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REVIEW

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Recent advances in the development and applications of luminescent bacteria–based biosensors

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Abstract

Luminescent bacteria–based biosensors are widely used for fast and sensitive monitoring of food safety, water quality, and other environmental pollutions. Recent advancements in biomedical engineering technology have led to improved portability, integration, and intelligence of these biotoxicity assays. Moreover, genetic engineering has played a significant role in the development of recombinant luminescent bacterial biosensors, enhancing both detection accuracy and sensitivity. This review provides an overview of recent advances in the development and applications of novel luminescent bacteria–based biosensors, and future perspectives and challenges in the cutting-edge research, market translation, and practical applications of luminescent bacterial biosensing are discussed.

KEYWORDS
acute toxicity, bioassay, biosensors, environmental pollutants, luminescent bacteria

1 | INTRODUCTION

Luminescent bacteria are a group of unique bacteria that possess the capability to emit visible luminescence during their normal metabolic processes [1]. When exposed to external toxic and hazardous substances, these bacteria experience disruptions in their metabolic pathways, leading to a discernible decrease in luminescence intensity [2]. Based on this phenomenon, luminescent bacteria can be effectively used to measure environmental toxicity by gauging the alterations in bacterial luminescence, and such studies can be traced back to the early 1980s [3]. Since then, luminescent bacteria–based biotoxicity assays have proved to be simple, rapid, sensitive, adaptable, and reproducible, which have been widely utilized for the detection of various environmental pollutants in the past few decades [4–12].

As biomedical engineering technology advances, current luminescent bacteria–based biotoxicity assays have evolved toward enhanced portability, integration, and intelligence. Among these advancements, the use of luminescent bacteria to develop biosensors has attracted significant attention. In general, a biosensor characterized by high integration and portability serves as a rapid information acquisition and processing tool. It operates through specific interactions between biomolecules to achieve the recognition and detection of target substances [13]. Typically, biosensors consist of three fundamental components: (1) a molecular recognition element (e.g., enzymes,

antibodies, antigens, microorganisms, cells, and other biologically active substances), (2) a transducer element (e.g., photodiodes, oxygen electrodes, thermistors), and (3) a control element (typically inclusive of microprocessors, memories, and monitors), as shown in Figure 1 [14]. Luminescent bacteria can be thought of as microsensing devices that emit light signals on contact with a specific target substrate. Subsequently, the transducer element is tasked with detecting light signals and converting them into electrical signals. Furthermore, the control element is responsible for converting the collected analog electrical signals into digital signals and processing them accordingly. Eventually, the detection results are obtained, and the data are visualized and stored.

With the increasing environmental pollution, there is a growing imperative for monitoring various pollutants in modern society [15]. Currently, the detection and evaluation of environmental toxicity predominantly rely on a combination of physical and chemical analyses, as well as bioassays. Physicochemical analytical methods typically use various instruments for the rapid and sensitive determination of the type and concentration of pollutants [16]. For instance, UV–Vis. spectroscopy enables the rapid determination of organic pollutant concentrations in water, whereas gas chromatography accurately identifies the types and concentrations of volatile organic compounds in air [17]. At the same time, these methods often require the use of cumbersome analytical instruments and are operationally intricate. More notably, they fall short of providing a straightforward depiction of the toxic effects of pollutants on living organisms [18]. Alternatively, bioassay approaches offer a direct characterization of environmental quality and present evident advantages [19]. In particular, luminescent bacterial biosensors have gained increasing popularity in the detection of environmental pollutants due to their heightened sensitivity, rapid response times, cost effectiveness, and ease of automation. Furthermore, advancements in genetic engineering have facilitated the development of recombinant luminescent bacterial biosensors, thereby enhancing accuracy and sensitivity in detection. Thus, the aim of this review was to provide a broad overview of recent developments and applications of luminescent bacteria–based biosensors, as well as to analyze the prospects and obstacles for future cutting-edge research, market translation, and useful applications of this technology.

2 | LUMINESCENT BACTERIA

2.1 | Classification of luminescent bacteria

Luminescent bacteria are widely distributed, primarily inhabiting marine environments. The prototypical species of gram-negative (G^-) bacteria within this group is a facultative aerobic bacterium [20]. It exhibits a coccobacillus or bacillus morphology, and the size of the bacterium is 1.4 – 1.8 × 2.0 – 2.6 μm, devoid of spores and pods, with one or several terminal flagella imparting a tadpole shape. Typically existing singly and occasionally in pairs, these bacteria thrive in temperatures ranging from 20 to 30°C [21]. Under normal physiological and metabolic conditions, luminescent bacteria are capable of emitting blue-green visible light within the wavelength range of 450–490 nm [22]. Currently, scientifically recognized luminescent bacteria are classified into four genera: *Vibrio*, *Photobacterium*, *Shewanella*, and *Photorhabdus* (Table 1) [23]. Notably, *Vibrio fischeri*, *Photobacterium*

TABLE 1 Classification of luminescent bacteria.

Genera	Habitat	Luminescent bacteria
Vibrio	Ocean	*Vibrio harveyi* *Vibrio splendidus* biotype I *Vibrio fischeri* *Vibrio logei* *Vibrio orientalis*
Photobacterium	Ocean	*Photobacterium phosphoreum* *Photobacterium leiognathi*
Shewanella	Ocean	*Shewanella hanedai*
Photorhabdus	Fresh water	*Photorhabdus luminescens*

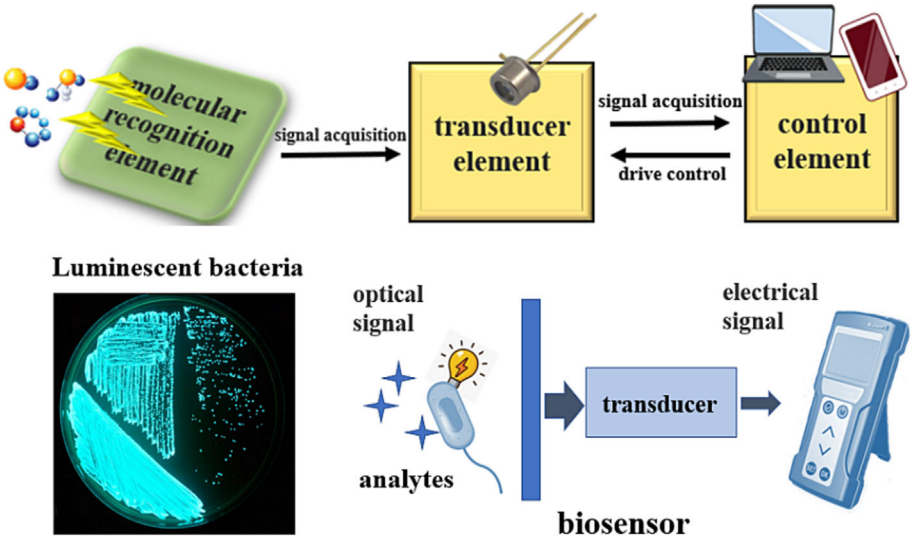

FIGURE 1 Architecture diagram of the luminescent bacteria–based biosensor system. Luminescent bacteria reduce or stop emitting light after exposure to toxic analytes; then the transducer collects and converts optical signals into electrical signals, followed by the control element for processing the signal.

phosphoreum, and *Vibrio qinghaiensis* are the most commonly used species for various applications [24]. Both *V. fischeri* and *P. phosphoreum* are marine bacteria, whereas *V. qinghaiensis* is a freshwater bacterium that was discovered by a Chinese scholar [25].

2.2 | Luminescent mechanism

In recent years, researchers have elucidated the same luminescent mechanism across various species of luminescent bacteria [26]. This intricate biochemical process involves the coordinated interaction of luciferase, reduced flavin acid ($FMNH_2$), a long-chain aliphatic aldehyde (RCHO), and molecular oxygen [27]. $FMNH_2$ assumes a pivotal role in luminescence reaction, primarily derived from flavin mononucleotide (FMN) and nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) through the catalytic action of NAD(P)H:FMN oxidoreductase [28]. The underlying reaction can be expressed by the following equation:

$$\mathrm{NAD(P)H} + \mathrm{FMN} + \mathrm{H^+} \xrightarrow{\text{NAD(P)H:FMN oxidoreductase}} \mathrm{NAD(P)^+} + \mathrm{FMNH_2}. \quad (1)$$

Bacterial luminescence is a process that involves the use of molecular oxygen, $FMNH_2$ and RCHO as substrates [29]. Under the action of luciferase, the reaction produces FMN, fatty acids, and water, accompanied by the generation of blue-green light [28]. The reaction equation is as follows:

$$\mathrm{FMNH_2} + \mathrm{RCHO} + \mathrm{O_2} \xrightarrow{\text{luciferase}} \mathrm{FMN} + \mathrm{RCOOH} + \mathrm{H_2O} + h\nu\,(\lambda = 450 - 490\,\mathrm{nm}). \quad (2)$$

A group of expressed luciferin genes (*lux*), including *luxC*, *D*, *A*, *B*, and *E*, and regulatory genes, *luxI* and *luxR*, are widely present in luminescent bacteria (Figure 2) [31]. The types and numbers of luminescent genes isolated from different luminescent bacteria vary. For example, the *luxF* gene is exclusive to *P. phosphoreum*, whereas the five genes *luxC*, *D*, *A*, *B*, and *E* are prevalent in all known luminescent bacteria [32]. *luxA* and *luxB* encode the α (40 kD) [33] and β (37 kD) [34] subunits of luciferase, respectively. The heterodimer formed is the bacterial luciferase with light-producing ability, which is highly specific for $FMNH_2$ during luminescence [35]. Furthermore, the genes *luxC*, *luxD*, and *luxE* are responsible for encoding the three essential subunits of the fatty acid reductase complex. These subunits are required for the synthesis of aliphatic aldehydes, namely reductase (54 kD), transferase (33 kD), and synthase (42 kD) [36].

2.3 | Luminescent bacteria–based bioassay

The luminescent process is closely related to bacterial metabolism [37]. This process is highly sensitive to external environmental factors, and any interference or disruption of bacterial respiratory or physiological processes can lead to a change in luminescence intensity. Exposure to external toxic and hazardous substances that disrupt the bacterial metabolic process inhibits the synthesis pathway and activity of luciferase. Consequently, the luminescence intensity is reduced [38]. On exposure to environmental samples for a certain period under specific conditions, the luminescence intensity of bacteria demonstrates an inverse relationship with the total concentration of toxic substances present in the samples [39]. The acute toxicity level of the environment can be determined by measuring the luminescence inhibition rate of luminescent bacteria after a 15-min exposure to the samples [40]. Biotoxicity is characterized by the relative luminescence intensity or luminescence inhibition rate, with the following formulas:

$$\mathrm{RI} = \frac{I}{I_0} \times 100\%, \quad (3)$$

$$\mathrm{IR} = 1 - \mathrm{RI}, \quad (4)$$

where RI is the relative luminescence intensity, I is the luminescence intensity of the sample, I_0 is the luminescence intensity of the blank control group, and IR is the luminescence inhibition rate. A commonly used indicator for evaluating the single toxicity of a sample is the EC_{50}, that is, the half-effect concentration, which is the concentration of a toxicant corresponding to a 50% inhibition value for a certain time of luminescent bacteria exposed to hazardous substances [30]. The magnitude of the EC_{50} value shows a negative linear relationship with the concentration of the toxicant [41].

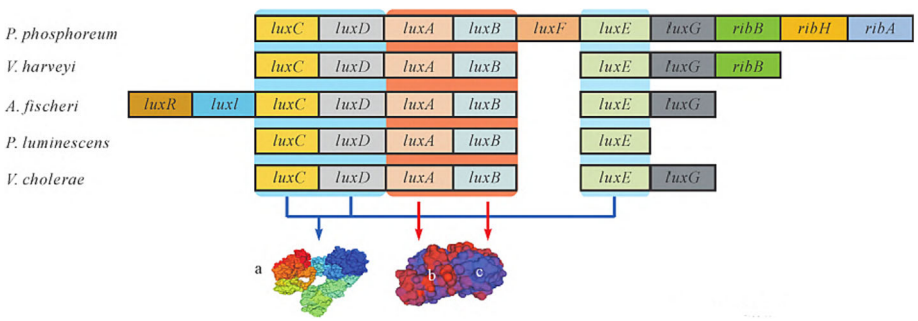

FIGURE 2 The *lux* gene arrangement of luminescent bacteria. *Source*: Reproduced with permission [30]. Copyright 2022 CAJEPH.

3 | DEVELOPMENT OF LUMINESCENT BACTERIA–BASED BIOSENSORS

The design of the sensing layer in luminescent bacterial biosensors is critical to the performance of the system [42]. Recent advancements in genetic manipulation techniques have made it possible for bacteria bioreporters to serve as the core sensing element, offering several advantages such as high sensitivity and low cost [43–49]. Most importantly, bioreporters can specifically respond to certain classes of compounds, making them highly valuable tools in various applications [50]. Crucially, the integration of the bacterial reporter with the inorganic transducer is imperative. The subsequent discussion provides a comprehensive summary of the research progress on the design principles and development of different types of bacterial reporters, as well as different integration methods employed in luminescent bacteria–based biosensors.

3.1 | Bacterial reporter

Currently, the most commonly used luminescent bacterial strains include wild-type luminescent bacteria (WLB) and recombinant luminescent bacteria (RLB), as presented in Table 2. WLB denotes luminescent bacteria that exist in their natural state without artificial modifications, such as *V. fischeri*, *P. phosphoreum*, and *V. qinghaiensis* [52]. Despite that WLB are suitable for comprehensive toxicity monitoring, recent efforts, both domestically and internationally, have focused on constructing various types of RLB capable of specifically identifying pollutants.

3.1.1 | WLB as reporters

WLB inhibit their luminescence process when exposed to toxic and hazardous substances. As early as 1978, scientists proposed using luminescent bacteria to assess the toxicity of environmental water samples [53]. Ma et al. assessed the acute toxicity of wastewater from various treatment units of oxidation ditch plants by using *V. qinghaiensis* sp. -Q67. The findings of their study revealed that filtering the effluent using microfiltration membranes enhanced the visualization of biotoxicity in water samples, thereby improving the reliability and precision of the toxicity test results [54]. Zhang et al. tested a mixture of 14 restricted detection substances specified in the hygienic standards for domestic drinking water using *V. qinghaiensis* sp. -Q67 [55]. Chen et al. developed a quick approach for detecting toxicity of Zn, Al, and copper contamination in soil from galvanizing plants using the same luminescent bacteria. The findings indicated that the mixture had a great inhibitory effect on bacterial luminescence. Although the concentrations of individual substances were all below the safety limit, their combined toxicity remained evident, potentially causing severe harm to organisms or humans [56]. Jiang et al. used *V. fischeri* to detect heavy metals (Ni^{2+}, Pb^{2+}, Cu^{2+}, Cr^{2+}, and Fe^{2+}) in the soil samples collected from five factories engaged in manufacturing [57]. Giovanella et al. applied *V. fischeri* to analyze the biotoxicity of diesel-contaminated soil [58]. Zeng et al. experimented to assess the inhibitory toxicity of five heavy metals (Cu^{2+}, Co^{2+}, Zn^{2+}, Fe^{3+}, and Cr^{3+}), as well as their binary mixtures, against *P. phosphoreum* T3 [59]. In addition, Wanjari et al. successfully isolated *Vibrio harveyi* and *Vibrio natriegens* from seawater samples. They then combined these luminescent bacterial strains to study their ability to detect radiation pollutants. The results suggested that various types of pollutants can be effectively detected for toxicity using biosensors based on WLB [60].

3.1.2 | RLB as reporters

Although WLB are suitable for the detection of combined toxicity, the increasing complexity of pollutants in the environment has made it necessary to not only respond to the combined toxicity but also provide information on different types of toxicants. RLB are selective for exogenous chemicals compared to WLB, which makes them promising for the detection of various cellular damages or specific compounds in the environment [61]. RLB are produced using recombinant DNA technology, and commonly used reporter genes include firefly luciferase (*luc*), bacterial luciferase (*lux*), and green fluorescent protein (*gfp*) [62]. In 1985, Engebrecht first used the *lux* gene as a bioreporter [63]. Subsequently, researchers applied the promoter-less *luxCDABE* to produce the broad-spectrum host range pUCD615 vector, which is widely used in the production of RLB [64].

RLB are classified as constitutive and inducible [65]. Constitutive types are usually based on strong promoters and are expressed efficiently under normal conditions. In contrast, when exposed to hazardous and detrimental environments, the expression level of the gene will noticeably decline to a measurable extent, which is directly proportional to the toxicity of the sample [66]. Inducible types, in which specific stress promoters are introduced into the reporter gene, require the presence of specific inducers to produce a corresponding response within the range of sublethal concentrations of the toxicant, and the intensity of light increases in proportion to the concentration of the toxic substances [67]. Yagur-Kroll et al. greatly improved the identifying sensibility, responding dynamics, and signal amplitude of biosensors by modifying the length of the promoter-containing DNA fragments, promoter random mutagenesis, targeted mutagenesis of consensus elements in promoters, and promoter replication [68]. Huang et al. constructed a set of bacterial biosensors using two reporter systems, *luxCDABE* and *gfp*. Their findings indicated that the *luxCDABE*-based construct is significantly more sensitive in detecting As (III) and Hg (II) than the *gfp*-based construct [69]. Wang et al. constructed a recombinant luminescent *Escherichia coli* strain used as toluene biosensors. They achieved this thorough inserting the T7, T3, and SP6 promoters between the *tol* and *lux* genes. Subsequently, the biosensor's sensitivity, selectivity, and specificity were assessed in relation to quantifying bioavailable toluene in river water and groundwater. They found that toluene-induced luminescence intensity was highest in *E. coli* expressing *lux* recombinantly using the T7 promoter [70].

TABLE 2 Current status of research on recombinant luminescent bacteria.[a]

Regulatory protein gene	Report gene	Host bacterium	Responsive substance/ mechanism	Constitutive/ inducible
cad	*luxCDABE*	*Escherichia coli DH5α*	Mercury	Inducible
cad	*luxCDABE*	*E. coli DH5α*	Cadmium	Inducible
mer	*luxCDABE*	*E. coli DH5α*	Mercury	Inducible
pbr	*luxCDABE*	*E. coli DH5α*	Lead	Inducible
pbr	*luxCDABE*	*E. coli DH5α*	Cadmium	Inducible
cue	*luxCDABE*	*E. coli DH5α*	Copper	Inducible
cue	*luxCDABE*	*E. coli DH5α*	Silver	Inducible
cop	*luxCDABE*	*E. coli DH5α*	Copper	Inducible
cop	*luxCDABE*	*E. coli DH5α*	Silver	Inducible
PcadA	*Gfp*	*Bacillus subtilis*	Cadmium	Inducible
uvrA	*luxCDABE*	*E. coli JM109*	DNA damage	Inducible
uvrA	*luxCDABE*	*E. coli JM109*	DNA damage	Inducible
alkA	*luxCDABE*	*E. coli JM109*	DNA damage	Inducible
alkA	*luxCDABE*	*E. coli JM109*	DNA damage	Inducible
recA	*luxCDABE*	*E. coli JM109/RFM443*	DNA damage	Inducible
recA	*luxCDABE*	*E. coli JM109/RFM443*	DNA damage	Inducible
dnaK	*luxCDABE*	*E. coli JM109*	Protein damage	Inducible
lac	*luxCDABE*	*E. coli Topl0*	Streptomycin sulfate	Constitutive
lac	*luxCDABE*	*E. coli Topl0*	Benzylpenicillin	Constitutive
lac	*luxCDABE*	*E. coli Topl0*	Gentamicin	Constitutive
lac	*luxCDABE*	*E. coli Topl0*	Oxytetracycline	Constitutive
lac	*luxCDABE*	*E. coli Topl0*	Streptomycin sulfate	Constitutive
lac	*luxCDABE*	*E. coli Topl0*	Benzylpenicillin	Constitutive
lac	*luxCDABE*	*E. coli Topl0*	Gentamicin	Constitutive
lac	*luxCDABE*	*E. coli Topl0*	Oxytetracycline	Constitutive
tfdRPDII	*luxCDABE*	*Ralstonia eutropha JMP134*	2,4-Dichlorophenoxyacetic acid	Inducible
tfdRPDII	*luxCDABE*	*R. eutropha JMP134*	2,4-Dichlorophenol	Inducible
tod	*luxCDABE*	*Pseudomonas putida Fl*	Toluene	Inducible
mopR	*luxCDABE*	*A.sp. DF4*	Phenol	Inducible
tetA	*Luc*	*E. coli K12*	Tetracycline family	Inducible
alkB	*luxAB*	*E. coli DH5α*	Octane	Inducible
PsaI	*lacZ*	*E. coli*	Naphthalene	Inducible
T7	*N/A*	*E. coli*	Arsenate, arsenite, antimonite	Inducible
Pdmp	*lacZ*	*E. coli MC4100*	Phenol	Inducible
Pdmp	*lacZ*	*E. coli MC4100*	2-Chlorophenol	Inducible
Pdmp	*lacZ*	*E. coli MC4100*	2,4-Dichlorophenol	Inducible
Pnah	*Lux*	*E. coli DH5α*	Naphthalene	Inducible
Tet	*Lux*	*E. coli K12*	Tetracycline	Inducible
arsR	*luxA, luxB*	*E. coli JM109*	Antimonite, arsenite	Inducible
fcb	*luxCDABE*	*E. coli RFM443*	*p*-Chlorobenzoic acid	Inducible
zntA	*luxCDABE*	*E. coli*	Salts of cadmium, lead, mercury, zinc	Inducible
copA	*luxCDABE*	*E. coli*	Copper and silver	Inducible
zntR	*Luc*	*E. coli MC1061*	Zinc	Inducible
smt	*luc FF*	*Synechococcus elongatus PCC7942*	$ZnCl_2$($CuSO_4$, $CdCl_2$)	Inducible

(Continues)

TABLE 2 (Continued)

Regulatory protein gene	Report gene	Host bacterium	Responsive substance/ mechanism	Constitutive/ inducible
cad	*luc FF*	*B. subtilis BR151, Staphylococcus aureus RN4220*	Cadmium	Inducible
cad	*luc FF*	*S. aureus RN4220*	Lead	Inducible
cad	*luc FF*	*B. subtilis BR151, S. aureus RN4220*	Antimony	Inducible
T5	*luc FF*	*E. coli MC1061*	Total toxicity	Constitutive
mer	*luc FF*	*E. coli MC1061*	Mercury	Inducible
ars	*luc FF*	*E. coli MC1061*	Arsenite	Inducible
ars	*luc FF*	*E. coli AW3110*	Arsenite	Inducible
cutA	*Lux*	*E. coli K-12*	Copper	Inducible

[a]This table was rearranged from reference [51].

3.2 | Design of luminescent bacteria–based biosensors

Since Karube introduced the concept of biosensors [71], advancements in detecting environmental pollutants have rapidly occurred, especially in the use of luminescent bacteria. Luminescent bacteria–based biosensors can be classified into two groups based on the state of bacteria: (1) biosensors based on immobilized luminescent bacteria and (2) biosensors based on luminescent bacteria suspension [72]. The following discussions provide an overview of the design principles for each type of biosensor.

3.2.1 | Biosensors based on immobilized luminescent bacteria

The immobilized luminescent bacterial biosensor uses cell immobilization technology to immobilize the luminescent bacteria into a matrix, which acts as a sensing element and is tightly combined with the subsequent transducer element [73]. The biosensor applies bioluminescence as an indicator of toxicity judgment, which organically combines cell immobilization, optical signal detection, and biotoxicity assay technology. Various immobilization strategies have been used to adhere entire cells to diverse substrates. Agar, agarose, and alginate gels are typically employed as carriers to immobilize luminescent bacteria [74].

Qi et al. successfully designed a dual-signal biosensor that can detect Cu (II) shock in real time. The complete setup of the biosensor is shown in Figure 3a. The authors inoculated 2 mL of luminescent bacterial solution into a bioelectrochemical system to form a biofilm shown in Figure 3b, which is the sensing element in the biosensor. The biofilm exhibited peaks for both reduction and oxidation, indicating that the bacteria might be both an electron donor and electron receiver, as shown in Figure 3c. Given the pronounced reduction peak, the authors initially examined the biofilm as a biocathode (electron acceptor). However, preliminary experiments showed that the biofilm as the biocathode could not transmit synchronous photoelectric signals. Further experimental results confirmed that the biofilm, when utilized as the bioanode, effectively facilitated the transmission of optical and electrical signals in biosensors. Subsequently, the response of the biosensor to different concentrations (1, 3, and 6 mg/L) of Cu (II) toxic pollutants was tested. By producing both optical and electrical signals, the research advances the exploration of the toxicity detection mechanisms in luminescent bacterial biosensors [75].

Agranat et al. established a miniaturized field-deployable biosensor module to detect the buried explosives. Figure 4a shows the schematic illustration of the biosensor system. The module is made up of three units: (1) a sensing unit for generating, detecting, and processing optical signals; (2) a digital unit for digitizing the analog signals; and (3) a communication unit for transmitting and receiving information. The bacterial reporter used in this study was *BS02*, based on a modification of the study by Yagur-Kroll et al. [77]. The authors found that previous Kroll's design was not sufficient to detect minuscule vapor concentrations above the target explosives; thus, they enhanced several components (as shown in Figure 4b). Using the Buchi Encapsulator B-390, the bacteria were immobilized in alginate beads by adding the bacteria–alginate–polyacrylic acid (PAA) solution to a $CaCl_2$ (0.1 M) solution that was being gently agitated. To ensure full gelation, the beads were agitated for a further half hour after coming into contact with Ca^{2+} ions. The optoelectronic circuit was engineered to operate at low-input light intensities while maintaining a good signal-to-noise ratio, centered around the wavelength of maximum bioluminescence emission (~490 nm). It adopted a "differential structure," as shown in Figure 4c. First, the authors tested the optoelectronic circuit and placed it under light intensity similar to bioluminescence. Preliminary experiments showed that the biosensor has high sensitivity and repeatability. They employed a network of six biosensors to find minuscule dinitrotoluene (DNT) quantities. These results demonstrated a DNT detection sensitivity of 0.25 mg DNT per kg sand [76].

Eltzov et al. exploited a transportable biosensor for measuring air quality in real time, as shown in Figure 5a. This device allows for

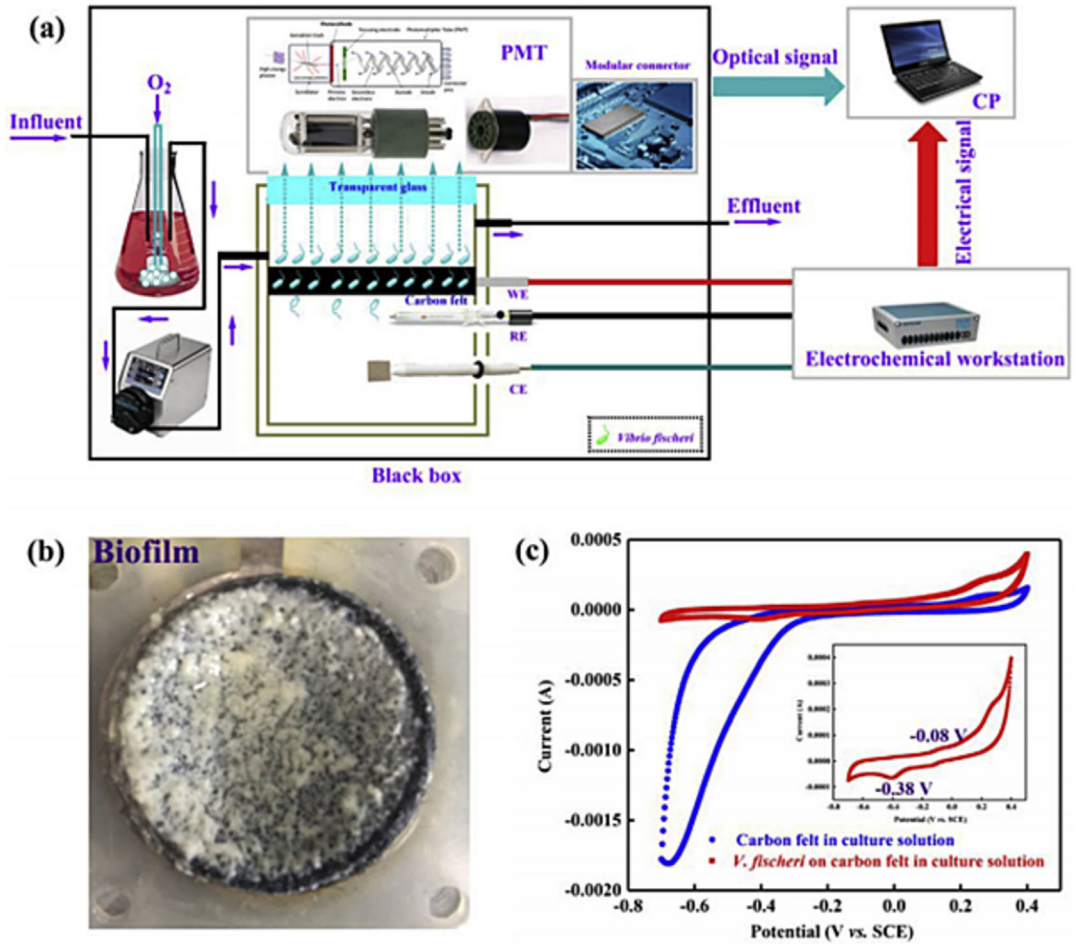

FIGURE 3 Luminescent bacterial biosensor for real-time online alert of Cu (II) shock. (a) Schematic diagram of dual-signal biosensor, (b) image of *Vibrio fischeri* biofilm, and (c) cyclic voltammetry of *V. fischeri* biofilm. *Source*: Reproduced with permission [75]. Copyright 2019 Elsevier B.V.

constant monitoring of air toxicity levels and is both affordable and user friendly. The bacterial reporter of the biosensor was *E. coli*, TV1061 [34]. This strain contains plasmid-borne fusions of the particular *grpE* [35] promoter and is susceptible to metabolic alterations, such as those caused by cytotoxic substances. The authors added a solution of 50-μL sodium alginate mixed with bacteria in a 1:1 ratio into a cylinder directly measuring 0.6 mm and also added 30 μL of 0.5 nM $CaCl_2$ solution to immobilized luminescent bacteria. Subsequently, the liquid light guide's proximal portion was situated forward of the photomultiplier tube (PMT) module, and the bacteria were positioned at its distal end (as shown in Figure 5b). Then, the authors exposed the biosensor to the office to detect pollutants in the indoor environment. After acetone (2 mL) or chloroform (5 and 10 mL) was added indoors, the bacterial luminescence of acetone increased by four times and that of chloroform increased by 25,000 times (Figure 5c). This study provides useful ideas and directions in the field of air quality testing [78].

Immobilized luminescent bacteria improve the stability of biosensors during storage and use [79–85]. The method simplifies the manufacturing process of the sensors, increasing economic and practical efficiency in many cases and allowing for a longer usage time.

3.2.2 | Biosensors based on luminescent bacteria suspension

In recent years, there have been many studies on biosensors with immobilized luminescent bacteria and a few studies involving bacterial suspensions [86, 87]. Thouand et al. designed a biosensor for detecting tributyltin (as shown in Figure 6). It consists of two parts: (1) the mini-bioreactor for providing a steady and reproducible environment and (2) the transducer and the control device. The recombinant *E. coli*: *luxAB* strain was regarded as the bioreporter. The chemical was detected by injecting the bacterial fluid through a probe. The biosensor enables real-time monitoring of bioluminescence, pH, and dissolved oxygen levels, while also facilitating temperature control through feedback regulation. The physiology of bacteria can be assessed by measuring the cell density; the respiration rate; and the levels of adenosine triphosphate, glucose, and acetate within bacteria [88].

Overall, biosensors based on luminescent bacteria suspension have some limitations. The ancillary and primary steps of obtaining reference preparations from freeze-dried bacteria can affect the rapidity of the assay. In contrast, biosensors based on immobilized luminescent bacteria are more economical and standardized.

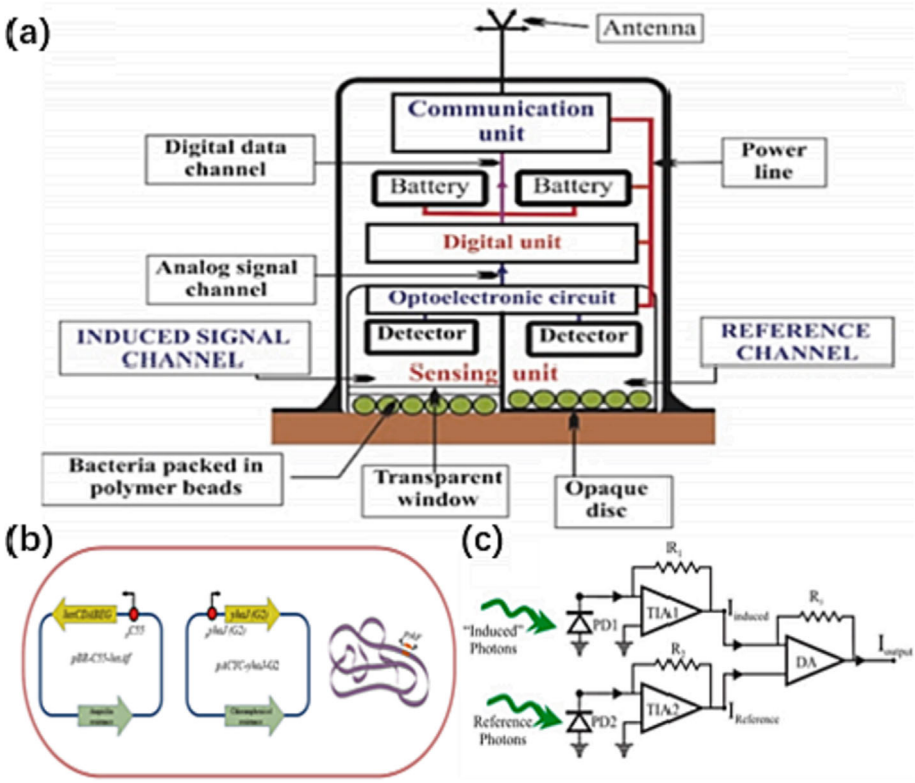

FIGURE 4 Luminescent bacterial biosensor for detecting buried explosives. (a) The biosensor module structure; (b) schematic design of the biological sensing-reporting element; and (c) schematic illustration of the optoelectronic circuit, which consists of a differential amplifier (DA) with a feedback resistor (R_3), photodiodes (PD1 and PD2), transimpedance amplifiers (TIA1 and TIA2), and feedback resistors (R_1 and R_2). *Source*: Reproduced with permission [76]. Copyright 2021 Elsevier B.V.

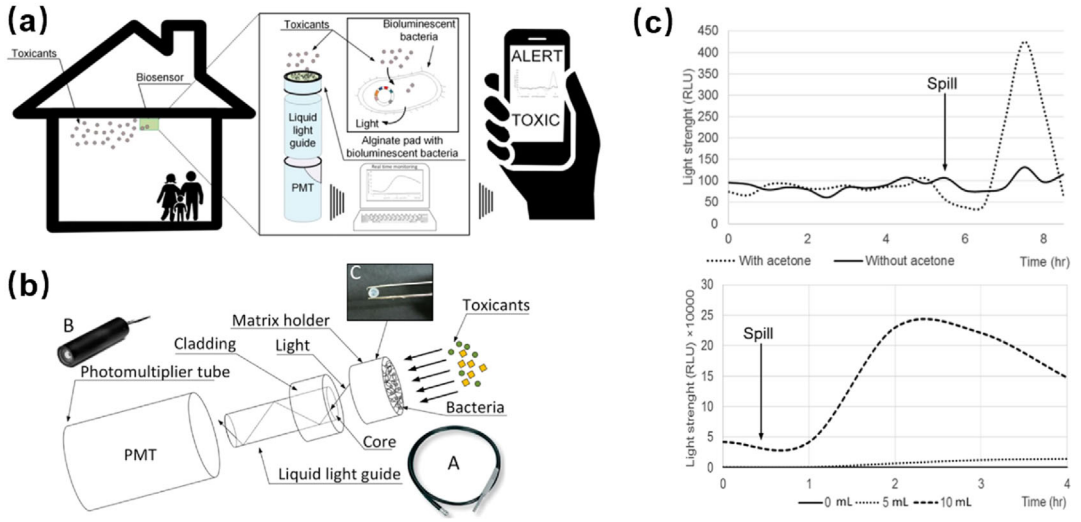

FIGURE 5 Luminescent bacterial biosensor for real-time air quality monitoring. (a) Schematic diagram of the working principle of the biosensor, (b) structure of the biosensor, and (c) response of the biosensor in a genuine office setting to exposure to 2 mL of acetone and 5 and 10 mL of chloroform. *Source*: Reproduced with permission [78]. Copyright 2015 American Chemical Society.

4 | APPLICATIONS OF LUMINESCENT BACTERIA–BASED BIOSENSORS

Luminescent bacterial biosensors are effective tools in environmental monitoring, offering an efficient and sensitive means of pollutant detection. These biosensors are primarily employed to detect harmful substances such as heavy metals, organic pollutants, antibiotics, and pesticide residues in various environments, including water, soil, air, and food.

4.1 | Water quality detection

Contaminated water must be treated promptly to prevent the gradual accumulation of toxic substances, which can lead to severe environmental pollution. In recent years, luminescent bacterial bioassay methods have made significant progress in the field of water toxicity monitoring, such as wastewater, groundwater, and drinking water.

Luminescent bacterial biosensors are commonly used in various types of wastewaters to obtain toxicity data and to study changes in

toxicity during certain treatment processes. Sazykin et al. applied a battery of luminescent bacterial biosensors to evaluate the toxicity of Rostov-on-Don and Munich wastewaters. The experimental results showed that both Munich and Rostov-on-Don wastewaters exhibit toxicity primarily due to membrane-damaging substances. Genotoxicants, promutagens, and substances that induce oxidative stress are important contributors to the synthetic toxicity of wastewater. Of these, the highest overall toxicity of Rostov-on-Don wastewater was observed in October 2012, and the highest overall toxicity of Munich wastewater was observed in July 2013, as shown in Figure 7a [89].

Toxicant concentrations in surface water and groundwater are usually low, requiring more sensitive test methods, for which biosensors based on RLB are more suitable. Trang et al. constructed an arsenate-sensitive RLB, *E. coli* DH5α (pJAMA-arsR) strain, and then analyzed arsenic in 194 samples of groundwater obtained from the Red River and Mekong River delta regions using *E. coli* DH5α and atomic absorption spectrometry, respectively. Comparative results showed that the bioluminescence sensitivity reached 7 μg As/L (the World Health Organization sets a 10-μg/L standard for the detection of arsenic in groundwater) [92]. Eltzov immobilized the RLB on the

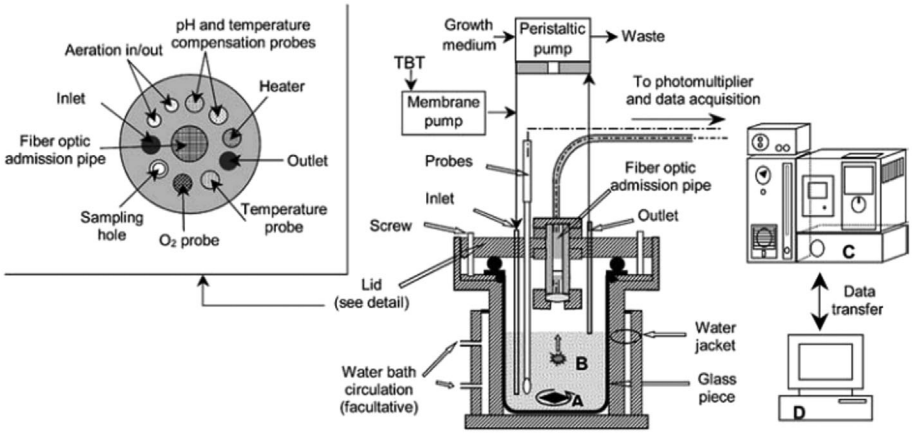

FIGURE 6 Schematic principle of the biosensor. A is for stirring, B is bioluminescent *Escherichia coli* (Ec:*lux*AB TBT3 strain), C is photomultiplier tubes and acquisition/conditioning equipment, and D is a graphical interface. *Source*: Reproduced with permission [88]. Copyright 2003 Springer-Verlag.

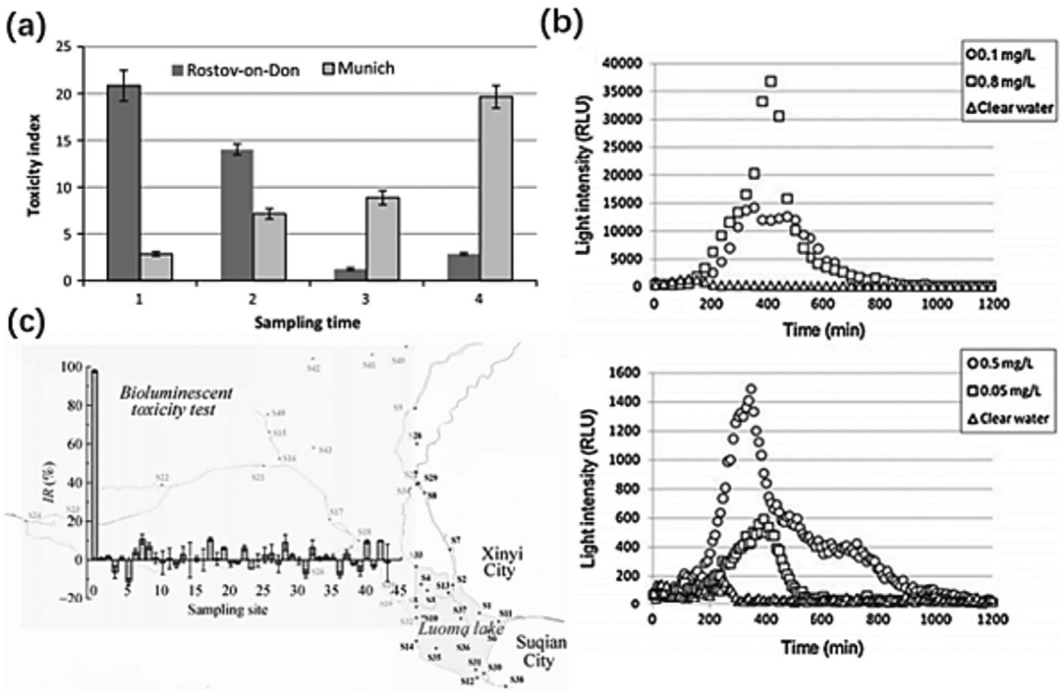

FIGURE 7 (a) The overall toxicity of Rostov-on-Don and Munich wastewaters. 1 represents October 2012, 2 represents December 2012, 3 represents May 2013, and 4 represents July 2013. *Source*: Reproduced with permission [89]. Copyright 2016 Islamic Azad University (IAU). (b) Biological reports on the response of bioluminescent strains to different harmful substances. The figure above represents the response of the strain DPD2794 exposed to several doses of mitomycin C in 7.5% (v/v) LB medium for $t = 60–120$ min. The figure below represents the response of the strain TV1061 to varying *p*-chlorophenol concentrations in 7.5% (v/v) Luria-Bertani (LB) medium in tap water at $t = 60–120$ min. *Source*: Reproduced with permission [90]. Copyright 2009 Elsevier B.V. (c) The acute toxicity evaluation of luminescent bacteria at 43 sampling sites in the Lake Loma basin. *Source*: Reproduced with permission [91]. Copyright 2020 Higher Education Press.

optical fiber to make an online monitor of toxic pollutants in water, which was used to detect genotoxic substances in groundwater and surface water, and the monitoring test of the former proved that its continuous working time could reach up to 24 h. However, there was a phenomenon of the loss of functionality of the bacterial strain in the latter test. The authors attempted to demonstrate the dose-dependent effect by exposing bioreporter bacteria to toxic compounds in 7.5% LB medium. Different quantities of *p*-chlorophenol were introduced to strain TV1061, and different concentrations of mitomycin C were introduced to strain DPD2794. Figure 7b presents the findings [90].

In addition, luminescent bacterial biosensors have been used in the toxicity assessment of drinking water. Yi et al. conducted an acute biotoxicity evaluation using an acute bioluminescence inhibition test with *V. fischeri* in the drinking water source of Luoma Lake in East China. The environmental parameters of water samples, including pH, organic matter, turbidity, hardness, and dissolved oxygen, were assessed to determine their appropriate ranges for conducting bioluminescent bacterial toxicity tests. The acute toxicity evaluation of luminescent bacteria at 43 sampling sites in the Lake Luoma basin is shown in Figure 7c. The bioluminescence inhibition of the samples measured ranged from −11.21% to 10.80% [91].

4.2 | Soil toxicity detection

Heavy metals are the most essential substances contaminating soil [93], and the luminescent bacterial biosensors provide a rapid and sensitive method for evaluating heavy metal contamination in soil and sediment [94]. Ivask et al. designed a fiber-optic biosensor for measuring Hg and As, attaching alginate-immobilized recombinant bacteria to the fiber optic, which was evaluated on 10 native soil and sediment samples [95]. Babapoor et al. used *E. coli* MC106 with pmerRBPmerlux plasmid for detecting mercury. The biosensor allows for the quantitative analysis of mercury in soil [96]. Dawson et al. used a luminescent bacteria–based biosensor to evaluate the acute toxicity and biodegradation of BTEX (benzene, toluene, ethylbenzene, and xylene) compounds in soil in the long term, and the efficiency of the chemical and biological approaches was compared [97]. Zhang et al. developed a luminescent bacterial biosensor and magnetic nanoparticles (NP), which enable the accurate detection of the biotoxicity of composite pollutants in soil without destroying soil integrity [98].

4.3 | Air pollution detection

Biosensors are miniaturized and portable, are capable of real-time monitoring, and provide numerous benefits for analyzing air toxicity. Eltzov et al. developed a portable biosensor that can monitor air quality in real time. This device has been tested in an indoor environment and has proven to be effective in detecting the presence of chemicals [78]. Although this method is relatively simple, there are still some limitations. For example, factors such as the speed of gas filling, the length of time in contact with the bacterial solution, and the amount of gas can affect the experimental results. As a result, the use of luminescent bacteria in detecting the toxicity of air pollution is rather restricted.

4.4 | Other applications

Currently luminescent bacteria–based biosensors are widely used in water quality and soil-integrated toxicity detection, in addition to the aforementioned aspects, and also in NPs, tetracycline drugs, toluene, and other biotoxicity detection.

Zhang et al. detected the acute toxicity of NP mixtures with *V. fischeri*, and their findings revealed that the toxicity of NPs to bacteria was in the order of CuNPs > ZnONPs > ZnNPs > CuONPs [99]. Pikkemaat et al. employed luminescent bacterial biosensors to analyze more than 300 poultry samples for tetracycline residues and compared with those obtained from microbial inhibition tests [100]. Wang et al. constructed recombinant luminescent *E. coli* for the development of a toluene biosensor. They evaluated the effectiveness, particularity, and sensitivity of the biosensor for determining the amount of bioavailable toluene in river water and groundwater [70]. Mwinyihija et al. conducted ecotoxicological screening of dust sampled from tanneries in Kenya using a luminescent bacteria–based biosensor. The results of the experiments indicated that the biosensor showed toxic responses to both solid- and liquid-phase analyses of the tannery dust [101]. Roda et al. employed a luminescent bacterial biosensor to measure mercury levels in urine samples from individuals with and without dental amalgam restorations. The findings of the study revealed that the biosensor was highly sensitive, precise, and selective for mercury and methylmercury [102].

5 | CONCLUSION AND OUTLOOK

Luminescent bacteria–based biosensors have emerged as a promising tool for toxicity detection, due to their high sensitivity, rapid response time, and user-friendly operation. These biosensors have gained widespread attention in the scientific community due to their capacity to accurately detect a broad spectrum of toxic substances [103]. The integration of optical fiber facilities, sensing modules, and microfluidic technology has further enhanced the versatility of luminescent bacterial bioassays, enabling their adaptation to intricate testing environments. Nonetheless, inherent to the characteristics of prokaryotic organisms, luminescent bacterial assays exhibit certain limitations [104]. Enhancements in the adaptability of luminescent bacteria; the stability of RLB; and advancements in activation, preservation, and immobilization technologies could address these limitations. Meanwhile, regulations and standards are needed to ensure the accuracy and reliability of luminescent bacteria–based biosensors. Successful improvements in these areas would position luminescent bacteria–based biosensors to be widely utilized, offering a more efficient and accurate means of detection in the realms of environmental monitoring, food safety, and biomedicine.

AUTHOR CONTRIBUTIONS

Yingying Li: Writing—review and editing; writing—original draft. **Yuankun Zhao:** Writing—review and editing. **Yiyang Du:** Writing—review and editing. **Xuechun Ren:** Writing—review and editing. **He Ding:** Writing—review and editing. **Zhimin Wang:** Writing—review and editing; supervision; conceptualization; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Luminescence at https://analyticalsciencejournals.onlinelibrary.wiley.com/journal/15227243?journalRedirectCheck=true.

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