixed organic and inorganic chemicals, studies undertaking the detection of those mixed pollutants are rare. As such, if the technology is to be better applied to the determination of real environmental samples, further efforts are required to improve sample processing, determination conditions, and reporter bacteria stability and sensitivity.

Combined assessment of different environmental samples

Although many systems have been well tested in the laboratory or under ideal artificial conditions, the practical application of EMWCBs is difficult for many reasons. Regulatory requirements may limit or exclude the environmental release of recombinant DNA, and the complexities of real samples outside the laboratory restrict the actual performance of EMWCBs. Although we are currently far from the release of those biosensors into the environment, there have been significant efforts to evaluate the various samples from soil, water, atmosphere, and other environmental systems. Differences among the treatments of samples and sensor strains need to be taken into account, as the viability of reporter cells; the signal detection methods and their sensitivities will not be equal, especially when performing combined assessment. This section highlights the recent applications of EMWCBs in different environmental samples and readers are referred to Table 1 for a more detailed list. Note that the majority of the combined chemical detection methods are still confined to the analyses of specific classes of compounds or groups of homologs.

Combined assessment in soil and sediment

Soil assessment is a primary field for the application of EMWCBs for environmental monitoring, bioremediation, and ecological analysis. Since King et al. (1990) performed pioneering work on the exploitation of a *lux*-based signal expression system for the salicylate and naphthalene detection, many similar tests for the detection of specific inorganic and organic compounds or groups of chemicals have been developed. At present, the use of EMWCBs to analyze combined chemicals in soil and sediment has mainly focused on the areas listed below. Firstly, there have been a number of studies related to determination and assessment of combined soil pollutants and ecotoxicity. Without any pretreatment, Hurdebise et al. (2015) tested a bacterial bioreporter *E. coli* $pP_{ZntA}gfp$ strain for the ability to determine combined Zn^{2+}/Cd^{2+} levels in contaminated soil samples from Belgium. Results of this direct assessment suggested that the detection limits of this method are close to those of most chemical methods and Zn^{2+}/Cd^{2+} ratio superior to 50× or inferior to 10× is the ideal detection range for these two metallic elements. Although flow cytometry and other analytical instruments were used, this study indicated that combined chemosensing or rapid environmental risk screening by bioreporters is feasible.

Secondly, soil conditions and environment complex and as such, combined chemicals, are always presented in different forms. The processes of adsorption, desorption, migration, conversion, aging, etc. constantly influence chemicals' bioavailability. Thus, how to specifically recognize or determine the actual amount of each of the combined chemicals has become another research area of greater interest. For example, based on the construction of a recombinant reporter system nonspecific for Zn, Pb, Hg, Cu, Cr, and Cd, microbial biosensors were exploited by Zhang et al. (2013a, b) to assess genotoxic levels of these metals in soils collected from Northern China. They also tested sensor specificities for different forms of heavy metals and provided supplements for geochemical characterization study associated with conventional analytical methods.

Thirdly, because of the different chemical properties of various soil substances, the pretreatment methods of soil samples were investigated and optimal conditions were tested. For example, water-soluble and particle-absorbed insoluble heavy metals can coexist in soil and sediments (Degryse et al. 2009). In contrast, PAHs, with low solubility in water, must be extracted by organic solvents before they can be evaluated by EMWCBs. Therefore, contaminated soil samples are always assessed in water or water-resolved organic solvent extractions. Thus, the bioavailability of certain chemicals will exist in a particular range, instead of an absolute concentration value as different solvents have different extraction capacities for organic contaminants in soil samples. EMWCB tests can also be performed directly on soil slurries or sediments (Zhang et al. 2013a, b; Song et al. 2014), but the soil/cell ratio need to be carefully chosen. Increased soil content may provide higher sensitivity and accuracy, whereas the soil particles may interfere with signal detection. On the other hand, lower soil/cell ratios may reduce interference; however, this will also lower the sensitivity or accuracy of sensor cells and the content of the targeted chemicals will be underestimated. Additionally, organic solvents (such as methanol and butanol), the extraction agents for pollutants, may damage the membrane of sensor cells, resulting in either an increasing fatty acid supply for the bacterial luciferase and erroneous overproduction of bioluminescence or may result in toxicity, with concomitant loss of cell viability and signal intensity (Xu et al. 2013). These areas need to be carefully considered prior to beginning the experiment to try to minimize the impact on sensor cells.

Combined assessment in water

Rapid urbanization and the formation of large cities have exacerbated the problems of ground and surface water pollution in many developing countries, such as China. The quality assessment of water quality by EMWCBs is another issue of great concern, as water is one of the most important resources and the basis of all life. Using EMWCBs to evaluate the combined chemicals in water samples is more appropriate than in other environmental matrices. Water-soluble materials, such as combined heavy metals or eutrophic matter, are always evaluated by EMWCBs to indicate the presence of toxic substances or the extent of water contamination. Non-water-soluble pollutants can also be assessed and illustrate their bioavailability. Readers can refer to other reviews to learn more about principle of the construction of a certain bioreporter or about the simple use of single-chemical detection in water samples (Eltzov and Marks 2011). Here, we will review the results of several successful studies and discuss noteworthy aspects that are related to application of EMWCBs for the assessment of combined chemicals in water.

Compared to the soil, the assessment of combined pollutants by EMWCBs in aqueous solutions is much simpler. Water samples can be measured either after simple filtration and centrifugation or directly assessed without any treatment, thus making it possible for the broad investigation of combined chemicals in water. Leusch et al. (2010) carried out experiments in seven distinct laboratories with well-established bioassays to measure estrogenic activity in samples from four separate water sources. Galactosidase, luciferase activity, or cell proliferation were detected and calibrated after genetically engineered yeast or mammalian cell lines were exposed to a combined estrogenic compound. Comparing five bioassay methods (estrogen screen of the yeast, MELN, ER-CALUX, E-SCREEN, and T47D-KBluc assays) (Legler et al. 1999; De Boever et al. 2001; Fenet et al. 2003; Wilson et al. 2004), the results obtained from ER-CALUX and E-SCREEN system correlated well with chemical analysis. Their quantification limit can reach 0.2 and 0.1 ng/L, respectively.

Due to the low concentration of pollutants in water samples, as well as the sensitivity limitation of EMWCBs, the exploration of different methods to identify multiple pollutants in water is indispensable. Kuncova et al. (2011) investigated the response of bioluminescent *Pseudomonas putida* strain TVA8 to 23 organic pollutant-saturated solutions under different temperatures, cell concentrations, and time courses of signal. A sensor cell-based device was also constructed to assess benzene, toluene, ethylbenzene, and xylene (BTEX)-contaminated water samples. Total pollution concentrations from 0.5 to 120 mg/L were determined, and the results of the sensor system showed good agreement with data from side-by-side chemical analysis.

One caveat to these studies is that current research results about combined assessment in water by EMWCBs cannot be treated as the same. Wastewater samples may have two or more substances than they were expected. Other uncharacterized substances may also affect the viability of sensor cells, thus make EMWCBs fail to respond to high concentrations of target chemicals. Dilution or other

pretreatment procedures may alleviate such toxic effect to some extent. Cells without sensing element and cells that constitutively express the signal should also be included as controls to determine the level of background signal and indicate cell viability.

Combined monitoring in gas phase

The determination of combined gaseous substances by EMWCBs can be directly conducted in a liquid-phase assay after chemicals have been collected in a solvent. Although there are only a few of studies that have performed this type of work, the reported findings suggest a promising future for gaseous detection by EMWCBs. For example, Cho et al. (2014) developed a recombinant *E. coli* biosensor carrying a *nahR*::*lacZ* element regulated by NahR protein, which is sensitive to naphthalene degradation. When tested in aqueous and gaseous systems, all suspended and coimmobilized sensor cell respond specifically to naphthalene and salicylate. The sensitivity of the sensor cells in the gas phase was approximately five to ten times higher than that in the aqueous phase. Radhika et al. (2007) and Dhanasekaran et al. (2009) employed a different method and constructed a yeast-based green fluorescent protein expression system controlled by a mammalian olfactory signaling pathway for the detection of the odorant 2,4-DNT and 2,4,6-TNT, which are often found in explosive residues. As the detectable gaseous substances are usually at low concentrations and must enter the liquid phase to be identified by biosensors, these systems are more suitable for chemosensing or monitoring instead of quantification by EMWCBs.

Combined chemosensing in environmental matrices in situ

While many types of EMWCBs have been constructed, their responses to different chemicals in culture have also been assessed. However, difficulties still remain in their use for the monitoring of combined chemicals and extensive application to in situ sites. First, in situ environments, such as the rhizosphere or phyllosphere, are so complex that all the monitored parameters are uncontrollable. Second, maintaining the viability of the sensor strain and avoiding interference from other factors are key points for in situ assessment. Even so, several groups have made some progress in this area. For example, Farhan Ul Haque et al. (2013) developed a bacterial whole-cell biosensor that was capable of detecting femtomolar levels of methyl halides on plant leaves. This study also showed satisfying results, and the 10-pM detection limit can compete with other analytical method. Other groups have developed a water-soluble gelatin capsule system for BTEX detection in situ soil (de las Heras and de Lorenzo 2011). The experimental plan was carefully designed to activate the sensor cells at the appropriate occasion and sites. This research may facilitate the implementation of large-scale environmental sensing and screening for the localization of combined chemicals.

Different types and forms of EMWCBs

According to the different origins of sensing elements, EMWCBs can be divided into specific and nonspecific biosensor. The key element of the specific EMWCB comes from the regulation gene and its controlling promoter of the contaminant resistant bacteria. For example, *merR-PmerT* and *cueR-PcueA* from *Pseudomonas* sp. are sensitive to Hg(II) and Cu(II), respectively. With relative high specificity, genetically engineered bacterial cells with *merR* and *cueR* construct can be used as detectors for combined Hg(II) and Cu(II) assessment with no or little interference with other heavy metal ions. The sensing element of the nonspecific EMWCB is derived from microbial cellular stress, DNA damage, heat shock, or SOS system, such as *recA*. They can be sensitive to any hazardous material and are usually used for rapid screening and genotoxicity testing of the combined chemicals.

EMWCBS can be divided into three types according to their functions, which are shown in Fig. 1. The most commonly used EMWCBs are those containing a series of genetically engineered microbial sensor cells, in which each of the reporters can be induced by a specific substance. These monofunctional EMWCBs constitute a group that can be used to test a variety of chemicals in a combined system (Fig. 1b). Multifunctional EMWCBs are another type in which different report gene systems are contained in a single microbial cell (Fig. 1c). For example, a dual-reporter systems with different fluorescent protein genes *(grpE::dsRed* and *recA::egfp*) were constructed by Belkin (2003). The recombinant *E. coli* strain, therefore, has the ability to monitor both toxic cellular stress (via the expression of a heat shock protein GrpE) and DNA damage (via *recA* gene expression). Although combined chemical evaluation was not mentioned in this article, the strain well expressed different protein signals in response to ethanol (a heat shock inducer) and nalidixic acid (a SOS activator). Currently, based on the principle of the incorporation of logic gates for regulated cell signaling and synthetic biology methods, EMWCBs that sense multiple inputs have been generated (Wang et al. 2011, 2013; Silva-Rocha and de Lorenzo 2014). The commonly studied "AND gates" will only be open and produce signal when both chemicals are detected. If either input is absent, then no signal will result (Fig. 1d). Although the research is still in the early stage of constructing and validating these sensor cells, such EMWCBs have potential applications for environmental assessment (Bereza-Malcolm et al. 2015). It is important to note that a better specificity and stronger sensitivity are required, whether for monofunctional or multifunctional

sensor strains. In addition, the multifunctional integrated sensor strains should have consistent cellular states, as the expression of variety of signals will physiologically stress the cells.

Instead of the free cells, different devices have been constructed to ensure the vitality of reporters, as well as make them easier to use. Although many reported biosensing studies have directly used the recombinant cells after genetic engineering, methods of reporter cell immobilization and silicon-based cell arrays have been developed and gathered more and more attention. For example, Yagur-Kroll et al. (2015) designed a novel miniature support material-based silicon chip, retaining live recombinant *E. coli* on a rigid planar surface of porous aluminum oxide (PAO), and this sensor array was used for online continuous water monitoring. In the presence of combined compounds (hydroquinone, cadmium chloride, paraquat, and nalidixic acid), optical signals (bioluminescent or fluorescent) were generated. The selective signal production confirmed the applicability of this strain and device in the evaluation of urban wastewater. It was also found that the freeze-dried biochips maintained their effectiveness as long as 12 weeks, suggesting a high level of quality assurance for the method. Compared to free cell biosensor, the immobilized microbial reporting system has potential advantages, such as low cost, high throughput, and ease to use. It is noteworthy that prerequisites, such as sensor cells vitality maintenance, selection of less signal interference solid support material, and mass transfer resistance, must be considered and resolved.

Conclusion

EMWCB technology provides a useful tool for combined chemical assessment and risk evaluation of contaminated samples from various environmental matrices. The miniaturization, low cost, easy cultivation, broad adaptability, and potential for a highly integrated silicon chips are advantages for the application of environmental chemosensing. Although the attributes of speed, sensitivity, and specificity need to be further improved, the elimination of antibiotic label from these recombinant reporter strains should also be considered, thus making them environmentally friendly for in situ assessment. Moreover, a suite of different bioassays and combined chemical analysis might be more appropriate to obtain a comprehensive understanding of combined chemical assessments. Although there is still not a fixed standard or a unified evaluation index (the absolute and relative values of signal strength are mixed), a massive amount of environmental assessment data has been generated. To determine whether any useful messages can be interpreted from this information, the work of data mining should be carried out immediately.

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Compliance with ethical standards

Conflict of interest Wei He declares that he has no conflict of interest. Sheng Yuan declares that he has no conflict of interest. Wen-hui Zhong declares that he has no conflict of interest. Md. Ashaduzzaman Siddikee declares that he has no conflict of interest. Chuan-chao Dai declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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