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Engineered Biosensors in an Encapsulated and Deployable System for Environmental Chemical Detection

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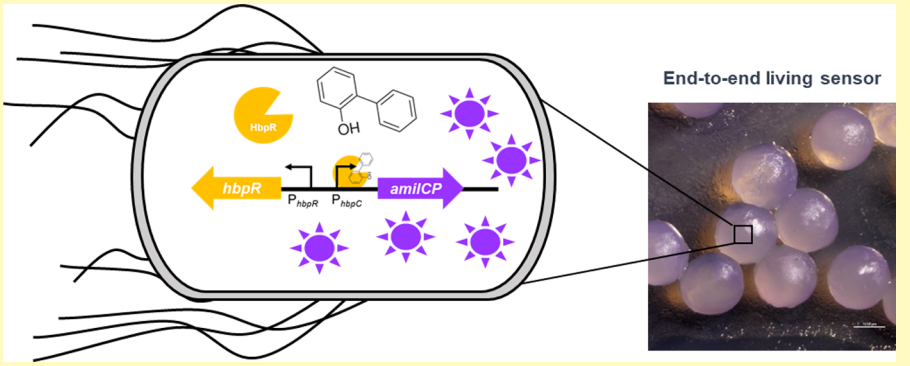

ABSTRACT: The long-term exposure of low levels of the fungicide, 2-phenylphenol (2-PP), to the environment presents a hazard to human and aquatic health. The cost and difficulty in large-scale production limit the use of existing sensors to detect 2-PP for applications such as personal protection and persistent environmental monitoring of large areas. While advances have been made in using whole cells as biosensors for specific chemical detection, a whole-cell biosensor system with robust biocontainment for field deployment and a strong visual reporter for readouts in the deployed environment has yet to be realized. Here, engineered biosensors in an encapsulated and deployable system (eBEADS) were created to demonstrate a portable, no-power living sensor for detection of 2-PP in the environment. A whole-cell living sensor to detect 2-PP was developed in *Escherichia coli* by utilizing the 2-PP degradation pathway with an agenetic amplification circuit to produce a visual colorimetric output. To enable field deployment, a physical biocontainment system comprising polyacrylamide alginate beads was designed to encapsulate sensor strains, support long-term viability without supplemental nutrients, and allow permeability of the target analyte. Integration of materials and sensing strains has led to the development of a potential deployable end-to-end living sensor that, with the addition of an amplification circuit, has up to a 66-fold increase in β-galactosidase reporter output over non-amplified strains, responding to as little as 1 μM 2-PP while unencapsulated and 10 μM 2-PP while encapsulated. eBEADS enable sensitive and specific in-field detection of environmental perturbations and chemical threats without electronics.

KEYWORDS: *whole-cell biosensor, 2-phenylphenol (2-PP), polyacrylamide alginate (PAA), hydrogel, transcriptional activation*

Exposure to low levels of toxic compounds can elicit negative long-term effects on human health; early indications of exposure may help mitigate these long-term adverse effects.[1−3] Chemical detection methods lack the sensitivity and specificity of mass spectrometry; however, mass spectrometry methods are costly and lack the portability of chemical detection.[4,5]

Advances in synthetic biology, pathway engineering, and materials engineering enable fieldable whole-cell living sensors. In particular, microbial sensors represent an attractive alternative to existing devices. Whole-cell living sensors allow the detection of an analyte and subsequent production of a readable reporter protein all within a cell.[6] They facilitate an entirely new paradigm of chemical sensing due to their favorable Size, Weight, and Power characteristics.[7]

Biosensors have been used in wearable medical monitoring,[8] health diagnosis,[9] and environmental monitoring.[10−12] Natural evolution of receptors have led to microbes' ability to detect and respond to known human health hazards, such as phenanthrene,[13] lead, copper,[14] and organophosphate pesticides.[15,16] These molecular components provide a basis for sensor engineering in living systems. One environmental pollutant for which there is currently no zero-power

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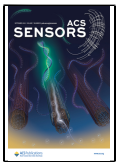

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monitoring technology is the fungicide, 2-phenylphenol (2-PP). This molecule is used to preserve citrus fruits and vegetables and treat lumber and is used in the manufacturing of other fungicides, dyes, resins, and rubber chemicals.[4,17,18] According to the EPA, regulatory tolerance levels for 2-PP are as low as 58.75 μM for certain fruits, and the LC50 for some aquatic organisms can range from 1.9 to 17 μM.[18] While 2-PP has been found in urine, its toxicity to humans is unknown, but it is toxic to aquatic life.[19] Current detection methods for 2-PP include high-performance liquid chromatography with UV,[20] electrochemical detection,[4] and gas chromatography with mass spectrometric detection.[21] These methods require extractions, are labor-intensive, require expensive equipment, or are low-throughput. Whole-cell living sensors allow for sensitive, specific, simple, and zero-power chemical detection without the use of electronics.

Whole-cell biosensors can be engineered utilizing transcription factors that sense specific analytes in naturally occurring biological pathways. The transcriptional activator, HbpR, senses 2-PP as well as other effectors, such as 2-aminobiphenyl, 2,2′-biphenol, and 2-benzylphenol.[22] HbpR is involved in the regulation of 2-PP and 2,2′-biphenol degradation in *Pseudomonas azelaica* HBP1 and has previously been used in whole-cell sensors to detect 2-PP using a bioluminescent output.[23] Here, we have developed a whole-cell biosensor utilizing HbpR to detect 2-PP with an output that is visible to human perception, the purple-blue colored reporter AmilCP. Outputs from simple single component sensing pathways are often weak and vary based on the type of reporter protein used.[24] To resolve this issue, we utilize components of the Hrp Type III secretion system from *Pseudomonas syringae* to make a genetic amplification circuit that significantly increases reporter signal and longevity. HrpR and HrpS components of this system are transcriptional activators that form a hetero-hexameric complex to activate transcription of the hrpL promoter.[25,26] This system has advantages over other amplification circuits such as using the quorum-sensing regulator LuxR, which requires continuous input from the signal.[27]

The use of whole-cell living sensors in a real-world setting requires biocontainment to prevent contamination of the environment with genetically engineered microbes. Effective biocontainment strategies should prevent the release of engineered organisms into the environment and satisfy regulatory requirements.[28,29] The focus of most biocontainment efforts has been on genetic approaches, such as engineered prevention of self-replication, kill switches, horizontal gene-transfer blocking, and synthetic auxotrophism. While promising, these strategies are yet to demonstrate scalable prevention of the engineered organism release into the environment.[28,29] Physical biocontainment offers a more reliable approach, but new materials that sustain long-term viability of cells, are analyte-permeable, maintain mechanical integrity, and enable easy deployment and retrieval are required. Here, we have designed polyacrylamide alginate (PAA) microbeads for the encapsulation of 2-PP whole-cell living sensors. We leverage the properties of this hydrogel to create a leakage-free matrix that is flexible, robust, and gas-permeable. PAA hydrogels are biocompatible due to their hydrophilic properties that allow nutrients and water to diffuse throughout the microbead to support cell viability.[28−30]

Previously, field-deployable whole-cell living sensors in hydrogel materials have been demonstrated for stand-off detection of chemicals in the environment.[29−31] While these demonstrations illustrated successful biocontainment, high cell viability over time, or remote sensing capabilities, each requires additional equipment to detect biosensor output.[29−31] In contrast, our design of whole-cell living sensor microbeads allows the user to detect the sensor's visual output when it is exposed to low quantities of 2-PP.

Here, we use PAA to encapsulate 2-PP whole-cell living sensors, engineered biosensors in an encapsulated and deployable system (eBEADS). These sensing strains employ an amplification circuit that enhances sensor output, resulting in a purple-blue signal that is clearly visible to the human eye when exposed to micromolar quantities of 2-PP. Our whole-cell living sensors survive in the beads for up to 2 weeks without leakage of the sensor strain. This approach allows one to find a portable, no-power sensing solution to detect 2-PP that is easy to deploy and scale and provides a physical biocontainment strategy that can be employed for other whole-cell living sensors.

METHODS

Materials. All chemicals including 2-PP, ethanol, isopropyl β-D-1-thiogalactopyranoside (IPTG), acrylamide, *N*,*N*′ methylenebisacrylamide (MBAA), alginic acid sodium salt from brown algae (alginate, medium viscosity), and calcium chloride were all obtained from MilliporeSigma (St. Louis, MO).

Media and Growth Conditions. Unless otherwise noted, bacteria were grown at 30 °C in Lysogeny broth (LB) media (10 g of NaCl, 10 g of tryptone, and 5 g of yeast extract per liter) with carbenicillin (100 μg/mL) and/or kanamycin (50 μg/mL) for selection of plasmids. Plasmids were directly transformed into chemically competent *Escherichia coli* NEB5α cells (New England Biolabs, NEB; Ipswich, MA). Strains containing plasmid for plasmid preparation were incubated at 37 °C.

Plasmid Construction. HbpR, its 654bp upstream region containing the hbpC promoter (Accession U73900), and lacZα were cloned into pAKgfp1[32] to make pBL_IMP_11. LacZα was replaced with the amilCP-reporting gene to make pBL_IMP_31. For the amplification plasmids, hrpR and hrpS were inserted downstream of the hbpC promoter and upstream of either the lacZα or amilCP reporter to make pBL_IMP_32 and pBL_IMP_28, respectively. A ribosome binding site (BBa_B0034, Registry of Standard Biological Parts) was inserted upstream of each hrpR, hrpS, and the reporter gene. For the second amplification plasmid, the entire hrpR, hrpS, and either lacZα or amilCP were cloned downstream of the hrpL promoter into the pVLT33[33] plasmid backbone to make pBL_IMP_33 and pBL_IMP_25, respectively. For the positive control plasmids that constitutively express the reporter, the hbpC promoter in pBL_IMP_28 was replaced with the tac promoter to produce pBL_IMP_26. All genetic components were amplified using Q5 polymerase and assembled using NEBuilder HiFi DNA Assembly Master Mix (NEB). All PCR primers and gBlocks were purchased from Integrated DNA Technologies (IDT; Coralville, Iowa).

Determination of β-Galactosidase Activity for Sensing Strains in Liquid Culture. Cultures were grown overnight to saturation and then diluted to an OD600 of 0.2. Cultures were incubated at 30 °C with shaking until an OD600 of 0.7−1 before inducing cultures with 2-PP dissolved in 100% ethanol. Cultures then continued to incubate for 3 h or 1 mL samples were taken every hour. For experiments where 2-PP was removed after incubating for 3 h, cultures were divided into two tubes and centrifuged. One-half of the cultures was washed with 1× PBS to remove 2-PP and resuspended in fresh medium with antibiotics and without 2-PP. The other half was resuspended in their original medium without washing. Aliquots were taken every hour. Cells were harvested, washed with 1× PBS, and resuspended in one volume of 1× PBS. 100 μL samples were used for analysis using the Beta-Glo Assay System (Promega; Madison, WI)

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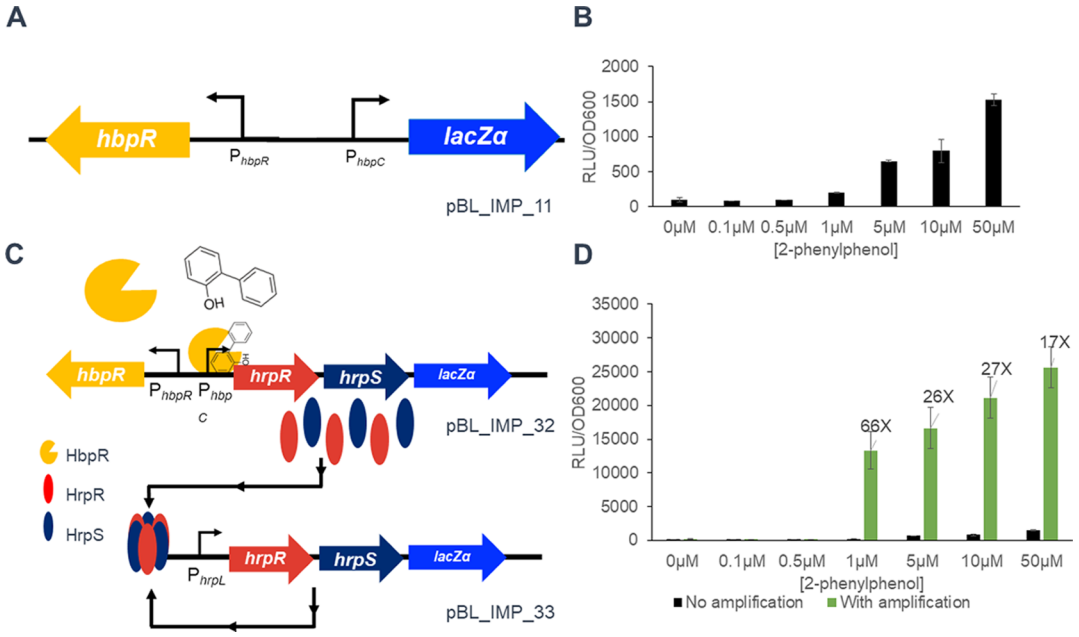

Figure 1. A) Schematic of an HbpR-based sensor. (B) β-galactosidase activity of 2-PP biosensor sensing strains after 3 h of treatment with increasing concentrations of 2-PP. (C) Schematic of an HbpR-based sensor with an amplification circuit. (D) β-galactosidase activity of a 2-PP biosensor sensing strain without the amplification circuit. Sensor strains were treated with 2-PP at OD 0.7−1. β-galactosidase activity is measured in relative light units (RLUs) and normalized to OD600. Error bars represent standard deviation for three biological replicates.

Table 1. Key for Plasmids Designed and Used in This Study

plasmid	plasmid backbone	cloned genes	description
pBL_IMP_11	pAKgfp1	PhbpR-hbpR; PhbpC-lacZα	2-PP-β-galactosidase
pBL_IMP_31	pAKgfp1	PhbpR-hbpR; PhbpC-amilCP	2-PP-amilCP
pBL_IMP_32	pAKgfp1	PhbpR-hbpR; PhbpC-hrpR-hrpS-lacZα	2-PPamplification circuit-β-galactosidase
pBL_IMP_33	pAKgfp33	PhrpL-hrpR-hrpS-lacZα	β-galactosidase amplification circuit
pBL_IMP_28	pAKgfp1	PhbpR-hbpR; PhbpC-hrpR-hrpS-amilCP	2-PP amplification circuit-amilCP
pBL_IMP_25	pAKgfp33	PhrpR-hbpR-hrpS-amilCP	amilCP amplification circuit
pBL_IMP_26	pAKgfp1	Plac-hrpR-hrpS-amilCP	IPTG induce amplification circuit-amilCP

according to the manufacturer's instructions. Statistical significance of the results was determined using a Student's two-tailed *t*-test. The limit of detection (LOD) was calculated using GraphPad Prism software and is defined as the blank plus three times the standard deviation of the blank.

Visual Reporter Demonstration with AmilCP on Plates. Strains containing AmilCP reporters were incubated overnight to saturation at 30 °C with shaking. 3 μL of the culture was pipetted onto 6 cm LB-agar plates containing 0.2 mM IPTG, 100 μg/mL carbenicillin, 50 μg/mL kanamycin, and 0, 1 μM, or 10 μM 2-PP. Images of the plates were taken at time points 0, 6, 12, 24, and 48 h with a Nikon SMZ25 stereoscope.

Fabrication of Hydrogel Microencapsulation of Sensor Strains. The sensing cells were entrapped in a semi-interpenetrating network of alginate and polyacrylamide hydrogel microbeads.[34−37] Alginate, acrylamide, and MBAA were dissolved in deionized water. The total weight percent of alginate and acrylamide was 33.3% (w/v) at a ratio of 1:50 (w/w) alginate to acrylamide. MBAA was added to a final concentration of 1.67% (w/v). The polymer solution was polymerized in a Branson 1800 sonicator bath containing water at 60 °C for 3 h and allowed to cool overnight.

Overnight cultures (grown as described above) were pelleted and resuspended into the polymer solution at an OD600 of 3 per mL. The polymer cell suspension was then extruded through a sterile 18G needle into a 1 M calcium chloride bath and allowed to cross-link for 30 min in the bath. The sensing strain microbeads were then filtered and rinsed twice with sterile deionized water.

Validation of Hydrogel Formulation for a Leakage-Free Matrix. Using the same encapsulation protocol as the sensing strain beads, positive control strain cultures containing plasmids pBL_IMP_25 and pBL_IMP_26 that constitutively express the reporter were used to fabricate triplicate samples. Negative control beads lacking cells were also fabricated. The sensing strain microbeads were cultured at 30 °C at 200 rpm in LB medium with no salt. OD600 measurements of the LB medium surrounding the beads were taken with a NanoDrop One MicroVolume UV−vis Spectrometer (Thermo Scientific; Waltham, MA) every 24 h for a 9 day period.

Characterization of Encapsulated Cell Viability. The encapsulation matrix's ability to support cell viability and long-term shelf life was assessed using the Live/Dead BacLight Bacterial Viability assay (ThermoFisher). Briefly, cells constitutively expressing AmilCP were encapsulated in hydrogel formulations as described above and stored at 4 °C or 25 °C without supplemental nutrients or liquid in a closed 50 mL conical tube in biological triplicate "batches". Cell viability was evaluated for each storage condition immediately after encapsulation, 24 h, and weekly for 1 month. At each time point and storage condition, a single bead from each replicate "batch" was stained with 1 mL of 3 μL/mL equal parts SYTO9 (live stain) and propidium iodide (dead stain) for 30 min, washed with 1× PBS, and imaged as an end point measurement using a Zeiss confocal microscope (LSM 900 Airy scan 2). Images were processed using ImageJ.

Demonstration of Sensor Induction in Hydrogel Microbeads. The encapsulated sensing strain's ability to detect low concentrations of 2-PP and produce a visible output signal was

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evaluated by time series of microscopy images. Briefly, the positive control strain and 2-PP with amplification circuit strain were encapsulated in the PAA formulation as previously described above. The microbeads were placed on LB agar plates containing 0.2 mM IPTG, 100 μg/mL carbenicillin, and 50 μg/mL kanamycin supplemented with 0, 1, or 10 μM 2-PP and incubated at 30 °C. Images of the sensing strain microbeads were taken at time points of 0, 6, 12, 24, and 48 h with a Nikon SMZ25 stereoscope.

RESULTS AND DISCUSSION

Sensing Strain Design and Function. To design a 2-PP whole-cell living sensor, we utilized components from the 2-PP and 2,2′-biphenol degradation pathway in *P. azelaica*. This pathway is regulated by the transcriptional activator HbpR, which belongs to the NtrC family of prokaryotic transcriptional activators.[22] In the presence of 2-PP, HbpR in the native-host induces expression from the hbpC promoter and drives the expression of enzymes for 2-PP degradation. In our 2-PP sensor, HbpR binds to 2-PP to activate the hbpC promoter, resulting in expression of the lacZα reporter gene (Figure 1A, Table 1). After 3 h in the presence of 2-PP, the reporter can be activated by as little as 1 μM of analyte. The LOD was calculated to be 0.863 μM for this sensor strain (Figure S1). There is no detectable activation at lower concentrations of 2-PP when compared to the negative control.

To enhance reporter production, an amplification circuit was added using HrpR and HrpS.[38] HrpR and HrpS are transcriptional activators that form a hetero-hexameric complex to activate the hrpL promoter.[38] Our amplification circuit consists of two plasmids. The first plasmid is derived from pBL_IMP_11 and contains the hrpR and hrpS genes downstream of the hbpC promoter, before the reporter gene (Figure 1C, Table 1). The second plasmid contains hrpR, hrpS, and a reporter inserted downstream of the hrpL promoter. When HbpR is activated by 2-PP, it drives the expression of hrpR, hrpS, and the reporter from the hbpC promoter on the first plasmid. The HrpR/HrpS complex drives the expression from the hrpL promoter on the second plasmid to produce more of the activation complex and reporter (Figure 1C).

Inclusion of the amplification circuit results in an increase in reporter activity for all concentrations greater than 1 μM 2-PP. These increases ranged from 17- to 66-fold after 3 h of incubation when compared to the non-amplification strain with the respective 2-PP treatments (Figure 1D). For concentrations below 1 μM, an increase in reporter production is not observed with the amplification circuit, suggesting that the overall sensitivity is determined by HbpR. The amplification circuit has a slight increase in background activity when compared to sensors lacking the circuit, but it is minimal compared to samples exposed to 2-PP.

In order to test the response of the reporter over time, the sensor strains were treated with various amounts of 2-PP, and the reporter activity was measured every hour for 5 h. After 2 h, there was an increase in reporter activity for the 5, 10, and 50 μM 2-PP treatments for strains that lack the amplification circuit when compared to the 0 μM 2-PP control (Figure 2A). For the strains with the amplification circuit, all of the 2-PP treatments show an increase in reporter activity after 2 h when compared to the 0 μM 2-PP control (Figure 2B). Treatment with 1 μM 2-PP in the strain with the amplification circuit shows the highest fold increase across throughout the 5 h time course when compared to the same treatment in the strains without the amplification circuit. The 5, 10, and 50 μM 2-PP treatments showed similar fold increases across the 5 h time course when compared to their respective strains without the amplification circuit. The 0 μM treatments for the amplification circuit containing strain did show a slight increase in activity over time, and there is minimal background β-galactosidase activity in this strain when compared to the strains without the amplification circuit. For both strains, the reporter activity continues to increase for 4 h before plateauing. Addition of the amplification circuit does not seem to affect the timing of the initial response of HbpR to 2-PP.

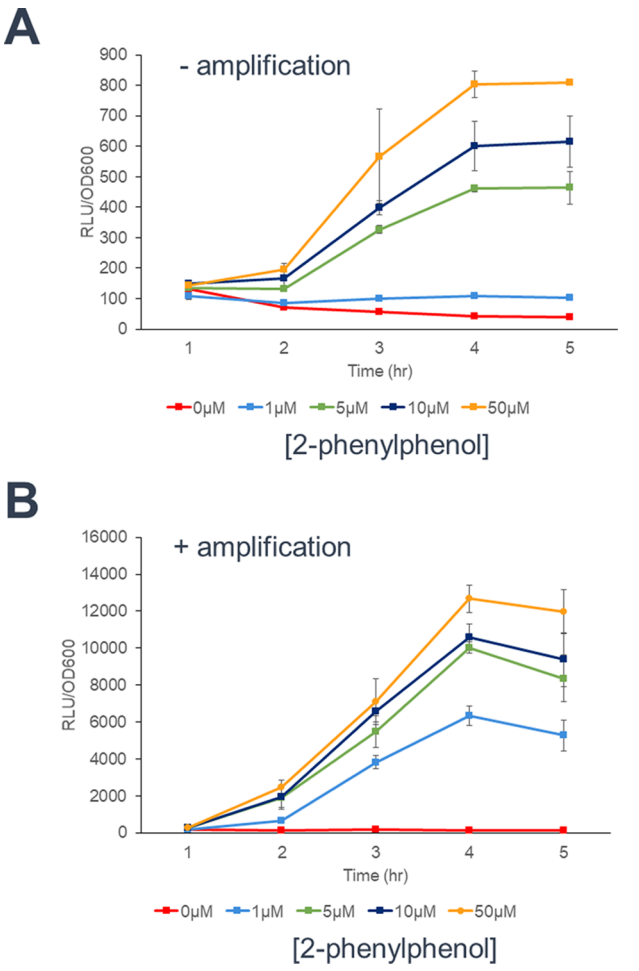

Figure 2. Time course of 2-PP biosensor sensor strain response (A) without an amplification circuit and (B) with an amplification circuit. Sensor strains were treated with 2-PP at OD 0.7−1. β-galactosidase activity is measured in relative light units (RLUs) and normalized to OD600. Error bars represent standard deviation for three biological replicates.

To demonstrate that this sensor strain could be used for visual detection without the use of any additional equipment, the lacZα gene in all plasmids was replaced with amilCP, a purple-blue chromoprotein from *Acropora millepora* (Table 1).[39] After 12−24 h on agar plates containing 10 μM 2-PP, the strains with the amplification circuit produced enough AmilCP, with the reporter becoming just barely visible. After 48 h on agar plates containing 1 and 10 μM 2-PP, the AmilCP reporter becomes faintly visible in the strain without the amplification circuit, while strains with the amplification circuit produce a more vibrant color (Figure 3).

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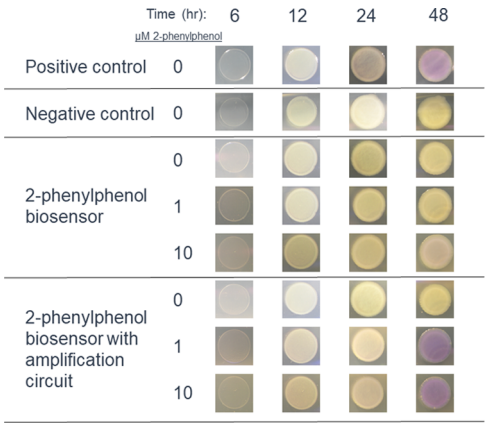

Figure 3. Images of the 2-PP biosensor sensor strain with and without the amplification circuit utilizing AmilCP (purple) as the reporter exposed to 0.2 mM IPTG and different concentrations of 2-PP over time on agar plates. The positive control strain contains pBL_IMP_26/25, which constitutively expresses AmilCP, and the negative control strain contains pAKgfp1/pVLT33. Images were taken at 0.3× magnification.

Addition of the amplification circuit allows for earlier detection of 2-PP by eye and produces a much more vibrant color than strains without the amplification circuit as seen on agar plates as well as in liquid culture (Figure S2). While the response time for the AmilCP reporter is slower than the 2 h response time for the more sensitive β-galactosidase reporter, use of the AmilCP reporter allows for reporter visualization without additional equipment or reporter substrates that could leech out of the hydrogel material. The increase in reporter production through genetic amplification makes it feasible for this living sensor to maintain a robust, visual reporter output when encapsulated.

Signal Persistence. An important consideration when designing a living sensor is the persistence of the reporter output. In some use cases, it is desirable to have a living sensor that does not need to be exposed to the analyte of interest for an extended period for production of a detectable signal output. To determine the continuous production of the reporter with the addition of the amplification circuit, the sensor strain was exposed to the analyte for a limited period of time and then removed as described in the Methods. For the strain without the amplification circuit, β-galactosidase reporter activity continued to increase for the samples that still contained 2-PP, whereas in the strains where 2-PP was removed, the signal immediately began to decrease (Figure 4A). After 24 h, these samples had similar activity to the 0 μM control (Figure 4B). For the strain with the amplification circuit, β-galactosidase reporter activity continued to increase for the samples that still contained 2-PP and then decreased at around t = 7 h (Figure 4C). After 2-PP was removed from the cultures, the signal began to slowly decrease over time. After 24 h, β-galactosidase activity was similar between the samples that had 2-PP and samples that removed the 2-PP (Figure 4D).

These results are consistent with what was expected based on the reporter design. For the non-amplification strain, once the analyte is removed, there is nothing to drive expression from the hbpC promoter, so the signal will no longer increase and will eventually decrease depending on the stability of the reporter protein. For the amplification strain, after removing the analyte, the HrpR and HrpS proteins are still present to induce expression from the hrpL promoter and continue to produce more HrpR and HrpS protein. This cycle should continue to produce the reporter protein as long as the degradation of HrpR and HrpS is not faster than the production of the proteins in the amplification circuit. Addition of the amplification circuit to this living sensor adds stability and does not require constant analyte input to sustain activation of the amplification circuit.

Validation of Hydrogel Formulation for a Leakage-Free Matrix. With a robust, visual reporting sensing strain developed, a containment system for environmental delivery

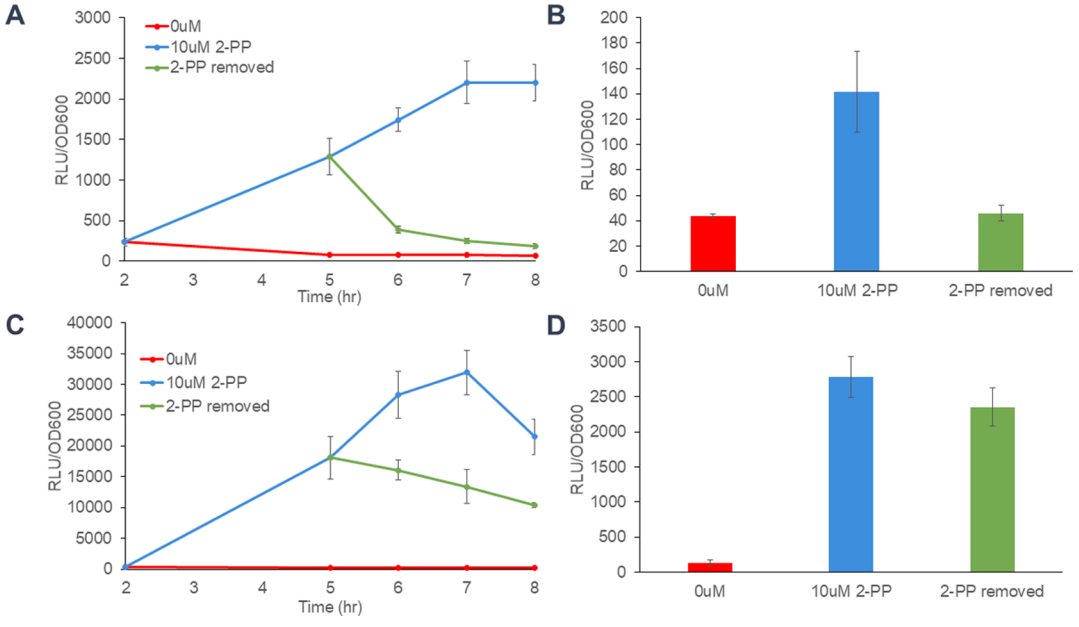

Figure 4. A) Time course of a 2-PP sensor without an amplification circuit over 8 h and (B) after 24 h. (C) Time course of the 2-PP sensor with an amplification circuit over 8 h and (D) after 24 h. Sensor strains were treated with 2-PP at OD 0.7−1. β-galactosidase activity is measured in relative light units (RLUs) and normalized to OD600. Error bars represent standard deviation for three replicates.

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was necessary. A PAA hydrogel was selected due to cell compatibility.[28−30] To validate the entrapment of the sensing strain cells in the semi-interpreting hydrogel network, microbeads containing sensing strain cells were incubated in salt-free, LB media at 30 °C. The salts were omitted from the LB medium for all encapsulation experiments due to issues with the ionic exchange of the calcium ion crosslinks with the sodium ions in the medium. The omission of salt eliminated this phenomenon, which causes degradation to the encapsulation material. The purpose was to control the variables of the leakage experiment to understand the material's ability to contain the cells. Future work is planned to optimize the material to eliminate the ion exchange in high-salt environments. The OD600 of the surrounding media was observed every 24 h for a 9 day incubation period. The OD600 remained zero for the duration of 9 days, indicating no cell leakage. If any cells were to have escaped, the OD600 would have reached 3+ within 24 h as observed with non-encapsulated, cultured cells (Figure S3). These results suggest the PAA hydrogel has a pore size that remains small enough to entrap the sensing strain cells even when the hydrogel is in its swollen state.

Characterization of Long-Term Microbial Cell Viability within Microbeads. Long-term viability of cells encapsulated in the PAA beads was evaluated in a series of live/dead assays on beads stored without additional nutrients at 25 and 4 °C over the course of 1 month. Confocal imaging of the beads showed that the majority of cells are alive (green) after 1 month of storage at both temperatures (Figure 5A). Quantitative analysis of the images illustrates that the relative percentage of live cells did not significantly change between storage conditions throughout the month ($p > 0.05$) and remained stable over the month in both conditions (Figure 5B). The initial viability of encapsulated cells starting at 43.9 ± 0.1% could be attributed to cell death caused by the shear stress of extrusion and high density of cells in the precursor solution during synthesis. However, the stability of relative % viability reflects that cells remain compatible with the encapsulation material over the month of storage time. While there was no significant change in viability over the month of storage, the % live does slightly decrease at day 7 and then increase afterward. The slight changes in viability can be attributed to the variability of cell dispersion between bead to bead in a batch. Overall, the quantitative and qualitative analysis of the live/dead confocal images confirm that the cells can survive up to a month in microbeads without additional nutrients in a laboratory environment. Future experiments will expand environmental conditions evaluated, such as humidity and temperature, to understand the encapsulation material's ability to preserve cell survivability in "real-world" conditions.

Induction of Encapsulated 2-PP Whole-Cell Living Sensors. To demonstrate that eBEADS can detect 2-PP at low concentrations, the sensor strain with the amplification circuit was encapsulated and exposed to 0 to 10 μM 2-PP for 48 h at 30 °C. The encapsulated strain showed a visual response within 24 h of exposure to 10 μM 2-PP (Figure 6). After 48 h, no response was observed in eBEADS that were exposed to less than 10 μM concentrations of 2-PP. These results demonstrate that eBEADS can detect as little as 10 μM 2-PP and produce a robust visual response. Future studies will be done to assess the eBEADS' performance in "real world" conditions by expanding temperature and humidity conditions.

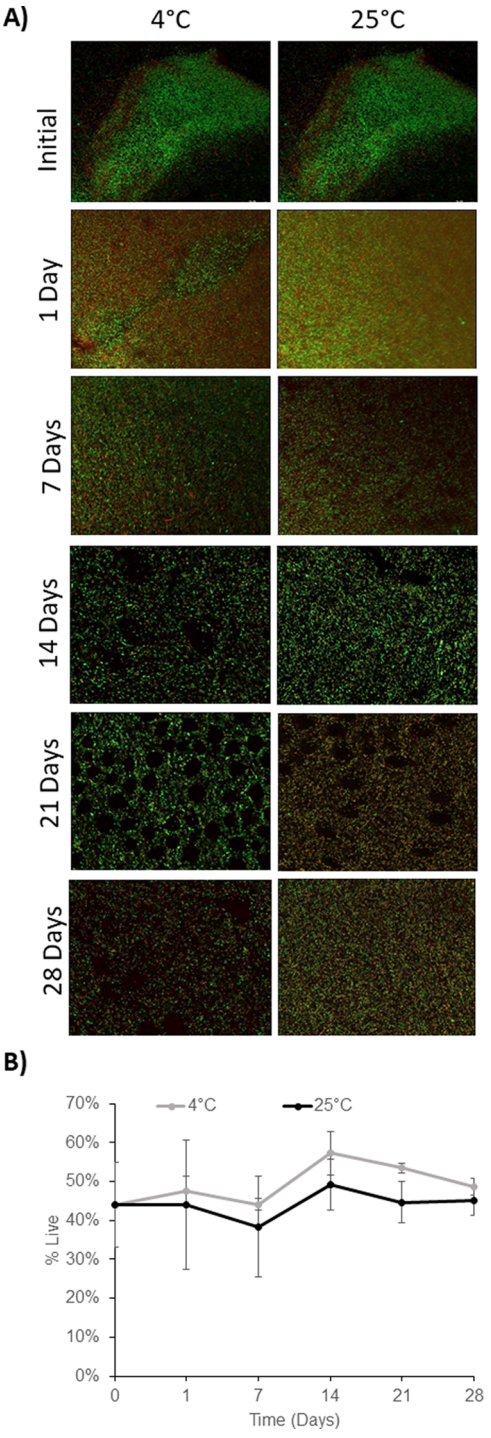

Figure 5. A) Compressed Z stack images of live/dead stained beads containing pBL_IMP_25/26 strain cells maintained over a month stored at 4 and 25 °C without supplemental nutrients in tightly closed conical tubes. Images taken with 20× objective. (B) Quantitative image analysis of confocal images confirms the steady viability of cells in microbeads stored at 4 and 25 °C without any additional supplements after a month. Error bars are standard deviation between three biological replicates.

CONCLUSIONS

eBEADS include an extensible system for deploying whole-cell living sensors with no need for equipment. Our engineered living sensor utilizes the HbpR transcriptional activator and an amplification circuit to detect as little as 1 μM 2-PP. This

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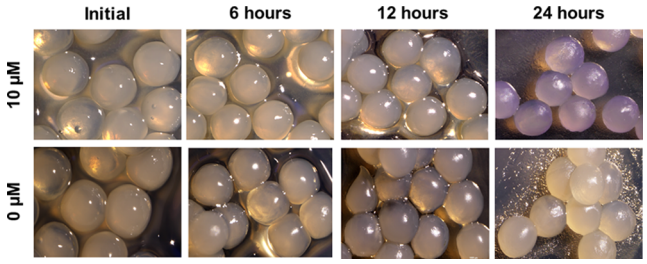

Figure 6. Images of whole-cell biosensors with an amplification circuit, encapsulated in the PAA microbeads, exposed over 24 h to 10 μM (top panel) and 0 μM (bottom panel) 2-PP. After 24 h of exposure (top right micrograph), the whole-cell biosensors demonstrate a visual color change in response to the analyte's presence. Images taken at 1.5× magnification.

whole-cell living sensor with the amplification circuit produces a long-lasting amplified signal up to 66 times over the non-amplification circuit strain treatments with minimal background. The signal amplification allows a timely and clear visual response, which is vital for in-field readouts. We demonstrate physical biocontainment and long-term viability of whole-cell living sensors in PAA microbeads. eBEADS is an end-to-end living sensor for the zero-power detection of 2-PP in the environment. This system serves as a tailorable platform to detect additional environmental pollutants and enables new sensor form factors to support applications such as remote deployment or wearables.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.2c00775.

Content on additional methods and results (PDF)

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■ ABBREVIATIONS

eBEADS, engineered biosensors in an encapsulated and deployable system; 2-PP, 2-Phenylphenol; IPTG, isopropyl β-D-1-thiogalactopyranoside; LB, Lysogeny broth; MBAA, *N*,*N*′-methylenebisacrylamide

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