J Ind Microbiol Biotechnol (2012) 39:45–53
DOI 10.1007/s10295-011-0997-5

ORIGINAL PAPER

Detection of dichloromethane with a bioluminescent (*lux*) bacterial bioreporter

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Received: 4 March 2011 / Accepted: 6 June 2011 / Published online: 19 June 2011

Abstract The focus of this research effort was to develop an autonomous, inducible, *lux*-based bioluminescent bioreporter for the real-time detection of dichloromethane. Dichloromethane (DCM), also known as methylene chloride, is a volatile organic compound and one of the most commonly used halogenated solvents in the U.S., with applications ranging from grease and paint stripping to aerosol propellants and pharmaceutical tablet coatings. Predictably, it is released into the environment where it contaminates air and water resources. Due to its classification as a probable human carcinogen, hepatic toxin, and central nervous system effector, DCM must be carefully monitored and controlled. Methods for DCM detection usually rely on analytical techniques such as solid-phase microextraction (SPME) and capillary gas chromatography or photoacoustic environmental monitors, all of which require trained personnel and/or expensive equipment. To complement conventional monitoring practices, we have created a bioreporter for the self-directed detection of DCM by taking advantage of the evolutionary adaptation of bacteria to recognize and metabolize chemical agents. This bioreporter, *Methylobacterium extorquens* DCM_{lux}, was engineered to contain a bioluminescent *luxCDABE* gene cassette derived from *Photorhabdus luminescens* fused downstream to the *dcm* dehalogenase operon, which causes the organism to generate visible light when exposed to DCM. We have demonstrated detection limits down to 1.0 ppm under vapor phase exposures and 0.1 ppm under liquid phase exposures with response times of 2.3 and 1.3 h, respectively, and with specificity towards DCM under relevant industrial environmental monitoring conditions.

Keywords Bioluminescence · Bioreporter · Dichloromethane · Lux · Methylene chloride

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Introduction

Dichloromethane (DCM, or methylene chloride, CAS No. 75-09-2) is a colorless organic solvent widely used in paint removers and various chemical processing applications. Its industrial prevalence, low boiling point (volatility), and high water solubility make it a frequently encountered environmental contaminant. Worldwide production approaches approximately 520,000 metric tons. It is estimated that 86% of discharged DCM is released into the atmosphere where it has a half-life of 79–110 days, while the remaining 14% accumulates in water, soil, and groundwater where, it can remain for over a millennium [1, 30].

A variety of adverse health effects occur during DCM exposure. Fatal intoxication due to DCM inhalation as well as numerous cases of non-fatal poisonings by both inhalation and oral ingestion have occurred [4, 10, 15, 17, 27]. Lightheadedness, nausea, and fatigue have been reported in the workplace as acute side-effects of short-term

exposures. Extensive studies conducted in mouse, rat, and human models have provided sufficient evidence for the U.S. Environmental Protection Agency (EPA) to classify DCM as a "probable human carcinogen". More recent re-evaluations have reduced the estimated risks but the mechanisms of DCM toxicity continue to remain unclear [8, 9, 28]. Once absorbed through the lungs, skin, or gastrointestinal tract, DCM is metabolized by two separate pathways, both yielding noxious by-products including carbon monoxide, carbon dioxide, hydrogen chloride, and formaldehyde [3, 9]. Among the many DCM metabolites, carbon monoxide and formaldehyde are of particular note because acutely, carbon monoxide is a known central nervous system depressant and is toxic to both the liver and cardiovascular system. Chronically, formaldehyde is a potent carcinogen. While most long-term health concerns involve the genotoxic effect of DCM metabolites, immediate and lethal DCM poisoning has been confirmed in several individuals after high-level occupational exposures [10, 15, 18]. Occupational safety standards are generally designed to minimize personal exposure through adequate ventilation. However, situations where breathing air is recycled or unventilated present cause for further precaution. For example, DCM is considered a high-priority airborne contaminant by NASA due to its known accumulation in Shuttle and International Space Station atmospheres, where Spacecraft Maximum Allowable Concentrations (SMACs) range from 100 ppm for 1 h exposures to 1 ppm over 1,000 day exposures [20]. More relevant to us here on Earth, the Occupational Safety and Health Administration (OSHA) has set permissible air concentration limits at 25 ppm as an 8-h time-weighted average (TWA) and 125 ppm as a short-term exposure limit (STEL). The odor threshold of DCM is approximately 250 ppm. U.S. EPA safe drinking water standards are set at 5 ppm.

The detection and monitoring of workplace and environmental contaminants can be achieved via whole-cell bacterial bioreporters that sense and respond to targeted metabolites. Bioreporter bioassays for numerous contaminants have been demonstrated in water, sediment, soil, air, and wastewater environments using genetically modified bacteria typically incorporated with colorimetric, fluorescent, or bioluminescent signaling elements [24]. For the bioreporter described herein, bioluminescence derived from the bacterial *lux* series of genes (*luxCDABE*) was applied to provide real-time, reagentless signaling and assessment of target analyte bioavailability. As a first line of detection for environmental contaminants, bioluminescent bioreporters offer a rapid, inexpensive, simple, and sensitive method of monitoring, unlike traditional time-consuming analytical techniques that require specially trained personnel and high operational cost. We developed a *lux*-based bioluminescent bioreporter specific to DCM by employing the evolutionary adaptation of the methylotrophic bacterium *Methylobacterium extorquens* (formerly *M. dichloromethanicum*) DM4 to scavenge for and metabolize DCM as a sole carbon source. The genes for DCM metabolism by strain DM4 are located within the *dcm* operon, which includes the *dcmA* and *dcmR* genes (GenBank accession #M32346) [12]. The *dcmA* gene encodes for a DCM dehalogenase while the *dcmR* gene encodes for a repressor that negatively controls the *dcm* operon. In the presence of DCM at low ppb concentrations, the repressor is inactivated and transcription ensues from the *dcmA* promoter, generating a 50–80-fold increase in dichloromethane dehalogenase [13]. Genetically engineering *M. extorquens* DM4 with the inducible bioluminescent reporter plasmid pCM66–*dcmA/R–luxCDABE,* containing a transcriptional fusion between the *luxCDABE* gene cassette from *Photorhabdus luminescens* and the *dcmA/R* gene of the *dcm* degradation operon, permitted quantifiable determination of DCM concentrations based on bioluminescence response profiles after DCM exposure.

Materials and methods

Bioreporter construction

M. extorquens strain DM4 (DSM No. 6343), a pink pigmented facultative methylotrophic bacterium, was used as the host strain for development of the DM4$_{lux}$ bioreporter. Genetic construction involved fusion of the *dcmR/A* genetic region (GenBank accession #M32346) of strain DM4 to the *P. luminescens luxCDABE* gene cassette (GenBank accession #M90093) downstream to which was ligated the *rrnB* T_1T_2 transcriptional terminator derived from the cloning vector pKK223-3 (GenBank accession #M77749) (Fig. 1). The 1,450-bp *dcmR/A* region was PCR amplified from *M. extorquens* DM4 genomic DNA using the forward primer 5'-TCTAGACCTCCAAGGCTTGAAC-3' containing a unique *Xba*I site (underlined) and the reverse primer 5'-GAGCTCCACGTTATCCTCCCTT-3' containing a unique *Sac*I site (underlined) and placed within a pCR4-TOPO vector (Invitrogen, Carlsbad, CA, USA). The *luxCDABE* gene cassette was PCR amplified from *P. luminescens* genomic DNA using the forward primer 5'-ATT AAATGGATGGCAAATAT-3' and the reverse primer 5'-GTCGACAGGATATCAACTATCAAAC-3' containing a unique *Sal*I site (underlined) and placed within a pCR-XL-TOPO vector (Invitrogen). The *rrnB* transcriptional terminator was PCR amplified from pKK223-3 using the forward primer 5'-GTCGACAAGAGTTTGTAGAAAC GC-3' and the reverse primer 5'-GTCGACCTGTTT TGGCGGATG-3' each containing a *Sal*I site (underlined) and placed within a pCR4-TOPO vector. The *luxCDABE*

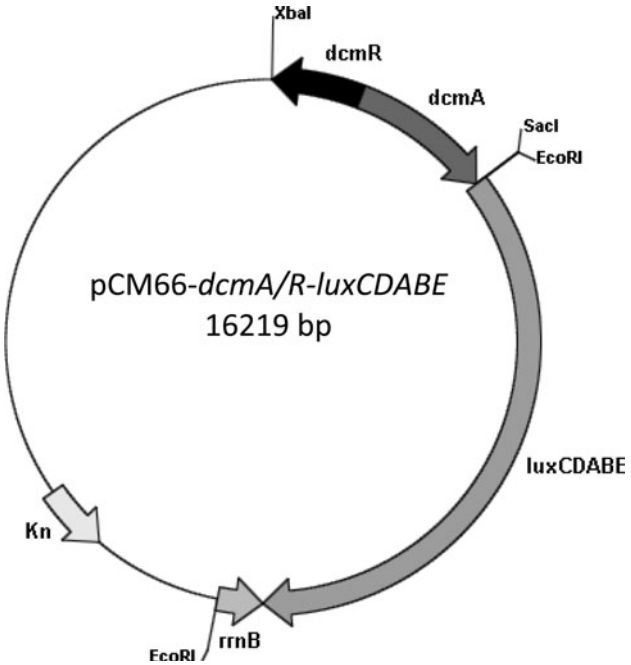

Fig. 1 The pCM66 vector [19] was used as the backbone for construction of the dichloromethane bioreporter DCM_{lux}. The *dcmR/dcmA* regulatory promoter region from *M. extorquens* DM4 was ligated upstream of the *P. luminescens luxCDABE* cassette (see text for specific details. *Kn* kanamycin resistance)

gene cassette was then ligated to the *rrnB* transcriptional terminator by cleaving the *rrnB* pCR4-TOPO vector with *Sal*I to remove the *rrnB* sequence and ligating it into the pCR-XL-TOPO-*luxCDABE* vector linearized via cleavage with *Sal*I with proper orientation confirmed by restriction mapping. This produced a pCR-XL-TOPO-*luxCDABE-rrnB* vector. DNA isolations were performed with Wizard Minipreps and Midipreps (Promega, Madison, WI, USA) and purified when necessary with the Geneclean Spin Kit (MP Biomedicals, Irvine, California, USA). PCR reactions were carried out in an MJ Research DNA Engine tetrad (Waltham, MA, USA) using Ready-To-Go PCR beads (Amersham Biosciences, Piscataway, NJ, USA). DNA was sequenced at all steps with the ABI Big Dye Terminator Cycle Sequencing reaction kit on an ABI 3100 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

To construct the bioreporter, each of the TOPO isolated gene sequences above were inserted into the multicloning site of the broad host range cloning vector pCM66 [19]. The *dcmR/A* genetic region was excised from its pCR4-TOPO vector with an *Xba*I/*Sac*I double digest and directionally inserted into pCM66 similarly cleaved with *Xba*I and *Sac*I. The *luxCDABE-rrnB* sequence was removed from its pCR-XL-TOPO vector via digestion with *Eco*RI and then ligated into the *dcmR/A*-pCM66 vector linearized with *Eco*RI. Proper orientation was confirmed by restriction digest mapping and sequencing. This finalized pCM66–*dcmA/R–luxCDABE* vector was electroporated into *M. extorquens* DM4 (2.5 kV, 400 Ω, 25 μF) with transformants plated on nutrient agar plates containing 20 μg kanamycin/ml. Exposing the plates to DCM vapor at approximately 50 ppm allowed bioluminescent colonies to be selected for using a Caliper Life Sciences IVIS imaging camera (Hopkinton, MA, USA). The brightest of these colonies, designated DCM_{lux}, was propagated for further analysis.

Growth conditions

The DCM_{lux} bioreporter, taken from −80°C frozen stock cultures, was maintained on nutrient agar plates containing kanamycin at 20 μg/ml to select for the *lux* reporter plasmid. The strain, being a slow grower, required incubation at 30°C for 4 days. Plates were refreshed from -80°C stock on a weekly basis. Liquid cultures were started from plates via inoculation into nutrient broth followed by incubation for up to 2 days at 30°C with shaking at 200 rpm to achieve an optical density at 600 nm (OD_{600}) of 0.15. Cultures were centrifuged at 10,000 rpm for 10 min and washed once in an equal volume of sterile phosphate buffered saline (PBS; in g/l, NaCl, 8; KCl, 0.2; Na_2HPO_4, 1.15; KH_2PO_4, 0.2). Cell pellets were then resuspended in 1/10th strength (0.1×) sterile minimal salts media (MSM; 1 × stock solution in g/l, KH_2PO_4, 0.68; K_2HPO_4, 1.73; $MgSO_4 \bullet 7H_2O$, 0.1; NH_4NO_3, 1.0; pH 7.0) to an OD_{600} of 0.25 for application in vapor and liquid-phase bioluminescent assays.

DCM vapor phase bioluminescent assays

For vapor phase testing, DCM_{lux} cultures were immobilized in 0.7% agarose in 0.1 × MSM. Approximate 10 ml cultures of strain DCM_{lux} were prepared in MSM at an OD_{600} of 0.25 as explained above. Liquefied 40°C agarose (2 ml) was added to 4 ml of this culture which was immediately and gently vortexed. From this mixture, 4 ml was pipetted into an 8-cm-long × 1.5 cm^2 20-ml total volume quartz flowcell. Once solidified (∼10 min), this formed an even, immobilized layer of DCM_{lux} culture with an upper surface area that could be exposed to DCM gas as it passed through the flowcell. DCM gas was purchased from Airgas Specialty Gases, Port Allen, LA, USA at 1,000 ppm. DCM concentrations were regulated using a mass flow controller/flow tubes (Aalborg, Orangeburg, NY, USA) and a diluting supply of Grade D breathing air (Airgas) to provide bioreporter exposures at 100, 50, 10, 1, and 0.1 ppm. The DCM/breathing air mixture was continuously metered through sterile, chemically inert 3.1-mm-diameter Masterflex PTFE tubing (Cole Parmer Instrument Co. Chicago, IL, USA) connected to a 900-cm^3 SPME glass sampling chamber,

then through the quartz flowcell positioned within the IVIS Lumina imaging chamber (Caliper Life Sciences), and finally to a waste outlet (Fig. 2). Bioluminescent imaging was monitored online in the IVIS Lumina with readings taken in photon counts per second (CPS) every 10 min over 4-h exposure durations using the integrated Living Image software package (Caliper Life Sciences) [5]. Control flowcells containing non-induced DCM_{lux} cultures exposed to breathing air without DCM additions were run simultaneously alongside test cultures to establish background bioluminescence. All DCM exposure experiments were performed in triplicate.

Paint stripper as a test medium

Bacterial cultures were grown and then immobilized in the quartz flowcell in the same manner as for the DCM vapor phase bioluminescent assays and exposed to dilute off-gas vapors from Crown brand Handi-Strip Semi-Paste Stripper (Packaging Service Company, Pearland, TX, USA). One milliliter of paint stripper was placed in an empty 3-l glass flask sealed with a Teflon-lined screw-cap lid. Metered breathing air was pumped continuously through an inlet located at the bottom of the flask to produce a DCM-contaminated air supply that was directed into the 900-cm^3 SPME sampling chamber, then to the flowcell in the IVIS imaging chamber, and finally, out to waste. Paint stripper exposures were performed over 4-h periods with IVIS images obtained every 10 min. Control flowcells containing non-induced DCM_{lux} cultures exposed to metered breathing air without paint stripper additions were run simultaneously alongside test cultures to establish background bioluminescence. All paint stripper exposure experiments were performed in triplicate.

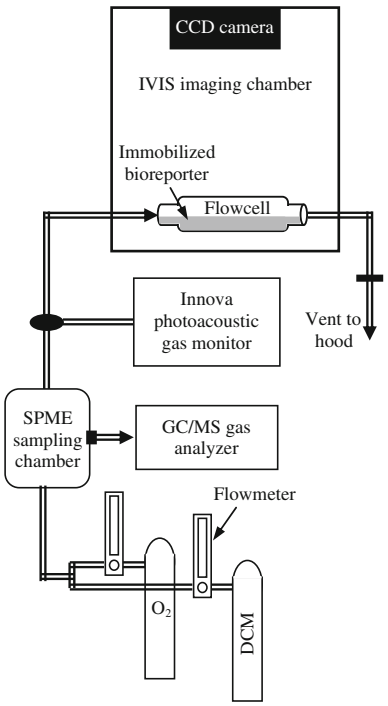

Fig. 2 Flow-through system for exposing the *M. extorquens* DCM_{lux} bioreporter to vapor phase DCM

DCM liquid-phase bioluminescent assays

A 13,000 ppm (0.15 M) stock solution of DCM was prepared by adding 490 µl of concentrated HPLC-grade DCM (Fisher Scientific, Fair Lawn, NJ, USA) to 49.5 ml sterile HPLC-grade H_2O. Cultures (100 ml) of strain DCM_{lux} were prepared in MSM at an OD_{600} of 0.25 and DCM from the stock solution was added to produce initial concentrations of 100, 10, 1, 0.1, and 0.01 ppm. Cultures were immediately placed on a magnetic stirrer and continuously mixed at room temperature ($\sim$20°C) while being circularly pumped at a flow rate of 340 ml/min through a sealed system consisting of the quartz flowcell positioned inside the IVIS Lumina imaging chamber. Experimental exposures were performed over 4 h with readings taken every 10 min. All associated tubing was sterile, chemically inert 3.1-mm diameter Masterflex PTFE. Control flowcells containing non-induced DCM_{lux} cultures (0.1 × MSM without DCM) were run simultaneously alongside test cultures to calibrate for background bioluminescence. All DCM exposure experiments were performed in triplicate.

Analytical measurements of DCM concentrations

All gas-phase DCM samples were analyzed using a Hewlett Packard gas chromatograph (Model 6890) equipped with a mass spectrometer detector (MSD, Model 5973N) with an inert source. A DB-5MS column with 10-m DuraGuard (30 m × 0.25 mm i.d., J&W Scientific, Folsom, CA, USA) was used for sample separation with helium gas as the carrier gas at a constant flow rate (1.0 ml/min) maintained by an electronic pressure control module. Manual injection was used for sample analysis. The oven temperature was held at 45°C for 10 min, and then increased to a final temperature of 300°C with a 10-min hold. The injection temperature was set at 280°C and the MS source temperature was 250°C. The MS was operated in the selected ion mode (SIM) and DCM (*m/z* 84) was monitored. Gas-phase DCM samples were collected with a SPME fiber assembly (75 µm carboxen/polydimethylsiloxane; Supelco, Bellafonte, PA, USA). Fibers were inserted for 15 min into the 900-cm^3 SPME gas-tight sampling chamber (Fig. 2) during the vapor phase bioluminescence assays ($n = 3$). For the liquid-phase bioluminescence assays, the SPME fibers were inserted for 15 min into the culture flask headspace ($n = 3$). DCM measurements were taken hourly with the SPME

fibers and immediately analyzed by GC/MS [23]. SPME fibers were cleaned daily by insertion in the 280°C GC injection port for 5 min.

Liquid calibration standards were prepared at six different initial DCM concentrations ranging from 0.0013 to 130 ppm in 4.0 ml glass vials containing 2 ml of solution and sealed with a Teflon-lined septum and screw-cap lid. These standards were stirred for 30 min prior to SPME fiber exposure to assure equilibrium distribution of the DCM between the gas and liquid phases within the vials [16]. The equilibrium headspace DCM concentrations were calculated using Henry's law constant for DCM (0.00196 atm-m^3/mol at 1 atm and 20°C) and plotted against the GC peak area measurements to establish a calibration curve [34]. This allowed direct measurement of the DCM exposure concentrations during the gas-phase bioluminescent assays. During the liquid-phase bioluminescence assays, the exposure concentrations were inferred using the equilibrium headspace DCM concentration and the Henry's law constant.

A factory-calibrated Innova Model 1412 photoacoustic gas monitor (LumaSense Technologies, Ballerup, Denmark) equipped with optical filter UA0980 was used to confirm the gas-phase DCM measurements made with SPME. Automatic sampling occurred every 2 min with a sample integration time of 20 s. This provided a manufacturer's specified lower detection limit of 0.05 ppm at 20°C and one atmosphere pressure. DCM concentrations measured with the Innova were maintained within 10% of the SPME vapor-phase measurements (data not shown).

Induction of *M. extorquens* DCM_{lux} by related aliphatic chlorinated industrial solvents

To characterize the specificity of the DCM_{lux} bioreporter, it was exposed to several related aliphatic chlorinated hydrocarbons commonly used as industrial solvents (trichloroethylene, tetrachloroethylene (otherwise known as perchloroethylene or PERC), 1,1,1-trichloroethane, and chloroform). Since testing each of these compounds at several different concentrations was too time-consuming using the flowcell system, they were instead tested for false-positive bioreporter induction in a higher throughput 96-well microtiter plate format in a Biotek Synergy2 microplate reader (Biotek, Winooski, VT, USA). The DCM_{lux} bioreporter was grown and resuspended in MSM to an OD_{600} of 0.25 as for the liquid-phase experiments above. The first column of wells in a black 96-well microtiter plate (Dynex Technologies, Chantilly, VA, USA) contained 200 µl of bioreporter culture at a final chemical exposure concentration of 8,500 ppm. Dilutions of 1:10 were then performed throughout successive wells in 200-µl volumes to achieve a lower concentration limit of approximately 0.85 ppm. Plates were prepared on three different days using three separately grown cultures ($n = 3$ among experiments) with each plate containing each compound in triplicate wells ($n = 3$ within experiments). Each plate contained triplicate wells of strain DCM_{lux} exposed to DCM at 10 ppm (positive control) and triplicate wells of strain DCM_{lux} not exposed to a chemical to establish background bioluminescence (negative control). Plates were sealed with Breath-Easy membranes (Diversified Biotech, Boston, MA, USA) and monitored for bioluminescence in the Biotek Synergy2 at room temperature every 10 min for 24 h.

Results

Bioluminescent response kinetics of the DCM_{lux} bioreporter to vapor-phase DCM and paint-stripper volatiles

The vapor-phase bioluminescent response profile of the DCM_{lux} bioreporter was established in a flowcell format at DCM concentrations of 100, 50, 10, 1, and 0.1 ppm (actual DCM concentrations as measured by SPME ($n = 3$) were 101.0 ± 0.03, 50.8 ± 0.02, 10.5 ± 0.05, 0.97 ± 0.03, and 0.14 ± 0.02 ppm). Significant bioluminescent responses (three standard deviations above control) were observed at all concentrations except 0.1 ppm and ranged from 1 h at 100 ppm to 2.3 h at 1.0 ppm (Fig. 3). Using the upper response time of 2.3 h, a linear bioluminescent response curve to increasing concentrations of DCM was generated ($R^2 = 0.99$) (Fig. 3, inset). The paint-stripper vapor-phase DCM concentration was estimated to be 34.5 ppm using this bioluminescent response curve. SPME measurement of paint stripper volatiles at the 12.3-h time point was 39 ± 3.55 ppm. The earliest significant response during the paint stripper exposure occurred at 1.2 h.

Bioluminescent response kinetics of the DCM_{lux} bioreporter to liquid-phase DCM

A recirculating pump-driven flow-through system was used to establish the response profile for *M. extorquens* DCM_{lux} upon exposure to liquid-phase DCM at concentrations of 100, 10, 1, 0.1, and 0.01 ppm (DCM concentrations inferred using SPME ($n = 3$) were 100 ± 5.71, 50 ± 2.45, 10 ± 0.70, 1 ± 0.001, 0.1 ± 0.30, and 0.01 ± 0.07 ppm). Significant bioluminescent responses (three standard deviations above control) were observed at all concentrations except 0.01 ppm (Fig. 4). Response times ranged from 0.5 h at 100 ppm to 1.3 h at 0.1 ppm. The bioluminescent response was linear ($R^2 = 0.99$) at the upper response time of 1.3 h (Fig. 4, inset).

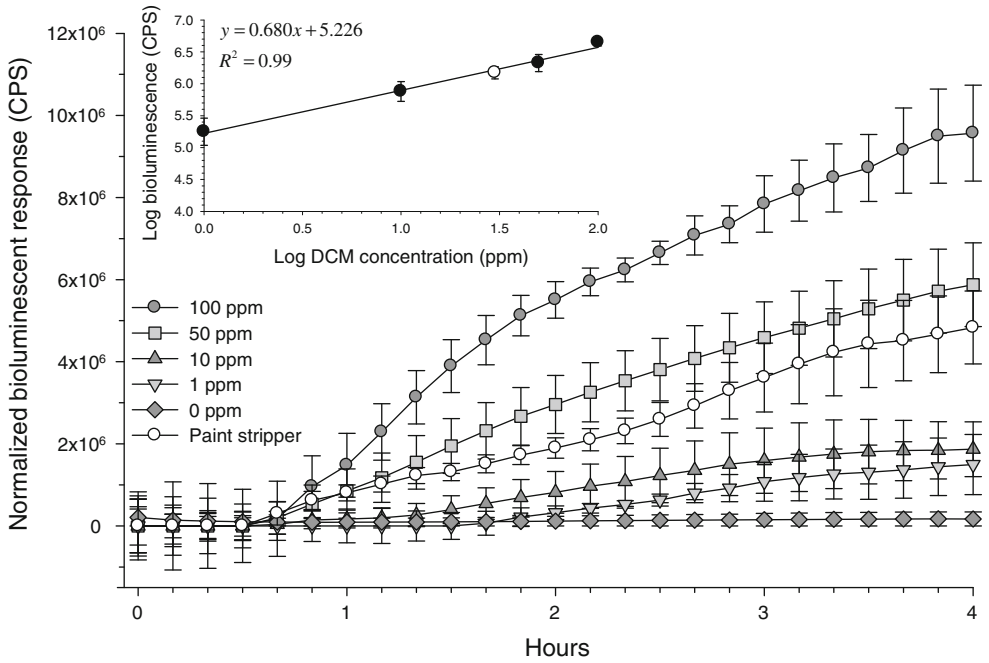

Fig. 3 Bioluminescent response profile in counts per second (CPS) of the *M. extorquens* DCM_{lux} bioreporter exposed to vapor-phase DCM at concentrations ranging from 1 to 100 ppm and to paint stripper. The normalized bioluminescent response was calculated by subtracting induced culture bioluminescence from background (un-exposed) culture bioluminescence for all concentrations. *Inset* Linearity of the bioluminescent response to vapor-phase DCM. Points were taken from the bioluminescent profile at the upper response time of 2.3 h and plotted over concentrations ranging from 1 to 100 ppm. The *white circle* represents the 2.3-h exposure response of the DCM_{lux} bioreporter to paint stripper volatiles

Induction of the DCM_{lux} bioreporter by related chlorinated solvents

M. extorquens DCM_{lux} was exposed in a 96-well microtiter plate to trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, and chloroform to assess the specificity of the bioreporter bioluminescent response to DCM ($n = 3$ within and between experiments). Exposure concentrations ranged from 8,500 to 0.85 ppm. No significant bioluminescent response (three standard deviations above control) was detected in any of the samples at any of the concentrations tested over the 24-h exposure period (Fig. 5).

Discussion

The bacterial bioluminescent bioreporter *M. extorquens* DCM_{lux} was constructed by fusing the 1,450-bp *dcmR/A* regulatory region from *M. extorquens* DM4 upstream of a promoterless *P. luminescens luxCDABE* gene cassette. The bioreporter proved capable of autonomously generating bioluminescence in response to vapor and liquid-phase DCM at lower tested detection limits of 1.0 and 0.1 ppm, respectively. These detection limits are well below the threshold levels for potential health risks established by OSHA for air contaminants (TWA of 25 ppm and STEL of 125 ppm) and EPA for safe drinking water (5 ppm). Response times at these lower detection limits ranged from 2.3 h under vapor-phase exposures to 1.3 h under liquid-phase exposures. Elevated DCM exposures (100 ppm) initiated bioluminescence within 1 h or less under both conditions. The parental *M. extorquens* DM4 strain can grow at an upper DCM concentration of approximately 850 ppm [14]. Thus, the bioreporter has sufficient robustness to tolerate much higher concentrations of DCM before succumbing to a toxic, disabling response. Testing at such high concentrations was not performed, however, since the purpose of the bioreporter was to signal low concentration, early warning contaminant exposures expected in workplace environments.

Volatiles emanating from enclosed commercially available paint stripper could be detected within 2.3 h at a concentration of approximately 35 ppm. This is well below the predicted DCM concentrations for consumer paint stripper applications that range from 170 to 453 ppm in residential, low-ventilation rooms [31]. Occupational exposures, for example, during aircraft paint stripping operations, produce DCM at concentrations estimated to range from 20 to 525 ppm [29, 33]. Thus, the DCM_{lux} bioreporter could therefore effectively pre-alert consumer and industrial workers to harmful DCM exposures.

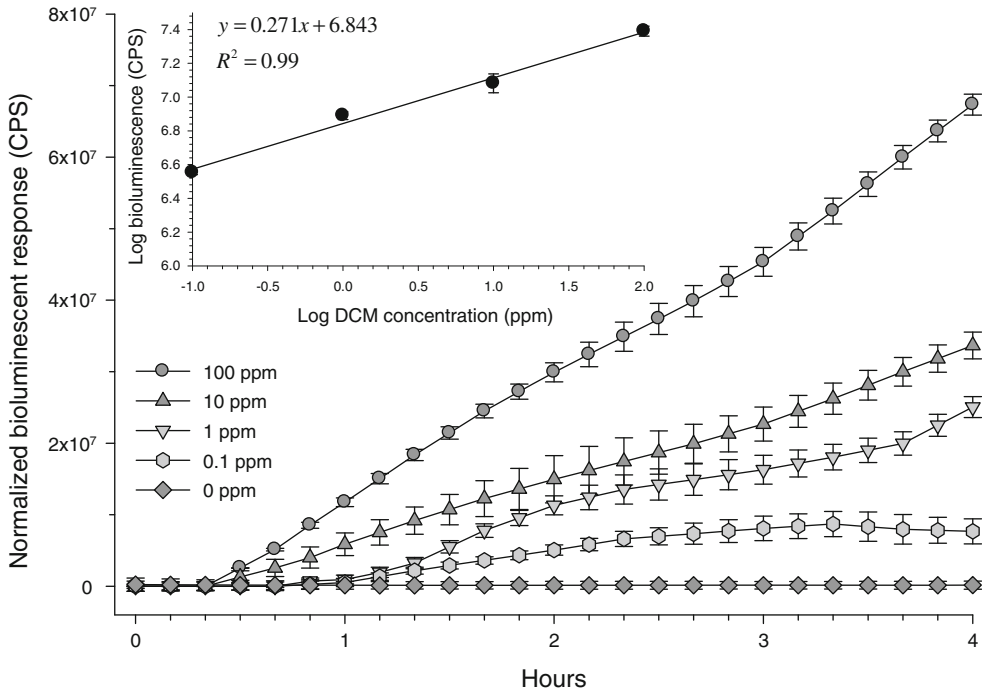

Fig. 4 Bioluminescent response profile in counts per second (CPS) of the *M. extorquens* DCM_{lux} bioreporter exposed to liquid-phase DCM at concentrations ranging from 0.1 to 100 ppm. The normalized bioluminescent response was calculated by subtracting induced culture bioluminescence from background (un-exposed) culture bioluminescence for all concentrations. *Inset* Linearity of the bioluminescent response to liquid-phase DCM. Points were taken from the bioluminescent profile at the upper response time of 1.3 h and plotted over concentrations ranging from 0.1 to 100 ppm

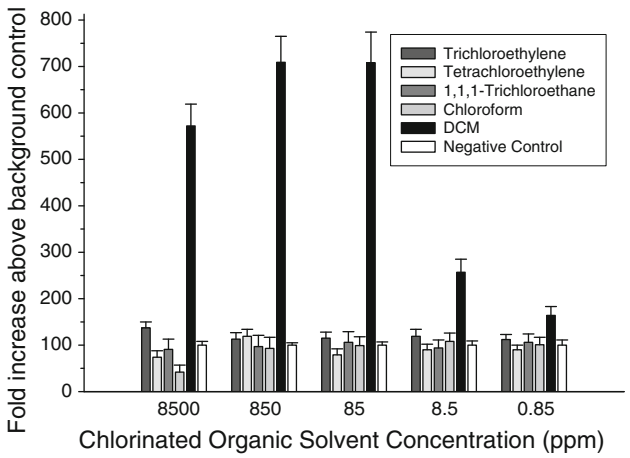

Fig. 5 Specificity of the peak bioluminescent response as fold increase above background control bioluminescence for the *M. extorquens* DCM_{lux} bioreporter exposed to potentially interfering liquid phase chlorinated organic solvents (trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, and chloroform) in a 96-well microtiter plate at final concentrations ranging from 8,500 to 0.85 ppm

No significant bioluminescence induction was observed from the DCM_{lux} bioreporter upon exposure to other common industrial solvents (trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, and chloroform), thus confirming its specificity for DCM and validating minimal false-positive signaling under the tested environmental conditions. *Hyphomicrobium* sp. DM2 (ATCC #43129), which also harbors a *dcmA* DCM dehalogenase gene [21], has been applied as a reporter in its native state in a multi-transducer flow-calorimeter/chloride-sensitive electrode biosensor format, with a detection limit of 10 ppb in water [13]. It demonstrated similar specificity but was predictively cross-sensitive to dihalomethanes besides DCM, such as bromochloromethane and dibromomethane. However, the minimal to nonexistent industrial and commercial application of these other dichloromethanes makes the DCM_{lux} bioreporter DCM specific within its intended monitoring environments.

In its current state, the DCM_{lux} bioreporter could be applied as a sensor within bioreactor treatment schemes designed for biological removal of DCM from air- and liquid-flow streams. Biotrickling filters, bioscrubbers, and similar bioreactor fabrications containing biodegradative microorganisms such as *M. extorquens* DM4 and *Hyphomicrobium* sp. DM2 have been shown to effectively reduce DCM concentrations in polluted air streams and industrial and municipal wastewater effluents [2, 11, 22]. The integration of DCM_{lux} bioreporters into bioreactors would serve not only to degrade DCM but to additionally report on its bioavailability, as has been previously demonstrated with, for example, the bioreporter *Pseudomonas putida* TVA8 and its targeted degradation and sensing of toluene

in a packed bed reactor [26]. The measurement of resulting bioluminescent signals within the bioreactor is then typically achieved via the connection of fiber optic cables that terminate to a photomultiplier tube (PMT) or direct connection of the PMT or other light gathering device to the bioreactor itself [7]. Smaller-scale chip-based biosensors can also be functionally applied for target chemical sensing using bioreporter interfaced microelectronic circuitry. Integrated circuit microluminometers and avalanche photodiodes have both been mated with living bioluminescent bioreporters to produce self-contained biosensors on a platform of only a few square millimeters [6, 25, 32]. The potential exists to similarly assimilate DCM_{lux} bioreporters into analogous on-chip detection schemes. The ability to effectively encapsulate these bioreporters into agar-based matrices and exploiting their slow growth rate to maximize longer-term viability attests to such potential suitability.

Acknowledgments Research support was provided by the NASA Advanced Environmental Monitoring and Control Program under cooperative agreement number NNJ04HF02A and the National Science Foundation Division of Biological Infrastructure (DBI) under award number DBI-0963854.

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