Highlights

- Whole-cell bioreporter based on *recA* promoter and GFP reporter was developed for formaldehyde bio-sensing
- Bioreporter cells were immobilized in calcium-alginate hydrogel beads
- The assay solution effect was shown to improve sensitivity of alginate beads for gas phase experiments
- The detection limits of bead immobilized bioreporter for liquid and gaseous formaldehyde were 7.5 μg/mL and 8.1 ppm, respectively

Formaldehyde Sensing in Air and Water Using Fluorescent Bacterial Bioreporter Cells

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Abstract

Formaldehyde is a genotoxic volatile organic pollutant and one of the causative agents of sick building syndrome. Despite of its hazardous carcinogenic effects, it has been still used in daily life products and household materials. Hence, determination of formaldehyde in ambient air and drinking water sources is crucial to prevent its adverse health effects. Whole-cell biosensors have emerged as bio-sentinels for environmental monitoring to assess pollution in air, water, and soil. Herein whole-cell bacterial bioreporter was developed based on a DNA damage response gene promoter and green fluorescent reporter protein, and the cells were entrapped in calcium-alginate hydrogel beads for sensitive detection of formaldehyde in air and water. Alginate bead-immobilized bioreporter could successfully detect formaldehyde in both solution and the gas phase at concentrations minimum of 7.5 µg/mL and 8.1 ppm, respectively. These detection limits are useful for monitoring cumulative doses of bioavailable formaldehyde and taking precaution to avoid acute toxicity of formaldehyde. This bioreporter system is simple, low-cost, performable at room temperature and free of sample pre-treatment. The findings of this study will facilitate future research for the creation of portable and user-friendly devices for on-site and real-time environmental formaldehyde detection.

Keywords: Formaldehyde, bio-sensing, bacterial bioreporter, green fluorescent protein, alginate bead

1. Introduction

Formaldehyde (FA) is a flammable, colorless aldehyde compound (molecular formula CH_2O) with a pungent unpleasant odor. Gaseous formaldehyde is one of the most toxic volatile organic compounds (VOCs) and regarded as a ubiquitous air pollutant. Formaldehyde has a high aqueous solubility, and the commercial form of FA is known as formalin which is an aqueous solution consisting of 30-50 % FA by weight [1,2]. Formaldehyde is a widespread chemical pollutant in air, water and soil because it is broadly produced and used in various industrial fields. It is used as antimicrobial and preservative agent in food industry, in the manufacturing of synthetic resins, binders, adhesives, plastics, paints, surface coatings, wood-based products (e.g., furniture, panels, particle board and plywood), flooring and building materials, as a coupling agent for textile finishing, and as a disinfectant and fixative in laboratories, mortuaries and in many consumers health and cleaning products. It is also released into the ambient air as a by-product in the combustion of organic compounds, on-site industrial and power plant emissions, forest or bush fires, automobile exhaust and tobacco smoke [3-6].

As one of the most hazardous substance, many organizations have set guideline levels for airborne formaldehyde. According to World Health Organization (WHO), short-term (30-minute) guideline of 0.1 mg/m^3 (0.08 ppm) is recommended as safety threshold limit of exposure for preventing significant sensory irritation in the general population and cytotoxic damage to the nasal mucosa [1]. The Occupational Safety and Health Administration (OSHA) has established the permissible exposure limit of formaldehyde in the workplace as an average of 0.92 mg/m^3 (0.75 ppm) for an 8-h workday, and also the maximum short-term exposure limit as 2.5 mg/m^3 (2 ppm) for 15-min period [7].

Gaseous formaldehyde is classified by International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) based on the sufficient evidence in humans for the nasopharyngeal cancer and leukaemia [3]. The major route of formaldehyde exposure is

inhalation. Acute or subsequent exposure is highly irritating to the eyes, nose, throat, and cause severe allergic reactions, lachrymation, sneezing, coughing, nausea, dyspnoea since FA is a highly reactive chemical and water-soluble that readily reacts with biological tissues, particularly the moist mucous tissues lining the respiratory tract and the eyes [8,9]. Chronic exposure to low-level formaldehyde causes respiratory diseases and allergic dermatitis which is also called sick building syndrome (SBS) as a result of outgassing from household materials and resins in furnitures, wallpapers or paints [10,11]. Moreover, chronic exposure of formaldehyde causes neurotoxicity, inflammatory and hyperplastic changes of the nasal mucosa, epithelial dysplasia, pulmonary damage, hematotoxicity, reproductive toxicity, and carcinogenicity. Formaldehyde exerts its genotoxic effects by reacting with DNA, RNA and protein to form adducts and cross-links such as DNA mono-adducts, DNA–DNA crosslinks, DNA–protein crosslinks (DPX) and DNA glutathione cross-link resulting in DNA strand breaks, chromosomal aberration, micronucleus formation and sister chromatid exchange [1,3,8,12,13].

As the knowledge and awareness of the harmful effects of formaldehyde become more obvious, the air quality and monitoring of the ambient and indoor air appear to be important issues to reduce the risk of damaging health effects of gaseous formaldehyde [14,15]. The most widely used traditional methods for the determination of the concentration of formaldehyde in air are based on spectrophotometry, with which sensitivities of 0.01–0.03 mg/m^3 can be achieved. Other analytical methods involve colorimetry, fluorimetry, high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis, polarography, conductometry, infrared detection and gas detector tubes [4,16-18]. These methods show certain limitations such as the need of expensive and bulky instrumentation and well-trained operators, operational complexity, non-portability, inutility in real-time or routine monitoring and finally lack of toxicity detection. Thus, the development of sensitive, selective and facile

methods for fast and cost-effective FA detection for rapid estimation of personal exposure is still a hot research topic [4,6,15,19]**.**

Various sensor-based approaches or combinations of different methods have been investigated for practical analysis of formaldehyde such as fluorescent probes [20], metal oxide films and semiconductors [21,22], piezoelectricity [23], quartz crystal microbalance [24,25], chemoresistive gas sensors [26], electrochemical [27,28], enzyme-based [29,30], and molecular imprinting [31,32]. The use of whole cell biosensors (WCBs) is another promising approach for detection of air pollutants. Unlike the aforementioned air monitoring technologies, WCBs (genetically engineered living cells) are able to assess the toxicity and bioavailability of the pollutant of interest [33,34].

Ever since the development of bacterial genotoxicity assay, the Ames test in 1973, in which strains of *Salmonella typhimurium* were used as mutagenicity or genotoxicity reporters [35], numerous and diverse genetically engineered bacterial bioreporters have been produced for environmental monitoring of specific or group of toxic pollutants in water, soil and air [36-39]. The basic principle of bacterial bioreporter is the combination of promoter-operator DNA region which acts as a pollutant sensing element and a downstream reporter DNA element which is translated into a detectable signal protein by using an appropriate bacterial host strain having a suitable genetic background allowing movement of the pollutant across the cell, recognition of the specific promoter and insignificant reporter background activity [40,41].

Since formaldehyde is a genotoxic chemical, one of the strategies of bioreporter construction for FA detection is to employ the DNA damage-inducible promoter of *recA* gene which produces the key regulatory protein of the well-defined bacterial SOS DNA repair system. The bacterial SOS regulon has more than 40 unlinked genes for DNA damage tolerance and error-prone replication (e.g., *dnaQ*, *uvrA*, *uvrB*, *recA*, *recN*, *sulA*, *umuC*, *umuD*) which are expressed in the cell at a basal level and controlled by the LexA transcriptional repressor protein

which binds SOS box (LexA-binding sites) of these inducible genes at various strengths. After bacterial cells are exposed to DNA damaging agents such as UV-irradiation or mutagens, the RecA proteins become activated by single-stranded DNA formation and catalyzes the self-cleavage of LexA protein thus allowing the high-level expression of SOS regulon genes including *recA* itself to repair the damaged DNA. Thus, RecA protein has an important role for initiation of the SOS response and has a broad involvement in many DNA repair pathways, including daughter-strand gaps, double-strand breaks, and error prone DNA damage survival mechanisms [42-44].

The aim of this study is to develop a bacterial bioreporter system for gaseous formaldehyde detection. A fluorescent bioreporter strain, *Escherichia coli* (pBR-PrecA), harboring a plasmid-borne transcriptional fusion between the *E. coli recA* gene promoter and *gfpuv* (green fluorescent protein) reporter gene was constructed. Thus, formaldehyde will induce the derepression of *recA* promoter which increase the expression of downstream GFP and increase the fluorescent signals to be measured. The testing of gaseous formaldehyde has been made feasible by immobilization of FA bioreporter into alginate hydrogel matrix permitting semi-direct contact between the sensor bacteria and FA gas. Sodium alginate was chosen as immobilization polymer since it is commonly used for cell immobilization due to its easy and gentle preparation conditions, its hydrogel environment enabling cells the maintenance of cell activity over a long period and providing a mechanical support and plasmid stability. Moreover, immobilization enables portability and long-term measurement in contrast to liquid culture [34,45]. Only limited research is available in the literature regarding gaseous formaldehyde detection by using WCBs. To the best of our knowledge, this is the first study for gaseous formaldehyde sensing by using alginate bead-immobilized *recA*-based fluorescent *E. coli* bioreporter. The developed alginate bead-immobilized bioreporter cells are able to detect

formaldehyde in both liquid and gaseous phases and offer a simple and cost-effective method for monitoring of toxic formaldehyde.

2. Materials and methods

2.1. Bacterial strains, media and chemicals

E. coli DH5α strain was used for promoter cloning experiments. *E. coli* MG1655 (ATCC 700926) was used as host strain for bioreporter construction. LB (Luria-Bertani) broth (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, pH 7.0) was used for bacteria propagation. Mineral salt supplemented medium (MSSM), (10 mM Na_2HPO_4, 5 mM KH_2PO_4, 34.2 mM NaCl, 12.5 mM NH_4NO_3, 2 mM $MgSO_4$, 0.2 mM $CaCl_2$, 35 µM $FeCl_3$, 0.1 % (w/v) casamino acids, 0.5 % (w/v) glucose, pH 7.0) was used as an induction and immobilization medium. Ampicillin (100 µg/mL) was used for plasmid maintenance. Formaldehyde solution (formaline), 37% technical grade (Applichem A2628) was used as the inducer. Formaldehyde (MW: 30.03 g/mol) concentration of formalin is 12.3 M. Alginic acid sodium salt (Sigma-Aldrich 71238) was used for alginate bead immobilization experiments.

2.2. Construction of the sensor plasmid

The formaldehyde sensing DNA sequence belonging to the promoter region of the *recA* gene (NCBI Gene ID: 947170) were obtained from *E. coli* MG1655 genome (GenBank Accession Number U00096.3). *E. coli* MG1655 genomic DNA was isolated by using Nanobiz DNA4U Bacterial Genomic DNA Isolation Kit (Turkey) for gram-negative bacteria and used as the template for amplification of the promoter region. The high-fidelity polymerase chain reaction (Hi-Fi PCR) of *recA* promoter was done by using Phusion DNA polymerase (Thermo Scientific, USA) using forward primer recAp-F (5'GTGACAGAATTCGTCACCTACAGTAACGAA GCC3') and reverse primer recAp-R (5'GTATGTGGATCCTACTCCTGTCATGCCGGG'3). The amplified 427 base-pair DNA fragment was then digested with the *EcoRI* and *BamHI* restriction enzymes and then inserted at the same restriction sites into formerly constructed

promoterless reporter plasmid ‘pBR-sGFP’ [46]. The final construct was confirmed using agarose gel electrophoresis and Sanger sequencing using the oligonucleotides which are forward primer Col-F (5’ATCACGAGGCCCTTTCGTCTTCAAGAATTC3’) and reverse primer Col-R (5’ACGCTGCCCGAGTTATCATTATTTGTAGAGCTC3’). The resulting sensor plasmid designated as ‘pBR-PrecA’ was chemically transformed into chemically competent *E. coli* MG1655 cells. The plasmid map was generated using Snapgene software (GSL Biotech, USA).

2.3. Induction of free bioreporter cells with liquid formaldehyde

The overnight-grown bioreporter cells were inoculated into 10 mL of mineral salt supplemented medium (MSSM) at a 1:20 (v/v) ratio in 50 mL conical tubes. Diluted aqueous solutions of formaline was added to bacterial cultures at final formaldehyde concentrations of 50, 100, 250, 500, 750 and 1000 μM (1.5, 3, 7.5, 15, 22.5 and 30 μg/mL) including a negative control (sterile distilled water). The tubes were incubated at 35 °C on a shaker at 180 rpm for 24 hours. The cell growth was monitored via measuring optical density at 600 nm by using Multiskan GO UV/Visible Microplate and Cuvette Spectrophotometer (Thermo Fisher Scientific, USA). The fluorescence measurements were done by applying 200 μL of each culture into 96-well standard black microplates (Greiner Bio-One) at different time points by using SpectraMax iD3 (Molecular Devices, USA) multi-mode microplate reader. The fluorescence measurements were taken at excitation and emission wavelengths of 395 and 509 nm, respectively, for the reporter green fluorescent protein, GFPuv [47]. Induced cells were observed using an EVOS Floid Imaging Station (Thermo Fisher Scientific, USA) under illumination of blue channel with excitation 390 ± 40 nm and emission 446 ± 33 nm.

2.4. Immobilization of bioreporter cells into alginate beads

Aqueous solutions of alginic acid sodium salt of 2% (w/v) were prepared one day before making beads for polymer stabilization. Overnight bioreporter bacterial culture was subcultured in

MSSM and exponentially grown bacteria were centrifuged at 3500 xg and 15 °C for 8 mins. Cell pellets were gently re-suspended in fresh MSSM to optical density at 600 nm (OD_{600}) of 0.4 (1 unit of OD_{600} = 8×10^8 cells/mL). The prepared bioreporter bacterial culture was homogeneously mixed with alginate solution in a 1:1 (v/v) ratio and gently stirred for 15 mins. The mixture was dripped from a 10 mL syringe with a 2 mm inner diameter spout that was mounted on a syringe pump into a 0.3 M $CaCl_2$ solution in a glass beaker with magnetic stirrer at a flow rate of 100 µL/sec. 1 mL of alginate-cell mixture generated an average of 30 beads [48]. The beads that spontaneously formed were stirred for additional 30 mins to ensure a complete gelation process. After rinsing with sterile distilled water to remove excess calcium chloride and unentrapped cells, the alginate beads were damp-dried on filter paper and stored at 4 °C until use.

2.5. Liquid formaldehyde detection by immobilized bioreporter cells

Thirty beads were placed in 50 mL conical tubes and 4 mL of 1:2 (v/v) diluted aqueous MSSM solution (0.5X MSSM) was added. Serial dilutions of aqueous formaldehyde solutions having final concentrations of 50, 100, 250, 500, 750 and 1000 µM including a negative control (sterile distilled water) were prepared on a daily basis. The tubes were incubated at 35 °C with gentle agitation for 16 hours. Fluorescence measurements were performed by placing three beads in the wells of 96-well microplate and data obtained by using SpectraMax iD3 multi-mode microplate reader at different time points at excitation/emission wavelength of 395/509 nm.

2.6. Gaseous formaldehyde detection by immobilized bioreporter cells

Between 25 and 30 alginate-cell beads were transferred to small plastic Petri dishes (40 mL in volume) and 3 mL of different assay solutions were added to beads in order to prevent desiccation and shrinkage of alginate hydrogel beads. These Petri dishes were placed into 5-L plastic storage boxes. Then, 20 mL of aqueous dilutions of formalin solutions having concentrations of 300 mM, 150 mM, 100 mM, 60 mM and 30 mM (FA concentrations of

including a negative control (distilled water) in a glass beaker were placed at the center of the storage boxes. Then the boxes were closed tightly and incubated under controlled temperature at 25 °C for 16 hours at 60 rpm shaking. Formaldehyde gas concentrations generated from the liquid-vapor equilibrium of diluted formalin solutions at 25 °C were determined by using Henry's law formula below [4,49] and using a H_{eff} = 3700 M atm^{-1}.

$$P(atm) = \frac{Concentration\ (M)}{H_{eff}(M.atm^{-1})}$$

$$ppm = P(atm).10^{6}$$

By applying these formula, FA gas concentrations (after saturation) in the boxes were calculated as 81, 40.5, 27, 16.2 and 8.1 ppm, respectively. At determined time points, three beads were quickly taken from the Petri dish without disturbing the system and placed into 96-well black microplate and fluorescence measurements were taken immediately as described above.

2.7. Testing different bead assay solutions

For vapor phase experiments, tested bead assay solutions include sterile deionized water, mineral salt supplemented medium (1X MSSM), 1:2 and 1:4 (v/v) diluted aqueous MSSM solutions (0.5X MSSM and 0.25X MSSM), 0.2 mM $CaCl_2$ solution, and 1X PBS (phosphate-buffered saline).

2.8. Gas specificity of immobilized bioreporter cells

For gas specificity test, some of common volatile toxic solutions were tested. 100 mM of aqueous solutions of glacial acetic acid, acetone, chloroform, isopropanol, methanol, and xylenes were prepared including negative control and 20 mL of each solution was placed in their respective boxes. Then the boxes were closed tightly and incubated at controlled temperature at 25 °C. At the end of 16 hours, three beads were placed into 96-well black microplate and then fluorescence measurements were taken immediately as described above.

2.9.Data analysis

All statistical analyses were conducted using the IBM SPSS Statistics 25.0 software package for Windows. The response of bioreporter cells was indicated by fluorescence intensity. Raw fluorescence intensities were expressed in the instrument's arbitrary relative fluorescence units (RFU). The (normalized) fluorescence response was calculated by subtracting induced culture fluorescence from background *E. coli* MG1655 (pBR-sGFP) (promoterless bioreporter) culture fluorescence for all concentrations at the corresponding time points. All tests were performed in triplicate and results were expressed as mean values with standard deviations which were represented by error bars in the graphs. The limit of detection or the detection limit was set at the lowest concentration of formaldehyde that is detected under the stated experimental conditions. The one-way analysis of variance (one-way ANOVA) was performed with the significance level of 0.05 ($p < 0.05$) followed by Tukey's post hoc comparison test between RFU values of induced and uninduced samples to determine the detection limits.

3. Results and discussion

3.1. Bioreporter strain construction

For the development of whole cell bacterial bioreporter for formaldehyde detection, a sensor plasmid, designated as pBR-PrecA, (Fig 1) containing promoterless green fluorescent protein gene (*gfpuv*) under the control of *recA* gene promoter, was constructed and then transformed to *E. coli* MG1655 host strain. As being one of the key SOS response genes that is responsible for error-prone DNA repair to survive sudden or extensive DNA damage [50], the application of the *recA* promoter is useful for development of efficient bacterial biosensors for genotoxic formaldehyde detection. Upon FA exposure, RecA proteins became activated in the cells causing derepression of *recA* promoters by the release the LexA repressor protein and the expression of reporter *gfpuv* was induced. The developed FA bioreporter, *E. coli* (pBR-PrecA), was tested against liquid and gaseous formaldehyde, and the bioreporter response was obtained by measuring green fluorescent protein (GFPuv) signals.

3.2. Induction of free bioreporter cells with liquid formaldehyde

In order to confirm the applicability of FA bioreporter cells, the liquid FA induction tests were done with FA concentrations between 50 and 1000 μM. Firstly, the growth of the bioreporter cells was monitored to assess the toxicity of formaldehyde on bacterial cells (Fig 2a). Bacterial cells tolerated liquid formaldehyde concentrations of up to 750 μM (22.5 μg/mL) and 1 mM FA (30 μg/mL) was found toxic at which no cell growth was observed.

Ptitsyn et al. [51] constructed a genotoxin bioreporter by fusing *cda* promoter upstream of the promoterless *luxCDABFE* genes and they also reported a small dynamic range of detection which is between 0.3 and 0.75 mM that formaldehyde concentration higher than 750 μM was highly cytotoxic and no light emission could be detected, similar to the present findings.

Fig 2b shows the time- and dose-dependent fluorescence response of the bioreporter to liquid FA presented during 16-h induction period. The detection limit was determined using statistically significant changes ($p<0.05$) in RFU compared with no induction control RFU. One-way ANOVA results showed that the FA bioreporter induction was significant after 4 hours ($p=0.00$) by all the tested concentrations of formaldehyde except 1 mM of FA which was very toxic and inhibited the cell growth. However, after 8 hours due to increased background fluorescence only 250, 500 and 750 μM showed a fluorescence response with a statistically significant change ($p<0.05$). The prominent response was obtained from 15 μg/mL (500 μM) of FA at 4-h and 8-h time points and 22.5 μg/mL (750 μM) FA induction continued to increase after 8 hours contrast to other concentrations which tend to decline after 8 hours.

According to World Health Organization [52] formaldehyde is not carcinogenic by the oral route and they did not set a guideline value in drinking water stating that the occurrence of formaldehyde is below the concentrations of health concern. However, US EPA has advised that the exposure to formaldehyde in drinking water health advisory limit of 10 mg/L for 1 day

or 5 mg/L for 10 days for 10 kg child. Moreover, The US EPA has also determined that a lifetime exposure to 1 mg/L of formaldehyde in drinking water is not expected to cause any adverse health effects [53]. Thus, since the developed FA bioreporter has detection range between 1.5 and 22.5 mg/L, it can be used detect these advised exposure limits.

Bacterial bioreporters constructed by the fusion of the *recA* promoter to a reporter gene has been regarded as an effective genotoxicity sensor for genotoxic agents as mitomycin C (MMC), nalidixic acid (NA), methylnitronitrosoguanidine (MNNG), dimethylsulfate, hydrogen peroxide (H_2O_2), bisphenol A, etc. and developed over the last two decades [54-60]. Formaldehyde has been also detected by *recA*-based bacterial genotoxicity bioreporters [61-65].

Kostrzynska et al. [62] constructed *E. coli* C600 cells carrying pRGW50 or pRGM5 plasmids based on *recA* promoter fused to wild type *gfp* or red-shifted variant *gfp* (mut3) to perform genotoxicity test. Besides nalidixic acid, MMC, MNNG, hydrogen peroxide they also tested formaldehyde. They report the detection limit for FA 305±51 μM and small dynamic range (200-800 μM) for *recA* induction. They also indicated that higher concentrations significantly diminished viability of cells. Kuang et al. [63] employed *E. coli* MG1655+pUA2699 carrying a *recA::gfp* fusion plasmid. They performed microtiter assay in M9 medium and tested 10 μg/mL of MMC, MNNG, H_2O_2, FA and NA. They reported much weaker fluorescence response from FA compared to MMC and reported that cells induced with FA concentrations of 0.1 mg/mL (3.3 M) had high fluorescence response with no lethal effect. Matejczyk [64] used *E. coli* K-12 MG1655 (pUA66) strain having *recA* promoter fused with gfp mutated gene – GFPmut2 variant. The tested concentrations of FA were between 50 and 1800 mg/ml which was too high compared to the present study. It was reported that 900 mg/mL (30 M) FA was found to induce highest fluorescence.

Fig 2c compares the uninduced bioreporter cells and induced cells with 500 μM formaldehyde (15 μg/mL). While uninduced cells had no visible emission, induced cells showed a bright fluorescence emission, and some of the FA exposed cells were seen as very long cells. The explanation for this phenomenon can be that as genotoxic FA causes cell replication to stall, RecA proteins are recruited to DNA at the stalled replication forks then activating SulA-mediated cell division inhibition. This leads to cell filamentation and increase in the ratio of elongated cells due to inhibition of cell division for the fast-growing *E. coli* cells [66,67].

3.3. Immobilization of bioreporter cells into alginate beads

Since the developed FA bioreporter was found to be applicable in formaldehyde detection according to the results of liquid culture broth induction, the bacterial bioreporter cells were entrapped in a suitable immobilization matrix for convenient gas detection. Immobilization enables portability, on-site detection ability and integration into mobile devices. By bioreporter immobilization, direct testing of the gaseous FA has been made possible in a way that liquid FA evaporated during incubation time and then diffused through the air directly to the entrapped bacteria [68,69].

Ca-alginate beads are widely used hydrogel matrices in which cells are passively and non-covalently entrapped into the gel matrix under mild physicochemical conditions. Moreover, alginate hydrogel beads have high porosity due to their open lattice structure and confer gentle environment that makes them an optimal choice for cell entrapment also enabling analyte diffusion and metabolite secretion. As described in Fig 3, the beads were produced by mixing bioreporter culture and sodium alginate solution and by extruding this mixture dropwise into calcium chloride solution where Ca-alginate beads are formed spontaneously by rapid crosslinking between negatively charged alginate polymers and positively charged divalent calcium ions [70,71]. The alginate immobilization optimized parameters in terms of final

alginate concentration of 1% (w/v) and bacterial cell density in the matrix of OD_{600} 0.25 [48] were employed in this study.

3.4. Liquid formaldehyde detection by immobilized cells

Prior to testing with gaseous formaldehyde, bead immobilized FA bioreporter was tested against liquid formaldehyde (aqueous dilutions of formalin). FA concentrations between 50 and 1000 μM were tested to obtain time- and dose-dependent fluorescence responses.

The fastest and highest fluorescence response was obtained with 500 μM (15 μg/mL) of FA within a 4 h-induction (Fig 4a). The lowest detection limit of liquid FA was obtained at 250 μM (7.5 μg/mL) within 8 hours and the highest FA concentration of 750 μM (22.5 μg/mL) was detected after 8 hours with a statistically significant change ($p<0.05$). As with the case of free cells, 1 mM FA showed lowered fluorescence compared to that of control with no FA treatment indicating its lethal effects. Fig 4b shows the comparison between the uninduced bioreporter beads (control) and induced beads with 500 μM formaldehyde (15 μg/mL). While for uninduced cells only excitation wavelength (blue) was visible, induced cells displayed a marked green emission due to GFP fluorescence.

There are few studies for immobilized *recA*-based bioreporter systems for liquid formaldehyde detection. Eltzov et al. [72] immobilized *E. coli* DPD2794 strain having plasmid-borne fusion of the *recA* promoter to a *luxCDABE* reporter operon into calcium alginate pads coupled to photodetector. They tested this strain against various chemicals including liquid formaldehyde between 10^{-6} and 10^{-14} M and the strain was not sensitive against formaldehyde which is probably due to very low tested concentrations.

3.5. Gaseous formaldehyde detection by immobilized cells

For gaseous FA induction, the experimental setup in Fig 5 was used. Firstly, the beads were placed in Petri dishes without a lid and assay solutions were added on them. The Petri dishes were then put in a 5-L storage boxes. The serial dilutions of formalin solutions and water

(negative control) were placed at the center of the boxes. Finally, the boxes were closed tightly and placed on orbital shakers for better evaporation of solutions and aeration of beads. Formaldehyde in the aqueous solutions was considered to evaporate completely during the experiment time and expected to diffuse into alginate beads to induce GFP expression in bioreporter bacteria.

3.6. Testing different bead assay solutions

Unlike the liquid induction tests, this time alginate beads were exposed to air for hours, so they were highly prone to dehydration and shrinkage. These hydrogel beads should be kept moist during induction to avoid loss of water, to sustain cell viability and bioreporter activity inside the beads. For these reasons, assay (preservation) solutions were added on the beads in a small amount to keep them hydrated. Different solutions were tested to find the most suitable assay solution presenting higher fluorescence performance and sensitivity.

As seen in Fig 6, MSSM and 0.5X (1/2 strength) MSSM provided the highest fluorescence responses whereas 0.25X MSSM had comparably low RFU values. The other solutions such as, water, calcium chloride and 1X PBS did not produce any response probably due to the fact that they could not support the metabolic activity of the cells to recover from the toxic effects of FA or to express sufficient amount of reporter protein. It should be noted that the main disadvantage of using alginate-based hydrogels is its tendency to dissolve in presence of low pH, high concentrations of non-gelling ions (e.g., Na^+, Mg^{2+}, and K^+), polyphosphates, citric acid and EDTA solutions [73]. Therefore, the composition of assay solution should be optimized to keep the integrity of hydrogel beads to withstand hours and to avoid releasing of immobilized cells. This case was observed with the tested 1X PBS assay solution which has a high sodium and potassium content.

3.7. Dose-dependent response of beads to different formaldehyde vapor concentrations

After determining the most suitable assay solutions that are MSSM and 0.5X MSSM, the immobilized bioreporter was tested in these assay solutions at different FA gas concentrations.

For MSSM assay solution, according to one-way ANOVA results, the induction of bioreporter was significant within 8 hours ($p=0.00$) and the detection limit was 8.1 ppm of gas FA. For 16-h incubation, gaseous FA at 27 and 40.5 ppm exhibited increased fluorescence (Fig 7a) which could be attributed to that these gaseous formaldehyde level in the box kept the cells stressed for a longer time, when compared to lower FA levels. When 0.5X MSSM assay solution was used, the sensitivity of bioreporter decreased to 16.2 ppm after 8 hours and the fluorescence responses were somewhat lower compared to that of MSSM for all data points (Fig 7b) which can be due to lower nutrient content of half-strength MSSM solution. For both assay solutions, gaseous formaldehyde of 81 ppm did not produce significant fluorescence response due to possible toxicity to immobilized cells.

3.8. Gas specificity of immobilized bioreporter cells

For assessment of gas specificity or selectivity of the FA bioreporter, aqueous solutions of commonly used volatile compounds, acetic acid, acetone, chloroform, isopropanol, methanol, and xylenes were tested.

As shown in Fig 8, for both MSSM and 0.5X MSSM assay solutions, among tested volatile compounds, acetone which is also an aldehyde, and methanol induced the bioreporter, but the fluorescence responses were less than half of those induced by formaldehyde. Upon most occurrences both outdoor and indoor, formaldehyde gas is the predominant form of environmental aldehydes [74], and the developed FA bioreporter showed a significantly higher selectivity for formaldehyde.

4. Conclusion

This study presents the development of an alginate-immobilized fluorescent bacterial bioreporter for both liquid and gaseous formaldehyde detection. The bioreporter cells entrapped

in alginate beads are able to detect liquid formaldehyde concentrations as low as 7.5 μg/mL within 4 hours and are able to indicate biologically harmful levels of formaldehyde. To our best knowledge, no studies have been conducted for immobilized *recA*-based bioreporter systems characterized for gaseous formaldehyde detection. The bioreporter described could detect gaseous formaldehyde levels as low as 8.1 ppm in air. Even though it has high gas detection limits compared to standard analytic methods, it is capable of detecting cumulative doses over 8 hours which can be considered similar to an 8-h working day. Moreover, it has a good specificity for formaldehyde and its detection limit can be improved by using more sensitive detection methods such as using very bright fluorescent proteins [75] or tandem fluorescent protein constructs [76]. The reported bioreporter system is simple, cost-effective, operates at room temperature and requires no sample preparation. It can be further developed to be used in handheld environmental monitoring kits or in remote-controlled sensor devices for on-site formaldehyde monitoring.

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FIGURE LEGENDS

Fig. 1. Complete map of formaldehyde sensor plasmid pBR-PrecA. Abbreviations used: *AmpR*, confers resistance to ampicillin; *ori*, pBR322 origin of replication; *bom*, basis of motility; *rop*, maintains plasmids at low copy number; *recAp*, DNA-damage responsive promoter; sGFP, promoterless gene for green fluorescent protein.

Fig. 2. a) Growth curve of bioreporter cells treated with different formaldehyde concentrations **b)** Fluorescence emission kinetics of FA bioreporter in response to different liquid formaldehyde concentrations. The average of triplicate measurements is plotted with standard deviations. Error bars are shown only when they exceed the size of the symbols **c)** Fluorescence micrographs of uninduced and 500 μM formaldehyde-induced bioreporter cells after 16-h treatment. Scale bars are 100 μm.

Fig. 3. Summary of experimental procedure describing formation of bioreporter immobilized Ca-alginate hydrogel beads. The figure was created with BioRender.com.

Fig. 4. a) Fluorescence emission kinetics of alginate bead immobilized FA bioreporter in response to different liquid formaldehyde concentrations. The average value of a triplicate of each induction is presented with standard deviation. Error bars are shown only when they exceed the size of the symbols **b)** The photo of uninduced and induced (with liquid 500 μM FA) beads. Images were taken by smartphone camera while the beads were placed on EVOS Floid Imaging Station (Thermo Fisher Scientific, USA) using blue excitation (390 ± 40 nm) with emission detection at 446 ± 33 nm.

Fig. 5. Overview of the basic experimental procedure of gaseous formaldehyde detection. The figure was created with BioRender.com.

Fig. 6. Effect of bead assay solutions on the gaseous FA detection performances of alginate-immobilized FA bioreporter. The average value from triplicates of each induction is presented for 16-h assay time with standard deviation.

Fig. 7. Fluorescence emission kinetics of alginate bead immobilized FA bioreporter in response to different gaseous formaldehyde concentrations assayed in **a)** MSSM and **b)** 0.5X MSSM. The average value of a triplicate of each induction is presented with standard deviation. Error bars are shown only when they exceed the size of the symbols.

Fig. 8. Gaseous selectivity of the FA bacterial bioreporter assayed in the MSSM and 0.5X MSSM. The gas concentrations of FA and other VOCs were at 400 μM. Measurements were performed following 16-h treatment.

FIGURES

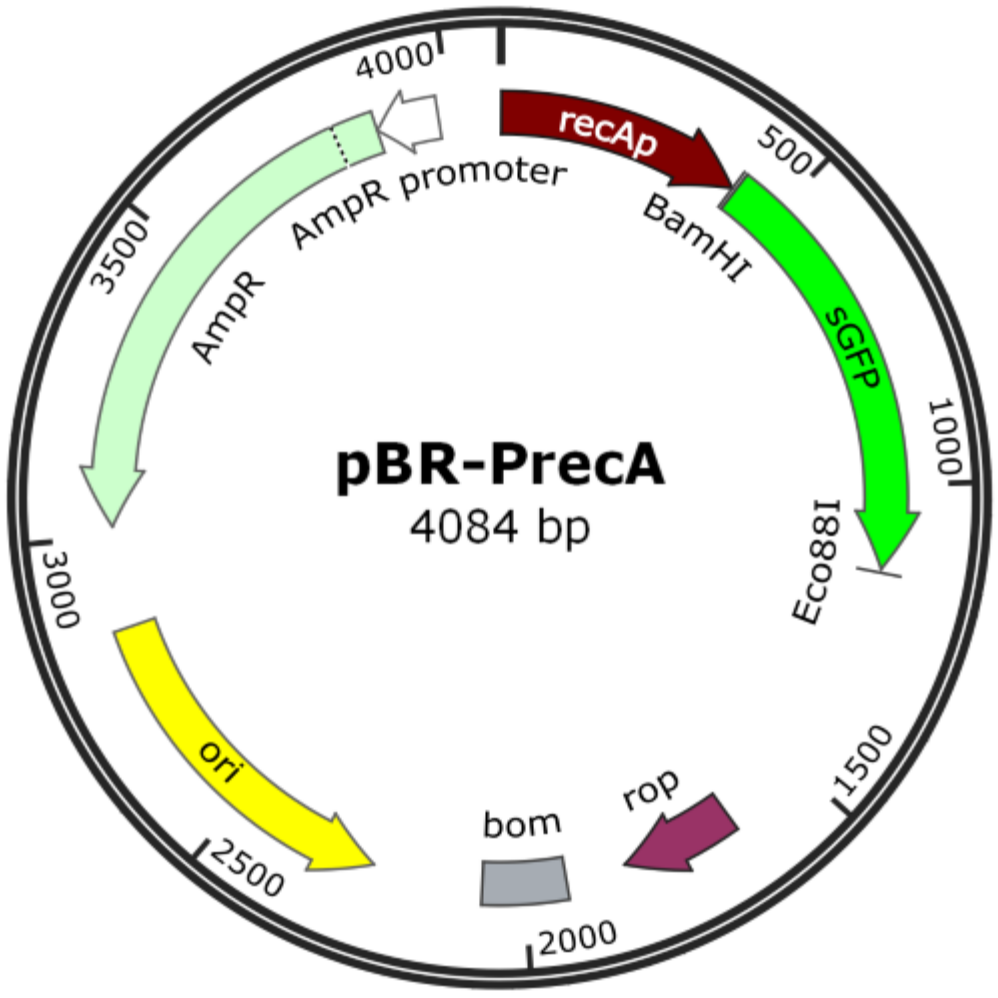


FIGURE 1

Fig. 1. Complete map of formaldehyde sensor plasmid pBR-PrecA. Abbreviations used: *AmpR*, confers resistance to ampicillin; *ori*, pBR322 origin of replication; *bom*, basis of motility; *rop*, maintains plasmids at low copy number; *recAp*, DNA-damage responsive promoter; sGFP, promoterless gene for green fluorescent protein.

FIGURE 2a

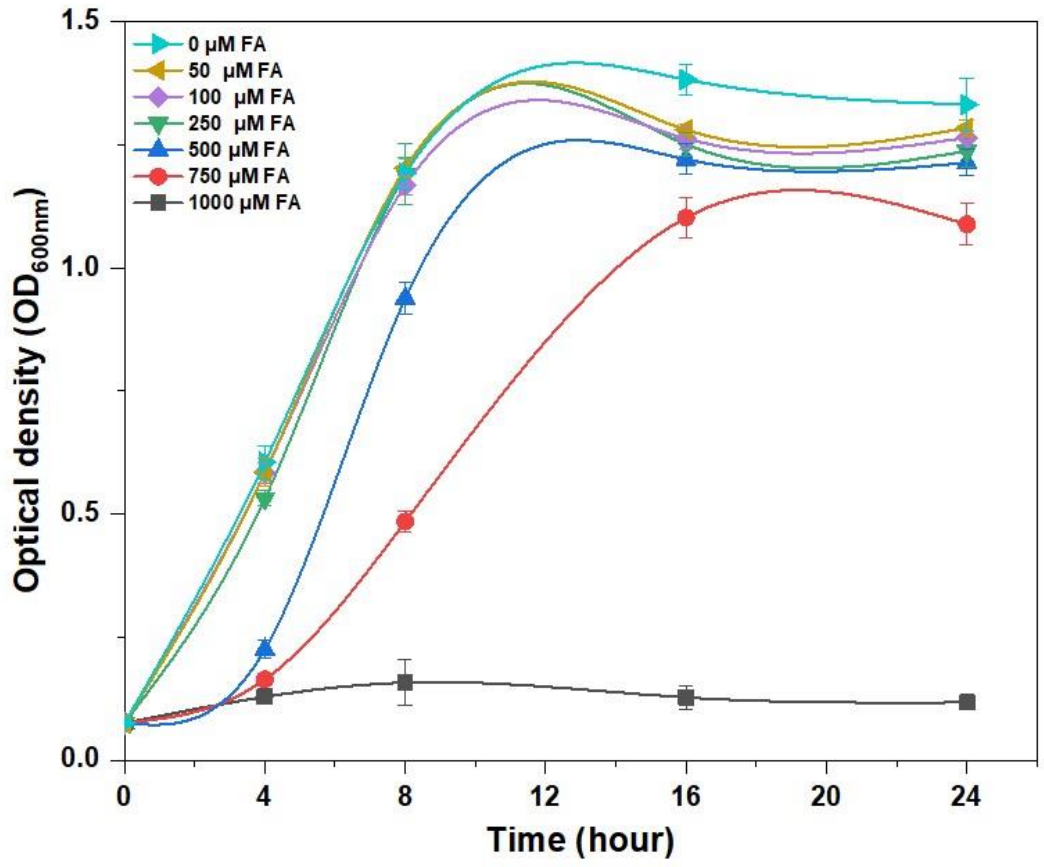
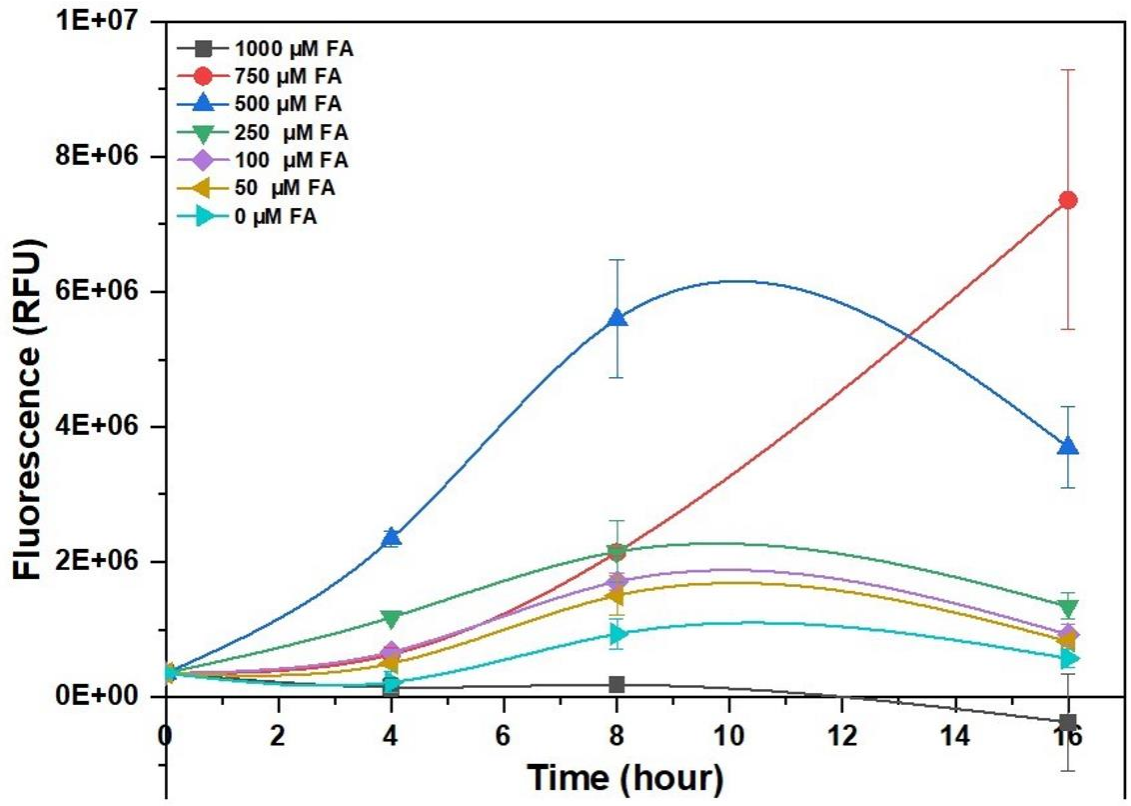


FIGURE 2b



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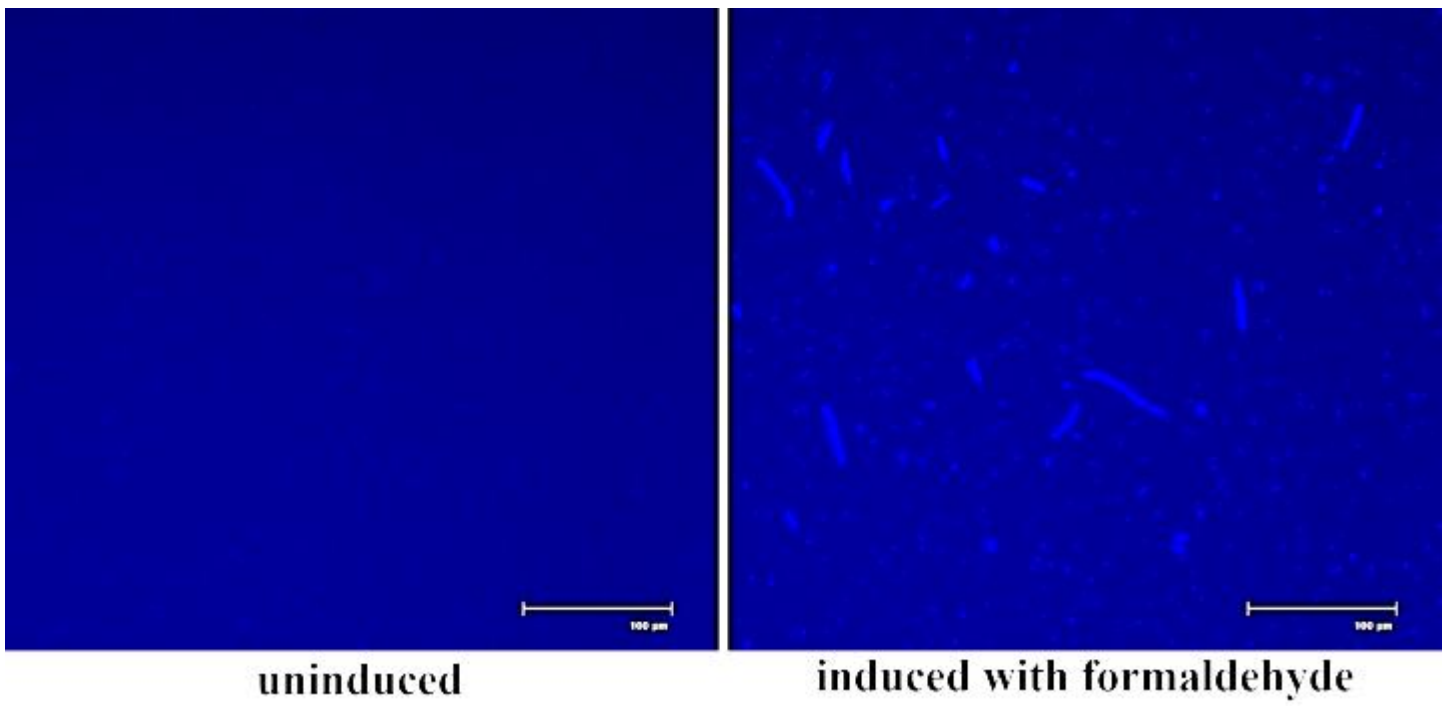

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FIGURE 3

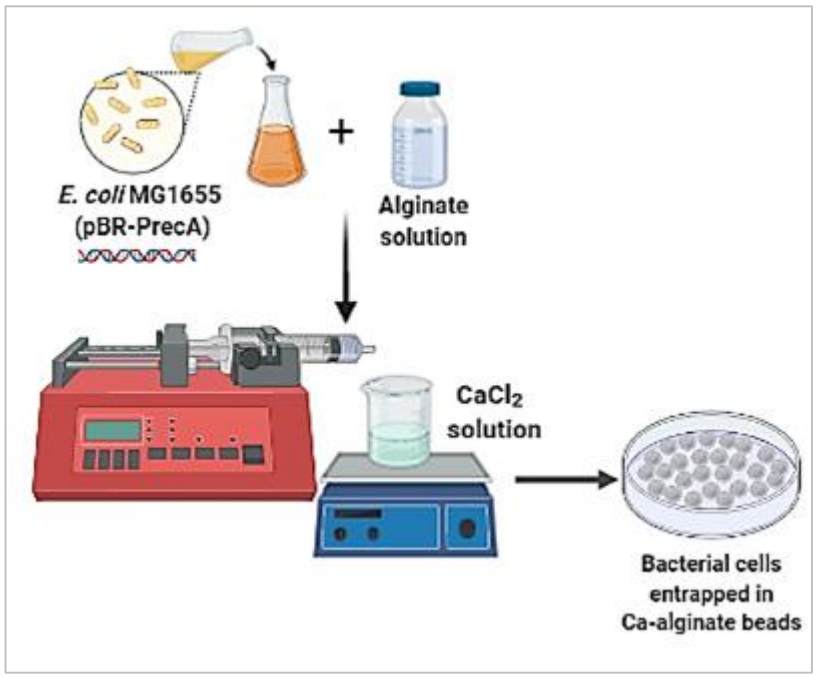

Fig. 3. Summary of experimental procedure describing formation of bioreporter immobilized Ca-alginate hydrogel beads. The figure was created with BioRender.com.

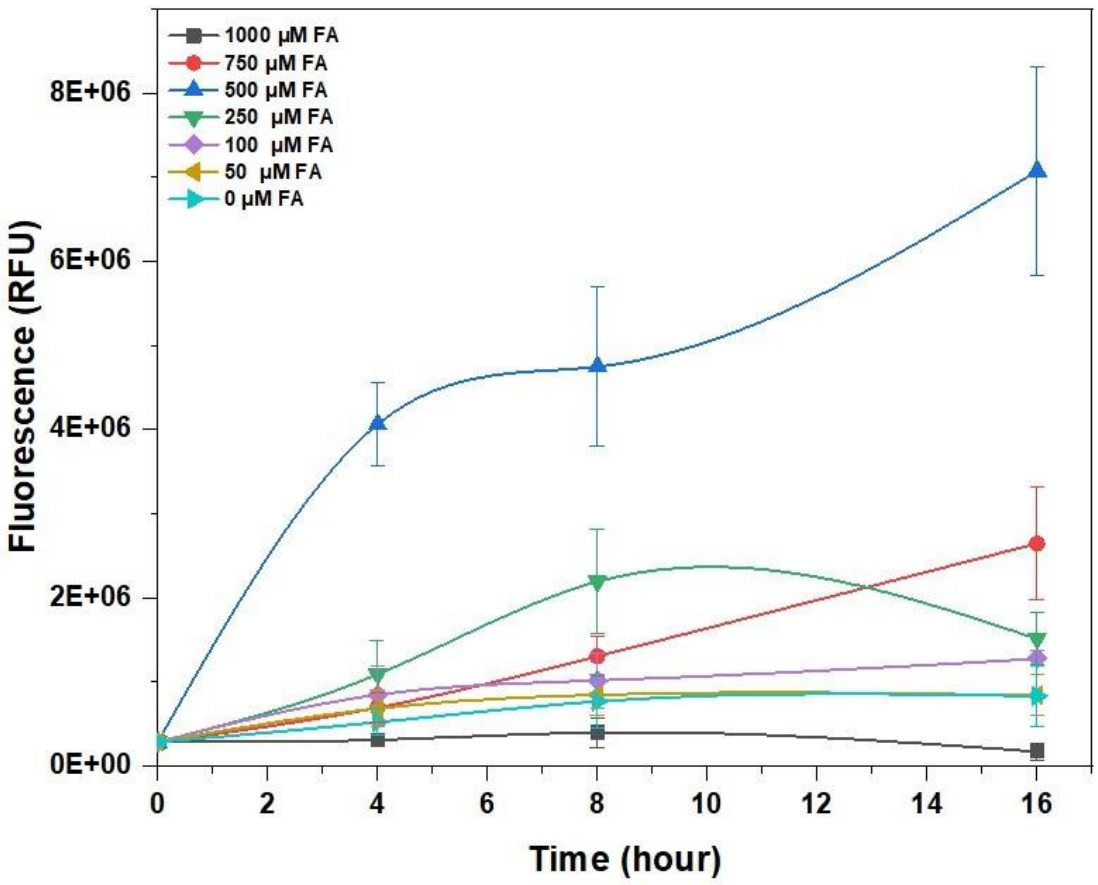

FIGURE 4a

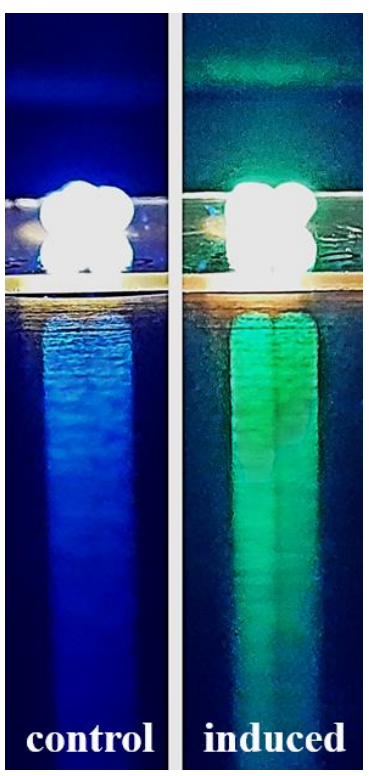

FIGURE 4b

Fig. 4. a) Fluorescence emission kinetics of alginate bead immobilized FA bioreporter in response to different liquid formaldehyde concentrations. The average value of a triplicate of each induction is presented with standard deviation. Error bars are shown only when they exceed the size of the symbols **b)** The photo of uninduced and induced (with liquid 500 µM FA) beads. Images were taken by smartphone camera while the beads were placed on EVOS Floid Imaging Station (Thermo Fisher Scientific, USA) using blue excitation (390 ± 40 nm) with emission detection at 446 ± 33 nm.

FIGURE 5

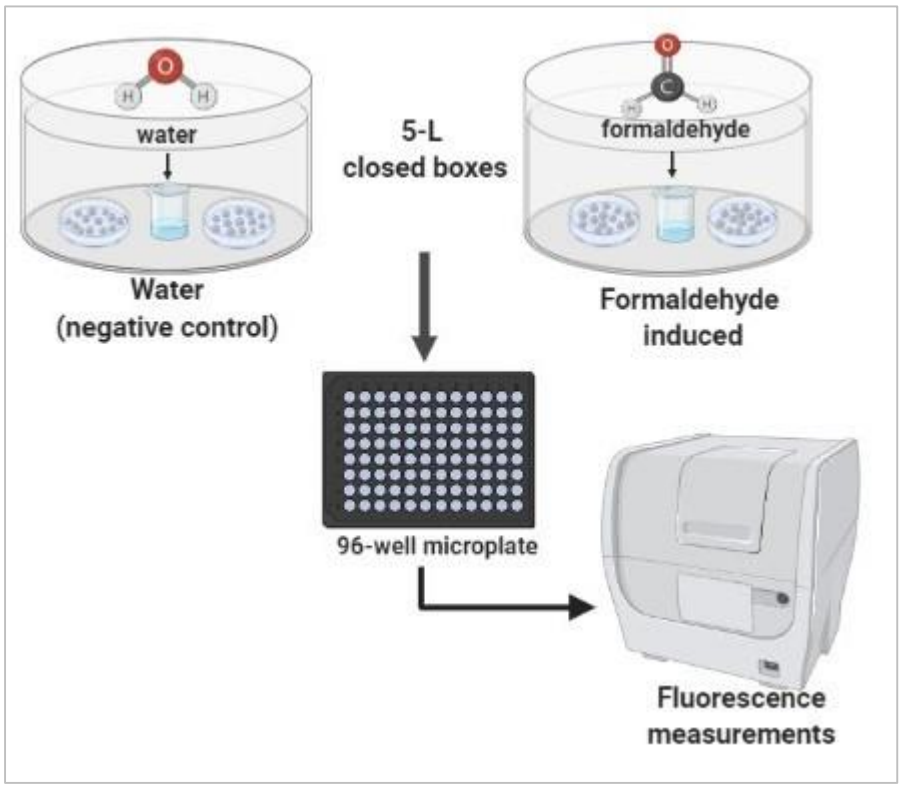

Fig. 5. Overview of the basic experimental procedure of gaseous formaldehyde detection. The figure was created with BioRender.com.

FIGURE 6

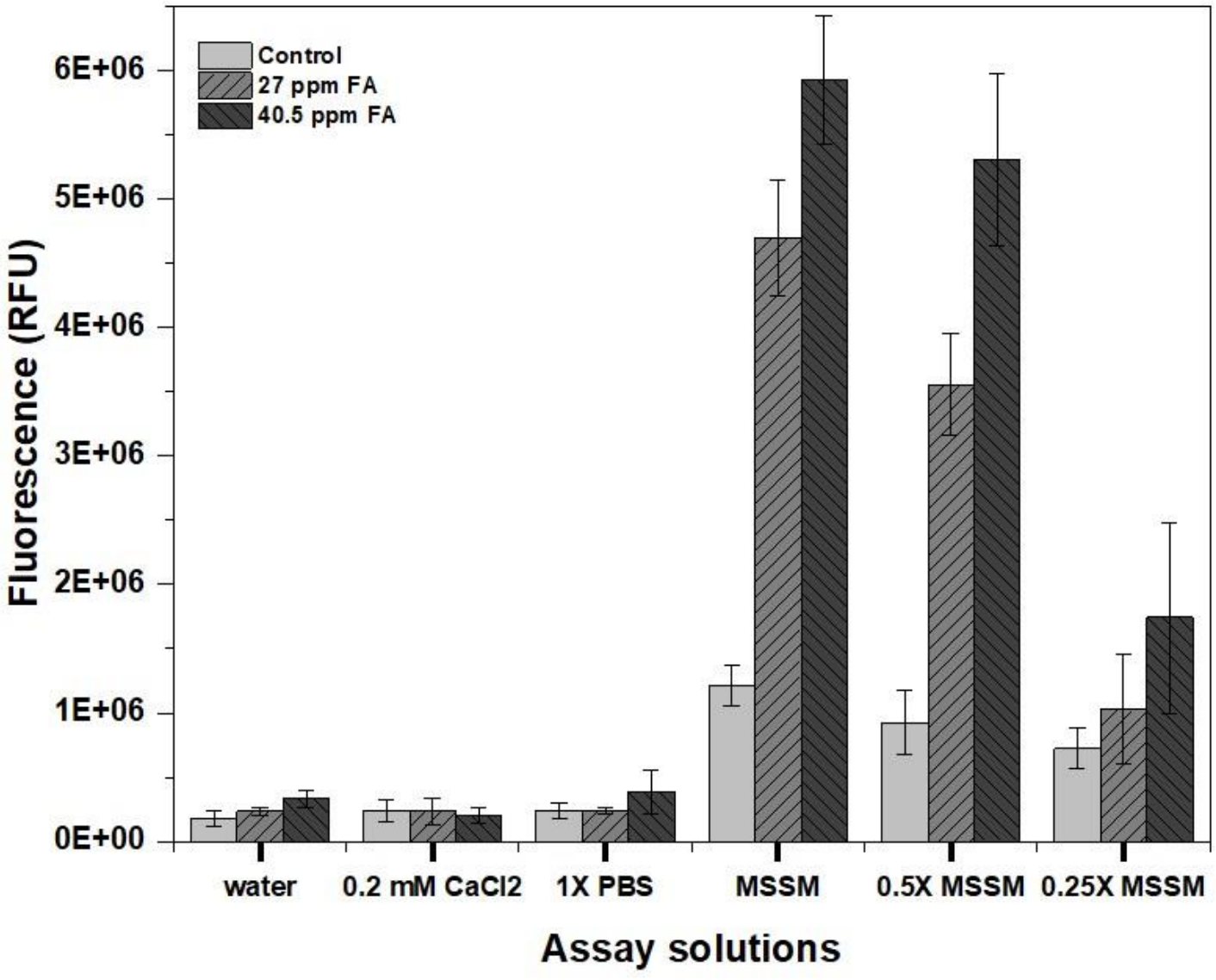

Fig. 6. Effect of bead assay solutions on the gaseous FA detection performances of alginate-immobilized FA bioreporter. The average value from triplicates of each induction is presented for 16-h assay time with standard deviation.

FIGURE 7a

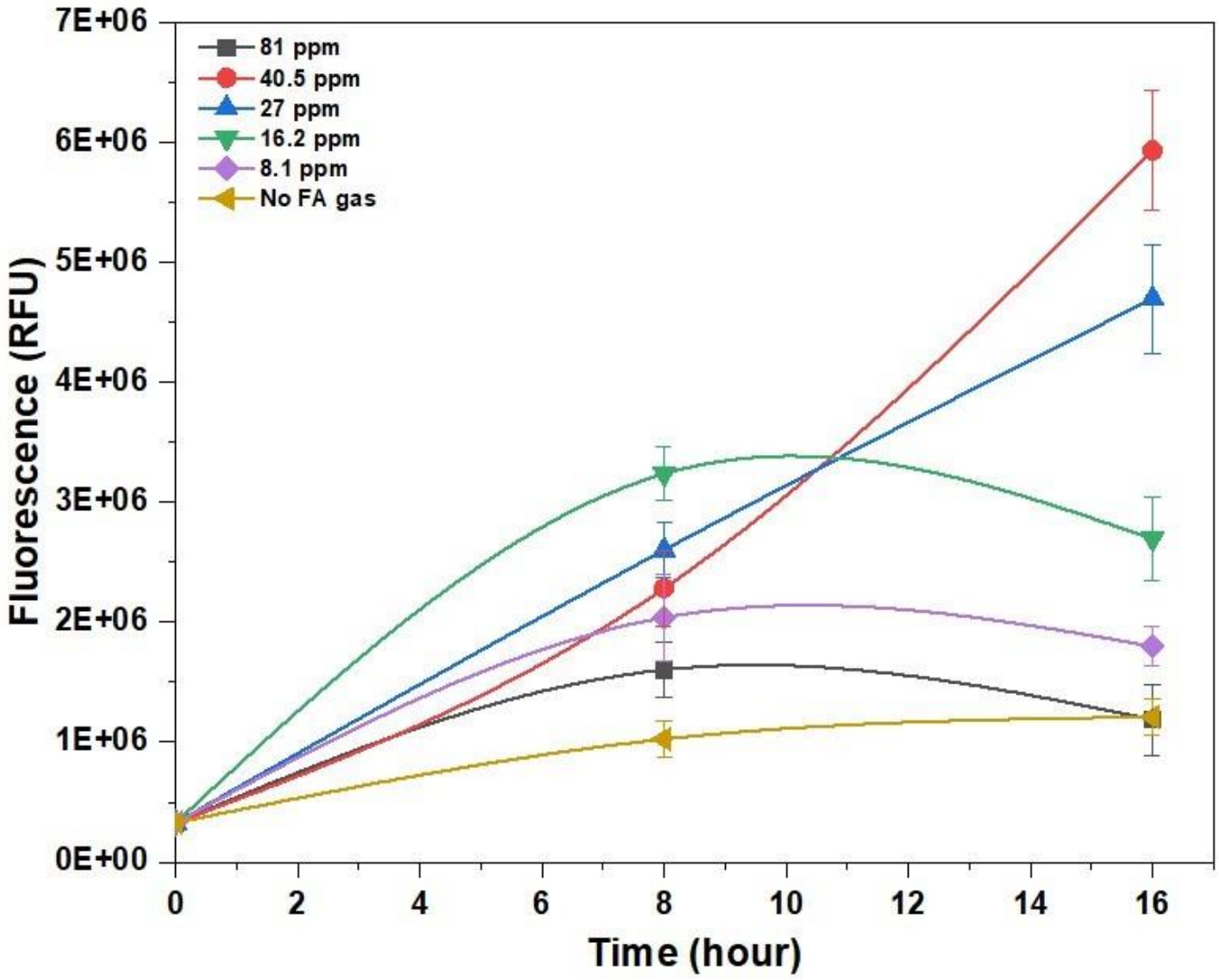

FIGURE 7b

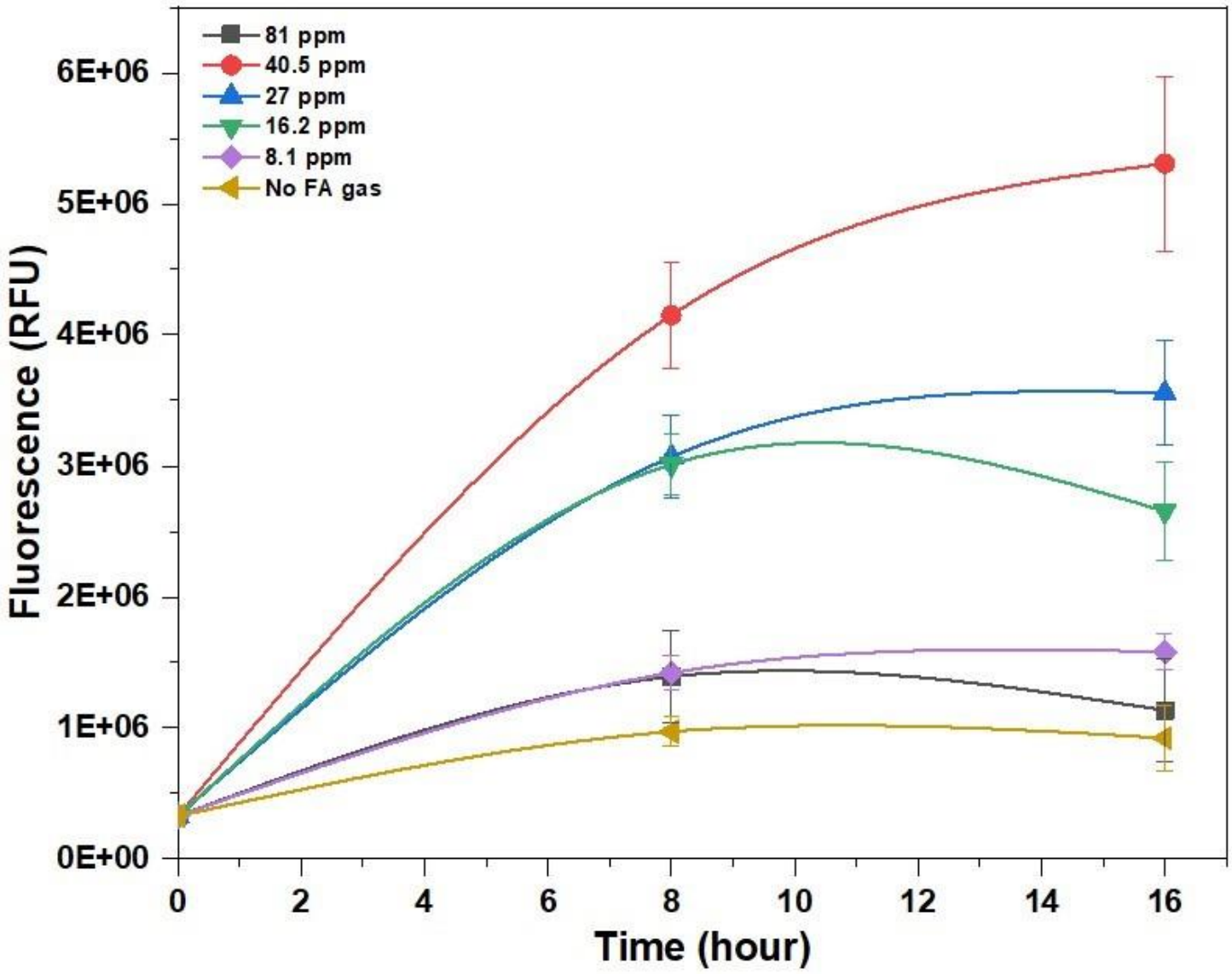

Fig. 7. Fluorescence emission kinetics of alginate bead immobilized FA bioreporter in response to different gaseous formaldehyde concentrations assayed in **a)** MSSM and **b)** 0.5X MSSM. The average value of a triplicate of each induction is presented with standard deviation. Error bars are shown only when they exceed the size of the symbols.

FIGURE 8

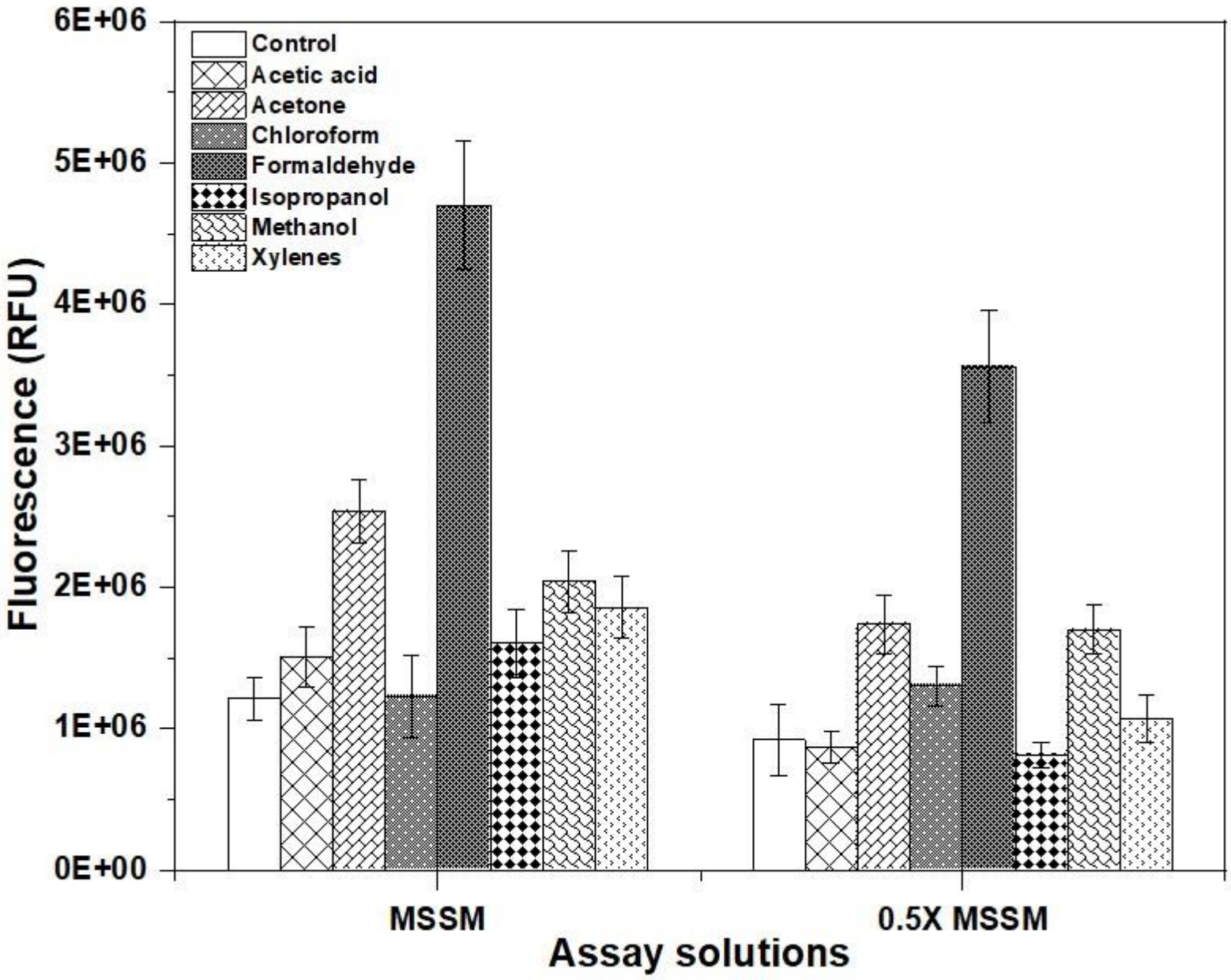

Fig. 8. Gaseous selectivity of the FA bacterial bioreporter assayed in the MSSM and 0.5X MSSM. The gas concentrations of FA and other VOCs were at 400 μM. Measurements were performed following 16-h treatment.