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REVIEW

Recent advances in synthetic biology–enabled and natural whole-cell optical biosensing of heavy metals

Ankur Singh[1] · Vipin Kumar[1]

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Abstract
A large number of scientific works have been published on whole-cell heavy metal biosensing based on optical transduction. The advances in the application of biotechnological tools not only have continuously improved the sensitivity, selectivity, and detection range for biosensors but also have simultaneously unveiled new challenges and restrictions for further improvements. This review highlights selected aspects of whole-cell biosensing of heavy metals using optical transducers. We have focused on the progress in genetic modulation in regulatory and reporter modules of recombinant plasmids that has enabled improvement of biosensor performance. Simultaneously, an attempt has been made to present newer platforms such as microfluidics that have generated promising results and might give a new turn to the optical biosensing field.

Keywords Biosensors · Heavy metals · Sensing module · Reporter module · Metal-induced transcription

Whole-cell platform for metal biosensing: a glimpse

Heavy metals (HM) have remained a matter of concern for water quality due to their ability to persist for long duration in nature. Metals like Cr, As, Ni, Zn, Cd, Cu, Hg, and Pb are well-known toxicants in both plants and animals; thus, it becomes an important aspect of water quality assessment and monitoring. Biosensors have emerged as a new-generation technology in HM testing due to its clear advantage over spectroscopic and chromatographic techniques, as they provide rapid, cheaper, on-site, real-time detection [1]. IUPAC defined biosensors as "A device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" [2]. They can be considered as a hybrid analytical device of chemical and physical sensing technique that uses biomaterials as a receptor component [3].

A living cell has an innate capability to sense a variety of external stimuli and respond by energy transduction, which can be physical, physiological, or in the form of genetic transcription. Thus, living whole cells in themselves can be termed as a complete biosensor [4]. However, the response of the cells has been too often difficult and minute to observe and interpret, leading to a need of a secondary transduction device that can convert signals to more understandable forms. The use of living cells as receptor element evolved due to the presence of multiple receptor molecules on the cell that can be used for detection of multiple analytes. This initially facilitated the development of low-cost sensors; however, only qualitative detection remained plausible [4]. With the growing influence of biotechnology and synthetic cell biology, the transduction capability of cells improved, paving the path for quantitative detection of metals. Currently, both the toxicity measurements and concentration determination of HM using whole-cell biosensors are an active field of research.

The cells used as a receptor in detection of heavy metals and their toxicity can belong to a wide group of organisms. Prokaryotic organisms, including bacteria and cyanobacteria, and eukaryotic microbes, such as yeast, multicellular fungi, algae, protozoa, and even plant cells, have been reported to be used in biosensing [5]. However, the use of prokaryotic bacteria is disproportionately high (~ 85%) compared with all other eukaryotic cells (~ 15%) [6]. This is due to the ease of growing and maintaining cutlers in the lab, their adaptability to survive in

✉ Vipin Kumar
vipinmicro1@iitism.ac.in

[1] Department of Environmental Science and Engineering, Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand 826004, India

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adverse conditions with minimum nutrients, and their ability to serve as a good chassis cell for genetic circuits developed by synthetic biology. Among the eukaryotic group, the yeast dominated the scientific study due to (1) unlike bacteria they can be stored in dry and active forms, (2) more tolerant to a harsher environment than bacteria, and (3) have advanced receptors such as the G protein–coupled receptor (GPCR) [7]. Despite several complications, the use of eukaryotic microbes other than yeast continues due to the high degree of resemblance in the metabolism between the microbe and higher organisms that face heavy metal pollution in the environment. This makes these biosensors more reliable and accurate when the results are extrapolated [6].

Whole-cell biosensors for heavy metals can be broadly categorized into two groups. The first group comprises genetically engineered whole-cell biosensors (GEWB), which essentially involve expression of certain crucial genes regulated and promoted by other segments of the cell genome. The expression of genes monitors for the detection of metal concentration. This method employs the tools of synthetic biology for sensor development. The genetic circuit serves as a program for specific detection of metal [1]. Sensitivity and selectivity of these sensors are often comparatively higher than those of its counter group [8]. The second group comprises natural whole-cell sensors (NWB), which involve the interaction of the metal directly with the cell metabolites, cell membrane molecules, and the cell wall. The functional groups present in the biomolecules of the cell play an important role in signal generation. Most of the electrochemical biosensors fall under this group and response time for such sensors is often extended. However, in recent studies, the combined role of both biotechnology and electronics has significantly increased to obtain small, portable, and self-reliant devices for HM monitoring.

The form of output signal generated by a microbial whole cell in response to HM is important to decide the type of external transducer that may be needed to transform the signal to more understandable forms. Visual biosensors that rely on color change due to pigments may be directly visualized while the fluorescence/luminescence-based signal might require a simple device like a luminometer. If the signal is produced in the form of electron transfer, a sophisticated device like a potentiostat is useful. It is important to note that the signal generated by the cells is often a function of the nutrient concentration of growth media and the growth stage of culture from which they have been procured [1]. For on-spot single-time testing, a colorimetric sensor is more suitable than an electron transport–based system, which is fitting for situations where continuous monitoring is required.

Genetically engineered whole-cell HM biosensors

Prokaryotic microbes have evolved over millions of years to thrive in environmental conditions that are toxic and uninhabitable for other evolved multicellular organisms. They have developed several mechanisms that enable them to face high concentrations of toxic metals: (1) development of selective barriers, (2) continuous removal of toxicants from the cell, (3) transformation of HM to less/non-toxic forms, (4) intracellular and extracellular sequestration, and (5) reduction in metal sensitivity of cellular targets [9]. Today, most of the HM biosensors will simulate the HM resistance mechanism of these microbes [10]. The mechanism of HM resistance is often driven by the genetic codes in the plasmids of these organisms. In the last few decades, biotechnology hugely invested in decoding the mystery of these plasmids and made it possible to manipulate the sequences of the plasmids by deleting the unwanted portion and adding desired sequences from another organism. This efficiency of genetic modulation has brought revolutionary changes in biosensing.

A GEWB for HM has two important components: the sensing module (responsible for identifying of HM target and regulation of transcription) and the reporter module (responsible for generating an output signal) (Fig. 1). However, the two can be mediated with genetic circuits to improve the various parameters of a biosensor [8]. The circuits can be formed of toggle switches, logical gate, or amplification circuits. For example, Wang et al. [11] used AND gate to develop circuits with two input-dependent transcription factors in delivering a single quantitative fluorescence signal. A three-metal biosensor for As^{3+}, Hg^{2+}, and Cu^{2+} was also developed using a consortium of cells with single- and double-transcription factors [11]. Jia et al. [12] used a positive feedback mechanism to amplify the signal generated by reporter *mCherry*. The *luxR* gene expressed by the presence of arsenic promoted its own expression and that of *mCherry*. The genetic circuits designed on principles of logic gates such as AND, NOT, OR, NAND, and NOR can be used in the customization of signaling response for target molecules (Fig. 2). This increases the predictability of the molecular system and can be exploited to develop multi-input sensors which require complex regulatory systems [13, 14].

Continuous evolution has occurred in the strategies to enhance biosensor parameters such as the limit of detection, detection range, and sensitivity of systems over the past decade. These strategies mainly include tuning of receptor protein concentration, tuning intercellular ligand density, and reducing leakiness [15].

The sensory module

The sensory module often consists of a set of sequences that code for proteins capable of detecting the presence of HM ions in the cell environment. These are the transcription factors that respond to HM and are also chief targets for modulation using genetic engineering for developing a sensor with improved sensitivity and selectivity. The transcription factors can be

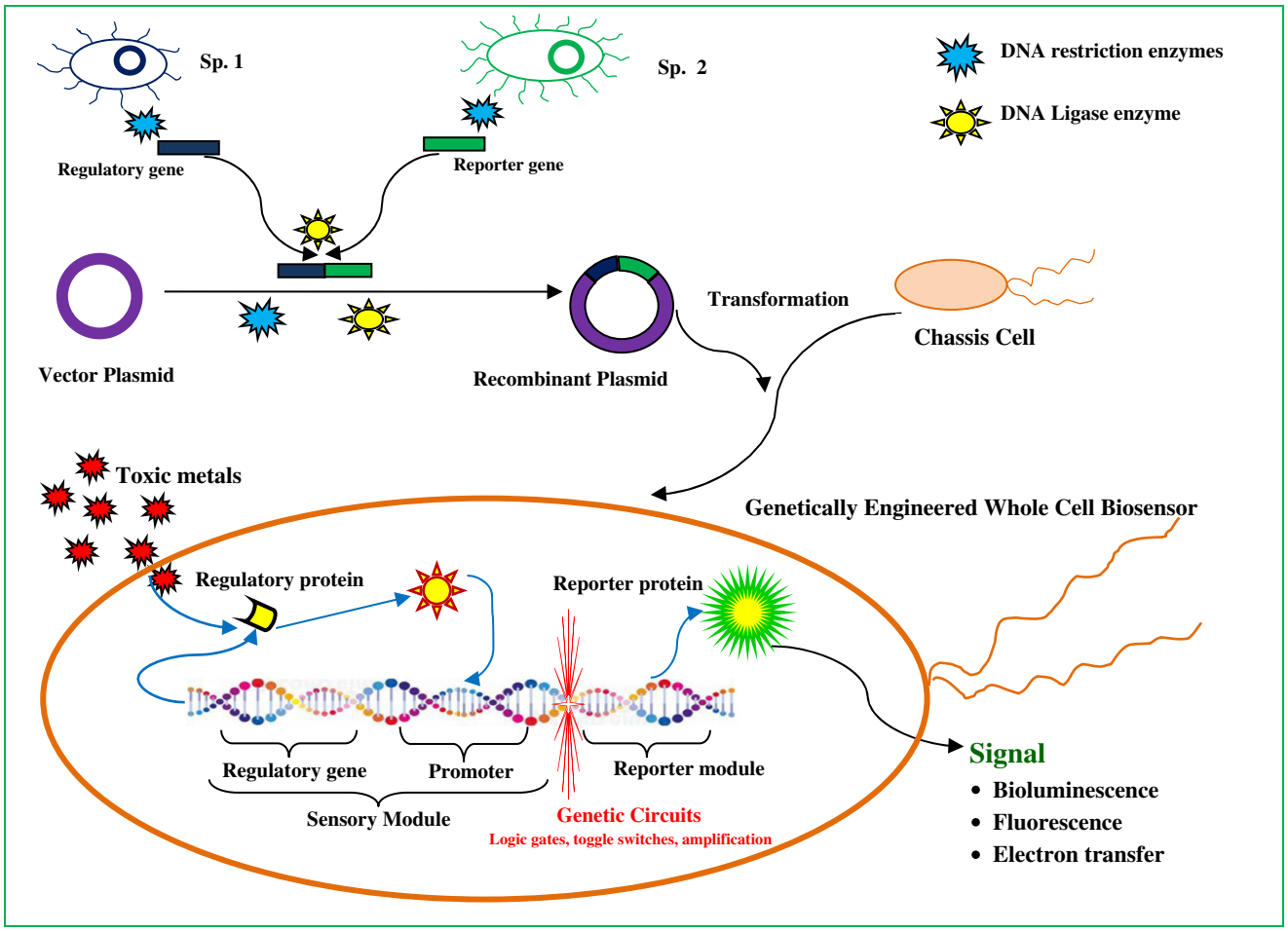

Fig. 1 Development of GEWB by the recombinant plasmid method, with a generalized flow of signals through the genetic circuit of the biosensor

organized in five groups—*MerR*, *ArsR/SmtB*, *DtxR*, *Fur*, and *NikR*—based on their structures and functions [8]. Among these groups, *meR* and *ArsR* that are responsible for metal detoxification are most commonly exploited. The *meR* group is known for their promotion activity while *ArsR* groups are suppressors in their native genomes.

The *MeR* family of genes consists of many members such as *cadR* (Cd resistance), *merR* (Hg resistance), *cueR* (Cu/Au/Ag resistance), *pbrR* (Pb resistance), and *zntR* (Zn/Cd/Pb resistance) and is known as the promoter of transcription (Table 1), making them useful in developing turn-on signal sensors, which have been observed to have an advantage of

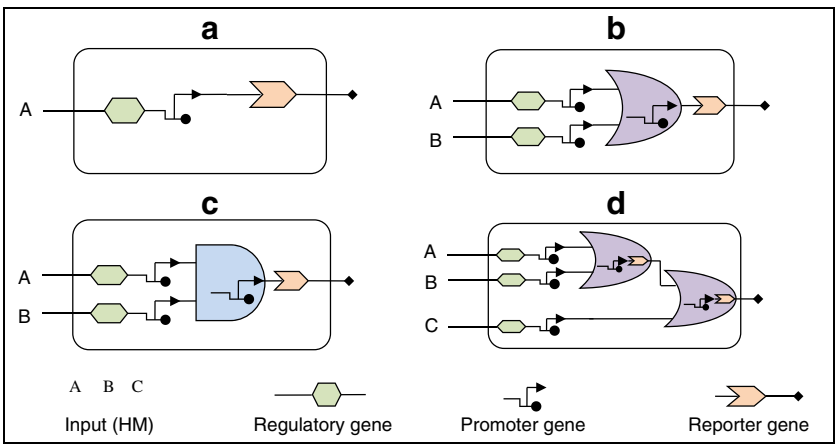

Fig. 2 Possible schemes of genetic circuit for HM biosensing based on logical gates: (**a**) most common signaling pathway with single input signal; (**b**) pathway with dual input and single output signal, reporter gene induced by either of the inputs; (**c**) single output signal induced by essential presence of both input signals; (**d**) possible circuit with multiple inputs, sensory system induced by presence of either of the target metals complementary to regulatory protein

Table 1 Recent sensory and reporter modules with corresponding chassis whole cell used for HM biosensing

S. no.	Promoter/regulator	Reporter gene	Host/chassis cell	Output signal	Reference
1.	Promoter gene *Pars* for *arsR* and promoter *Pmer* for *merR*	Operon *luxCDABE* and green fluorescent protein gene (*gfp*)	*Escherichia coli* DH5α	Increase in luminescence and green fluorescence	[10]
2.	Promoter *Pars* and *arsR* gene Promoter P*luxI* and *luxR* gene	Reporter *mCherry*	*Escherichia coli*	Increase in red fluorescence	[12]
3.	Zinc-responsive promoter (*PzntA*) and *zntA*	Riboflavin synthetic genes (*rib*B) and porin protein gene (*oprF*)	*Escherichia coli* BL21 (DE3)	Improved voltage production in fuel cell	[16]
4.	Promoter genes *PcadR* and sensory gene *cadR*	Enhanced green fluorescent protein gene (*egfp*)	*Escherichia coli* DH5α	Increase in green fluorescence	[17]
5.	Promoter gene *P479* with *MerR*	Red fluorescent protein gene (*RFP*)	*Escherichia coli* DH5α	Increase in red fluorescence	[18]
6.	Promoter *Pmer* with *merR* and *pbrR*	Enhanced green fluorescent protein gene (*egfp*)	*Escherichia coli* DH5α	Increase in green fluorescence	[19]
7.	Promoter *PT7* and *cadO*	Enhanced green fluorescent protein gene (*egfp*)	*Escherichia coli* DH5α	Increase in green fluorescence	[20]
8.	Promoter *CUP1*	Bifunctional purine synthetic protein gene (*ADE5,7*)	*Saccharomyces cerevisiae*	Production of red pigment	[21]
9.	Promoter *PrcnA* and regulator *RcnR Ni/Co* (mutated form)	Reporter *luxCDABE* gene	*Escherichia coli* TD2158 wild-type	Increase in luminescence	[22]
10.	Arsenical resistance operon repressor (*arsR*) and *ParsR*	Green fluorescent protein gene (*gfp*)	*Escherichia coli* DH5α	Green fluorescence promotion	[23]
11.	Promoter *Pcad* and *cadC* gene	Green fluorescent protein gene (*gfp*)	*Escherichia coli* DH5α (pNV12).	Increase in green fluorescence	[24]
12.	Promoter *PcadR* and *Pcad* transcription regulator (*cadR*)	Green fluorescent protein gene (*gfp*)	*Escherichia coli* DH5α	Increase in green fluorescence	[25]
13.	Promoter *Pars* and *arsR* gene	Beta-galactosidase gene *lacZ* gene	*Escherichia coli* DH5α	Increase in blue color	[26]
14.	Regulator *arsR* and promoter *ParsR*	Green fluorescent protein gene (*gfpmut3b*)	*Escherichia coli*	Increase in green fluorescence	[27]
15.	Chaperonin GroEL (*groEL*)	Beta-galactosidase gene (*lacZ*)	*Escherichia coli* Nova Blue	Decrease in blue color	[28]
16.	Continuously expressed *Ptet* on *pWH1274_lux*	Luminescent *proteins gene* (*luxCDABE*)	*Acinetobacter baylyi* ADP1	Inhibition of luminescence	[29]
17.	Regulator *CopSR* gene	Red fluorescent proteins gene (*RFP*)	*Ralstonia eutropha* and *Cupriavidus metallidurans*	Increase in red fluorescence	[30]
18.	Cd-responsive Promoter *PzntA*	Operon *luxCDABE*	*Escherichia coli JW3596*	Increase in green fluorescence	[31]
19.	Promoter *MTT1* and metallothionein protein coding gene *MTT5*	Green fluorescent protein gene (*gfp*)	*Tetrahymena thermophila*	Increase in green fluorescence	[32]

specificity over turn-off sensors [6]. Khan et al. [16] used the promoter of *zntR* to enhance the cell permeability leading to the release of ions in the extracellular environment, thus managing to increase the voltage produced in a microbial fuel cell–based biosensor. The *merR* promoters are known for their efficient response and hypersensitivity (Hill coefficient of ca. 2.6) to Hg ions and thus are commonly used [33]. Guo et al. [17] effectively used the *meR* module with *cadR* and *PcadR* in association with the reporter gene *gfp* for biosensing of Hg. Later studies show that an addition of promoter *P749* in the *merR* module causes continuous expression of *merR* leading to high concentrations of receptor protein that promotes the linear response of the sensor to Hg [18]. A similar enhancement of sensitivity can be observed with the concentration increase of *LuxR* [34] and *CadR* [17] receptor proteins obtained by upstream promoter sequences. However, contradicting results have also been reported where reduction in receptor protein concentration using a repressor (for *tetR*) seems to enhance the sensitivity of the system [34]. The effect of receptor protein concentration depends on the gene type and needs to be optimized for the most efficient signaling. The degree of expression of the receptor protein drives the sensitivity of the biosensor and can be understood by the "promoter-slope parabola principle" [17]. A too low concentration fails to generate an effective signal while overexpression of transcription factors can create a toxic intracellular environment leading to mutations [34].

Based on the *merR* and *arsR* modules, Huang et al. [10] constructed biosensors for simultaneous detection Hg and As in groundwater with two separate reporters, *luxCDABE* and *gfp*. In systems that contain original feedback loops of the *meR*, the background expression of the reporter gene can be

sometimes high and unwanted; thus, systems with an uncoupled reporter gene from feedback loop are preferable [19]. It has been demonstrated that sensitivity can be enhanced by uncoupling; however, the linear range of detection reduces compared with the uncoupled circuit [19].

The abovementioned sensory modules are located in the plasmid, but the sensory modules can even be placed directly in the cell genome of microbe. Kim et al. [20] tested various combinations of *cadC*-controlled T7 RNA polymerase gene in BL21(DE3) *ΔlacI* strains, developing new strains. The developed strains could detect Pb and Cd as low as 10 nM and 3 μM respectively [20]. Depending on the chassis cell chosen for biosensing, promoter module, and reporter module, the choice of plasmid vector also changes. The plasmids originally isolated from *Escherichia coli* have been continuously used as vectors with needed tinkering or modifications. This has generated a wide range of successful recombinant plasmids capable of biosensing metals, when transformed into a host cell. A large number of other plasmids have been used as a vector for biosensing development. For example, to transform yeast cells, Vopálenská et al. [21] used the vector pUG6 with the promoter gene *CUP1* that regulated the gene *ADE5,7* for developing a colorimetric biosensor for Cu.

The presence of unique receptor proteins provides specificity to sensors, but in nature, receptor proteins seldom have only a single recognizing HM. Therefore, to impart a selective selectivity to a system, the proteins can be modified by selective mutation. For example, *RcnR*, capable of responding to both Ni and Co, was mutated to respond only to Ni under the influence of promoter *rcnA* [22]. Another approach could be the use of multiple recognition proteins for the same ligand functioning under the logical operation of AND gate [35]. However, in such a heterologous AND-based genetic circuit, the performance is also a function of the plasmid copy number [36], and increasing the plasmid copy can result in behavioral changes in the composition of AND gate, leading to the unbalancing of inputs and undesirable disruptions. The study of Wang et al. [37] suggests that signaling can be regulated by chimeric proteins that can today be an easy transcript from augmented codes for different protein subunits or through mutations in the original regulatory sequences. Rewiring of the signals results in regulatory proteins with modified recognition ability of different molecules. Such systems can be modulated for recognition of multiple HM ions with the same two-component system of regulation, though the limited understanding of different protein interactions with HM remains a major constrain [37].

The reporter module

The reporter module of the biosensor consists of the sequences that generate an output signal in response and under the control of the sensory module. The reporter module functions as a transducer for the biosensor. Most of the GEWB fall in the category of optical biosensors; thus, the reporter modules consist of genes coding for optically active proteins, for example, the green fluorescence protein *gfp* [10, 17, 19, 23–25], red fluorescence protein *RFP* [12, 18, 30], and luminescent protein *LuxCDABE* [10, 22, 29, 31]. The role of the reporter in biosensor development is considered crucial as selection of reporter can influence the limit of detection and sensitivity of the system. For example, Huang et al. [10] observed that the reporter *luxCDABE*–based system outperformed the *gfp*-based system in his sensor for Hg and As.

The literature suggests a number of reporter genes have been used in the development of sensors, but the grounds for selection of a reporter module for a system were debatable for a long time. Through a comprehensive work on profiling of reporter genes, Lopreside et al. [38] showed that *NanoLuc* had better induction ability and response time in whole cell (*E. coli*) compared with other reporters like *gfp*, *mCherry*, *mScarlet-I*, *luxCDABE*, and *lacZ*. The limit of detection obtained by *mScarlet-I* and *gfp* was similar but the background fluorescence of *gfp* was high, making it unsuitable for direct visualization sensors.

The optical reporter genes like riboflavin synthetic genes (*ribB*) with the porin protein gene (*oprF*) can be significantly handy as they can enhance the electrogenic nature of the microbes [16]. Under the influence of zinc-responsive *PzntA*, the expression of *rib* and *oprF* lead to significant increases in cell membrane permeability and electron transfer ability due to the increase in riboflavin that acts as an electron shuttle mediator. Another common reporter module is the *lacZ* gene–based system; however, the *gfp* gene remains the most commonly used reporter in augmented genetic circuits for biosensors (Table 1).

Optically active proteins and enzyme reporters can be equally effective. The enzymes may catalyze the development of pigmentation, for example, the reporter gene *ADE5,7* encoding for an enzyme that catalyzes the AMP pathway leading to the formation of red pigment [20]. Tra-ngan et al. [28] used the *lacZ* reporter that encodes for enzyme β-galactosidase. The enzyme acted on substrate X-gal leading to products that dimerize to give a characteristic blue color.

The signal produced by the reporter module can be amplified to several folds using cascading genetic circuits, and this can dramatically affect the performance parameters of a biosensor [27]. Using this principle, Wan et al. [39] developed multilayered transcriptional amplifiers for a sensor with an optimized sensory protein level. The output signal generated was exponentially magnified resulting in a biosensor for As and Hg with sensitivity improved by 5000-fold and 750-fold, respectively, compared with the sensor without cascading amplification.

Host cells

A variety of host cells for expression of genetically engineered plasmids have been used; however, the choice of *Escherichia*

coli is more prevalent in the literature than any other cell. This is probably due to ease of the microbe to culture, transform, and gene transfer by conjugation. The full genome sequence of *E. coli* is already available enabling researchers to attribute the observations and performance to the corresponding codes.

Genetic engineering can conjure various combinations of sensory and reporter modules in the plasmid that can be tested and verified in test cells. However, the reporting efficiency of the augmented genetic circuit is equally affected by the intracellular environment of the chosen chassis cell. Bereza-Malcolm et al. [23] constructed a plasmid *BarsRgfp* for arsenic, transferred it to a range of gram-negative cells, and observed variations in the developed sensors. Despite the same detection limits with host cells *Enterobacter* sp. *NCR3* and *Escherichia coli DH5α*, the response time for the former (40 min) was doubled that of the latter (20 min) [23]. The study clearly suggests that despite the development of a desired recombinant plasmid, its expression in the different host cell may vary; this makes the test for the best suited chassis cell necessary to ensure the functioning of the sensor at its best.

Acinetobacter sp., another fully sequenced soil bacterium, can also serve as a good choice for chassis cell due to its non-toxic nature and resistance to high salt concentrations. For example, Cui et al. [29] transformed *Acinetobacter baylyi* ADP1 with a recombinant plasmid *WH1274_lux* to get a luminescence-based toxicity biosensor for HM. The results showed good correlation with results of toxicity tests obtained by a fish exposure method. Other gram-negative bacteria such as *Ralstonia eutropha* and *Cupriavidus metallidurans* have also been reported to serve as good chassis cells [30]. In general, the choice of chassis cell is mostly a gram-negative bacterium as these microbes are equipped well for stress tolerance. However, occasionally gram-positive bacterium models such as *Bacillus subtilis* have shown an equally effective biosensing ability for HM [40]. In the yeast group, *Saccharomyces cerevisiae* is considered to be a model member for testing the recombinant genes [21]. Amaro et al. [32] used a ciliate organism model (*Tetrahymena thermophila*) as a chassis cell for Cd biosensing based on fluorescent output signals.

Optical biosensors

HM biosensing can rely on the optical transduction ability of the whole cell. Cells that can produce specific proteins with luminous [22, 41], florescent [18, 20, 23–25, 32, 42], or pigmentation [21, 26, 28, 30] properties can be used for developing a viable biosensor for HM (Table 2). In recent past, microfluidic platform-based sensor development is on the rise. The microfluidic platform has exterminated the hindrances produced due to the matrix and is also effective with minute sample volume [45]. These systems are also reported to reduce the use of chemical analysis and cost while improving the sensitivity for HM detection [46]. The microfluidic platform can function as a microchemostat that can improve the expression of the reporter gene (*gfp*) due to continuous availability of fresh nutrients and dynamically improve the sensitivity of the system [44, 47]. The optical probe-like sensor developed by Verma et al. [45] was disposable and relied on the colorimetric changes in phenol red that resulted by pH change. They immobilized *Bacillus sphaericus MTCC 5100* with phenol red in glass capillaries using sodium alginate hydrosol gel. The urease enzyme produced by the microbe broke down urea leading to pH changes due to production of ammonium ion [45]. The HM presence inhibited the activity of urease, thus producing a variation in color changes of phenol red. Halkare et al. [43] developed surface plasmon resonance (SPR)–based detection of HM using *E. coli B4* immobilized on inner walls of a gold nanoparticle–coated optical fiber. The HM interaction with functional groups of cell wall of microbe produced variation in the refractive index around them that was analyzed by a spectrometer [43].

The use of specific substrates such as ferozine [48], X-gal [26], and chlorophenolred-beta-D-galactopyranoside/CPRG [49] that can produce a chromogenic signal in response to cell exudates is an effective strategy. Yang et al. [48] developed a visual biosensor to monitor HM toxicity using an acidophilic iron-oxidizing microbe strain Y10. In the presence of HM, the Fe^{+2} oxidizing capability of microbes was hindered, producing a variation in color intensity. Ferozine assay which forms a purple complex with ferric was used to determine the remaining Fe^{+2}.

Biosensing based on the extracellular enzyme inhibition by HM has remained an unspecific mechanism. The method can be turned to a selective and more reliable system if enzyme production itself is regulated by a metal-dependent genetic circuit. In the study of Huang et al. [26], the activity of the *lacZ* gene that produces β-galactosidase was regulated by operon (*Pars*) and *arsR* gene, thus producing the enzyme in accordance with As concentration. The activity of this enzyme was monitored using X-gal that produced a blue color of varying intensities in response to a concentration gradient of As. Zhang et al. [49] monitored activity of β-galactosidase produced by encapsulated *E. coli* in PVA hydrogel for toxicity measurement. They used CPRG as a chromogenic agent that turned from red to yellow by the β-galactosidase activity.

Toxicity monitoring has been effective with immobilized cells, which are more stable and easier to transport to the testing sites. Sodium alginate can act as a good entrapment matrix of cells. The cells entrapped by Eltzov et al. [50] were genetically engineered bioluminescent strains and the induction of the cells in the presence of HM was monitored by a system of photomultiplier tubes. In bacterial systems, respiration is inhibited by HM toxicants; thus, respiration rate can

Table 2 Whole-cell biosensors developed using different optical transduction mechanisms

S. no.	Biosensing microbe	Test sample	Method	Response time	Target metal	Limit of detection	Linear range of detection	Stability of sensor	Reference
1.	*Escherichia coli DH5α*	Cosmetics dissolved in water	Fluorescence (green)	2 h	Hg^{+2}	0.03 μM	50 nM to 10 μM	-	[18]
2.	*Escherichia coli*	Synthetic solution	Fluorescence (green)	10 h	Cd^{2+} and Pb^{2+}	10 nM	2 nM to20 mM	-	[20]
3.	*Saccharomyces cerevisiae*	Ground water	Colorimetric (red color development)	18 h	Cu^{2+}	1 μM	1 to 100 μM	50 days stored at 4 °C	[21]
4.	*Escherichia coli* TD2158 wild-type	Synthetic HM solution	Bioluminescence	30 min	Ni^{+2}	80 nM	1–40 μM	-	[22]
5.	*Escherichia coli DH5α*	Synthetic HM solutions	Fluorescence (green)	40 min	Arsenite	0.01 μg/mL	0.5 to 2.0 μg/mL	-	[23]
6.	*Escherichia coli DH5α*	Milk samples	Fluorescence (green)	30 min	Cd^{2+}	5 μg/L	5 to 15 μg/L	-	[24]
7.	*Escherichia coli DH5α*	Synthetic HM solutions	Fluorescence (green)	40 min	Cd^{2+}	0.5 μg/mL	0.5 to 10 μg/mL	-	[25]
8.	*Escherichia coli* DH5α	Ground water	Colorimetric (blue color development)	3 h	As^{3+}	10 μg/L	10 to 500 μg/L	9 days at 4 °C	[26]
9.	*Escherichia coli*	Water from natural reservoirs	Colorimetric (blue color development)	18 h	Cd^{2+}	3.0 μg/mL	3 to 50 μg/mL	30 days stored at 4 °C	[28]
10.	*Escherichia coli*	Tap water and pond water	Colorimetric (yellow pigment)	4 h	Cu^{2+}	87.3 μM	0–1000 mM	-	[30]
11.	*Tetrahymena thermophila*	Synthetic HM solution	Fluorescence (green)	2 h	Cd^{+2}	0.44 μM	-	-	[32]
12.	*Escherichia coli*	Water extracted from soil	Bioluminescence	15 min	Hg^{2+}	0.24 μg /L and 0.37 μg/L	0 to 70 μg/L and 0 to 500 μg/L	-	[41]
13.	*Escherichia coli B40*	Spiked tap water and demonized water	Localized surface plasmon resonance	10 min	Hg_2^{+2} and Cd^{+}	0.5 ppb	0.5 to 2000 ppb	-	[43]
14.	*Escherichia coli* DH5α	Synthetic waste water	Fluorescence (green) with microchemostat platform	3 h	Pb^{2+}	2 nM	2 nM to 20 μM	1 month stored at 4 °C	[44]
15.	*Escherichia coli* DH5α	Synthetic HM solution	Fluorescence (green)	5 min	Hg^{2+}	-	0 to 40 nM	-	[42]

become an indicator of HM concentration. Wasito et al. [51] used the respiration rate of alginate-immobilized *E. coli* strain *ATCC 25922* to monitor toxicity. Conversion of K_3 [Fe (CN) $_6$] to K_4 [Fe (CN) $_6$] during respiration was reduced in the presence of HM which was important for the formation of Prussian blue that was spectrophotometrically monitored. Methods involving effective integration of optical transduction with the bioelectrochemical systems can reduce the requirement of sophisticated instrumentation. Yu et al. [52] integrated a Prussian blue strip (cathode) with a microbial fuel cell, converting to a self-powered device capable of optical monitoring of toxicity due to HM. The color intensity of Prussian blue varied with the respiration of microbes on bioanode due to the variation in the generation of electrons. A spectrophotometric monitoring of Prussian blue cathode notified the HM concentration.

Challenges for whole-cell biosensing of HM

Heavy metals are known to exist in multiple forms, including free ionic forms, complex forms, and compound forms, adsorbed on particulates [53]. Though ionic forms are harmful, the toxicity of organic complexes and compounds like methylmercury is also well known. Biosensors developed in most cases do not account for the forms other than the ion form. This is due to inertness, large size of the complex molecule, and variable properties of the complex. Thus, the development of a biosensor that incorporates all the forms of the metal in the analysis without a need of pretreatment of the samples is still highly challenging. The genetically engineered whole-cell sensors that depend on the HM-induced transcription require the transport of HM into the intercellular environment for signaling. This process can be time consuming, especially in extremely low concentrations of analytes, and adds to the response time of the sensor. Thus, a sensory system that can respond to extracellular HM is required to make the sensing process more efficient.

Several studies on HM biosensors developed seem to lack an analysis on the stability of the developed whole-cell biosensor. The designed genetic circuits can be burdensome for cell and hamper the metabolism and growth process [54]. In many cases, the preservation of the developed biosensor requires near 0 to − 4 °C which can be challenging to maintain if the field scale application is to be made possible. The shelf life of the sensor in cases like that of Huang et al.'s [26] may be very small (below 10 days), which implies that tedious work of the genetic manipulation needs to be repeatedly performed if continuous supply of sensors is needed for long duration. Sangal et al. [55] showed that sensor stability can be retained in spores for over 12 months in spore-forming bacteria such as *Bacillus subtilis*; however, more investigation is needed in this direction. Such restricting bottle neck issues need to be solved to transcend academic success with industrial investments.

Designing new genetic pathways for biosensing and the performance of the developed biosensors are connected in a closed loop. More exploration of gene combinations slowly complicated the possibility of a next possible improvement strategy for biosensors. Simultaneously, the risk of exposure of genetically manipulated microbes, leading to detrimental ecological and environmental effects, also increases. Although several strategies of control such as toxin-antitoxin encoding, kill switches, and auxotroph organism have been promising [54], the risks of auto-mutations and horizontal gene are still high that can lead to the disruption of microflora areas leading to unpredicted challenges. The moral obligations and legal issues related to the development and use of GEWB need to be thoroughly scrutinized and revised for providing a wider window for the development of biosensors.

Conclusion

Important selected aspects of whole-cell biosensors have been reviewed in this work and the following conclusions can be drawn. Despite a large number of research works on the recombinant whole-cell biosensors, the choice of chassis cell seems to be limited and seems to revolve around the model organisms, though better results have been reported with non-model microbes [23]. Cells such as microalgae that have multiple reaction points, including photoreaction points, can be explored for developing biosensors with smaller reaction times [56].

The complex transcription and metabolic pathway of the cell can act as redundant for the biosensing. In this context, the development of Sim cells (without a native chromosome) that can host only desired combinations of genes has evolved as new hope [57]. These cells lack not only the ability to self-replicate but also the ability of gene transfer to other natural microbes, thus making them environmentally and ethically sound.

Most of the optical biosensors developed through synthetic biology use the metal-triggered transcription response mechanism. This mechanism, though quiet and efficient, is time consuming. The response time may vary from a few minutes to hours in some cases. To overcome this problem, biosensors based on cellular metabolism can be helpful where the changes can occur in shorter duration, though the mode of transduction might need to be changed.

Most of the biosensors developed in the past few years have reported good limits of detection and sensitivity; however, the study of the robustness of the system in multiple natural environments is seldom done. In actual water samples such as industrial effluents and waste water, the presence of toxicants can severely impact the performance. Thus, robustness studies

should be carried out essentially to determine the usability of the developed sensor in different environments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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