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A method for detecting perfluorooctanoic acid and perfluorooctane sulfonate in water samples using genetically engineered bacterial biosensor

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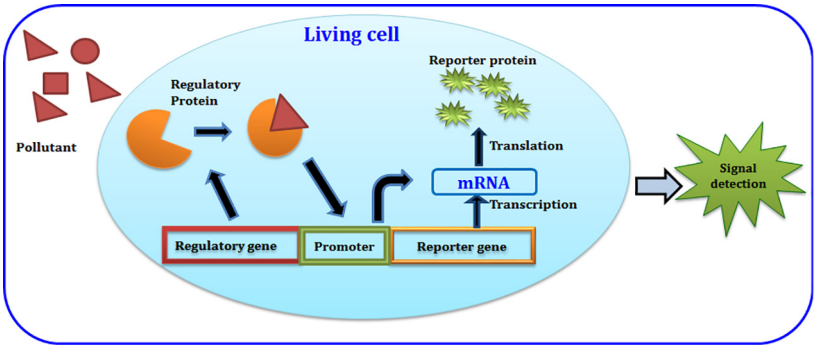
HIGHLIGHTS

- Bacterial biosensor was developed for detection of PFOA and PFOS.
- Defluorinase and *gfp* gene were used as regularity and reporter gene.
- The biosensor response was achieved from 10 ng/L to 1000 ng/L within 24 h.
- The developed biosensor was successfully applied in field water samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple, reagent and pre-treatment (i.e. dilution, sample purification and pH adjustment) free approach based genetically engineered bacterial biosensor is developed and demonstrated for the detection of perfluorinated compounds in water samples. The bacterial biosensor was developed by integrating two genes called regulatory (defluorinase gene) and reporter gene (green fluorescence gene) through genetic engineering techniques. The as-developed bacterial biosensor was employed to detect perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in water samples upon induction of regulatory gene and expression of green fluorescence protein. The induced fluorescence emission by the biosensor was visualized using fluorescence microscopic images. The specificity of biosensor was evaluated with different types of organic pollutants such as chlorinated compounds, polyaromatic hydrocarbons and pesticides etc., in both presence and absence of PFOA and PFOS. The biosensor was employed to detect the perfluorinated compounds at nano gram level in both standard solutions and natural water samples like river water, wastewater and drinking water with an analysis time of 24 h. The detection of PFOA and PFOS by the developed-bacterial sensor is validated by liquid chromatography coupled with mass spectrometer. The developed biosensor has demonstrated a rapid and sensitive detection of PFOA and PFOS in various water samples.

1. Introduction

PFCs (Perfluorinated compounds) are ubiquitous environmental pollutants which are mainly from the anthropogenic sources. Among PFCs, PFOA and PFOS are of considerable interest due to their extensive

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applications in the industrial and commercial products (Zhang et al., 2014). Table 1 shows the physiochemical properties of PFOA and PFOS compounds. The major sources of PFOA and PFOS in the environment have been identified as residues of unreacted PFOA and PFOS compounds in commercial products and untreated wastewater from the industries. The occurrence of PFOA and PFOS in the environment is influenced by industrial and human activities which are shown by positive correlation of PFOA and PFOS in the environment. Both group of compounds were found in water (Qi et al., 2016; Sunantha and Vasudevan, 2016; Yamazakia et al., 2016; Zhu et al., 2017), soil (Kim and Kannan, 2007), air (Stock et al., 2004), food products (Haug et al., 2010) and human blood (Inoue et al., 2004).

Our previous studies reported the occurrence of PFOA and PFOS compounds in surface water (Noyyal River and some lakes in Chennai) at Tamil Nadu, India (Sunantha and Vasudevan, 2016). Fig. S1 represents the concentration of PFOA and PFOS compounds in different water samples. Fujii et al. (2007) identified that the PFOA and PFOS concentration in tap water in China are found to be in the range from 1.1–109 ng/L and 1.5–13 ng/L, respectively. The authors were also listed the number of countries where the PFOS and PFOA compounds found to be prevalent in their tap water. The countries which are polluted with these aforementioned pollutants are, Thailand (0.1–1.9 ng/L of PFOS and 0.2–4.6 ng/L of PFOA), Sweden (1.3 ng/L of PFOA), Canada (0.2 ng/L of PFOA) and Malaysia (0.1 ng/L of PFOS). Wan et al. (2017) measured 12 perfluoroalkyl acids in water samples from coastal region of Shandong peninsula in China. The concentration in water ranged from 23.69 to 148.48 ng/L for PFOA and from 10.49–61.64 ng/L for PFOS. Choi et al. (2017) reported that the presence of PFOA and PFOS in agricultural sites at the concentration level ranged from 0.001–0.007 μg/L and 0.001–0.22 μg/L, respectively.

As an environmental contaminant, PFOA and PFOS have following features such as extreme resistant to degradation, enhanced bioaccumulation in food chains, and longer half–life. The half-life of PFOA and PFOS in human being is estimated as 3.8 and 5.4 years, respectively. The exposure to PFOA and PFOS could cause adverse effects on living forms. The PFOA and PFOS are known as endocrine disruptors, which have effects on developmental, reproductive, neurological and immune system (Koskela et al., 2017; Staples et al., 1984). The United States Environmental Protection Agency (US EPA) review suggested that these compounds show the effects on human health such as low birth weight and thyroid problems (Li et al., 2017; US EPA, 2012). According to US EPA, the maximum permissible amount of PFOA and PFOS in drinking water is 70 ng/L (US EPA, 2016). However, when the concentration exceeds the permissible limit it causes adverse effects to human and animal through bioaccumulation. In such situation, monitoring of PFOA and PFOS is primarily important and hence the rapid sensing and detection device for PFOA and PFOS is the need of the hour.

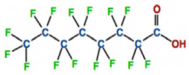
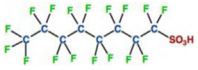
Many well-known methodologies for PFOA and PFOS determination are available that mainly requires either expensive and complicated instruments like liquid and gas chromatography or the use of relatively large amounts of organic solvents such as hexane and methanol for the extraction of PFOA and PFOS compounds. Various research articles have highlighted the importance of sample preparation and detection limit of pollutant (Bellan et al., 2011; Demeestere et al., 2007; Martin et al., 2004; Petrovica et al., 2010). Therefore, there is a critical need to develop new and improved methods for perfluorinated compounds detection. To detect the these compound, several approaches have been attempted by researchers to develop a solvent-free methods for the past few years (Karimian et al., 2018; Ranaweera et al., 2019; Shoemaker et al., 2019). One of the best approaches is mainly based on the use of genetically engineered bacteria, which produces a measurable signal when the bacterium comes in contact with biocomponents (Rodriguez-Mozaz et al., 2005, 2007). A whole-cell electrochemical biosensor was generated for detection of organochlorine pesticide by monitoring the change in the conductivity using pulsed amperometry with a detection limit of 2 ppt (Prathap et al., 2012). Several biosensors were developed for the detection of polychlorinated biphenyl (Liu et al., 2010), polyaromatic hydrocarbons (Werlen et al., 2004), phthalates (Gu et al., 2002), heavy metal (Liu et al., 2019) and pesticides (Zhao et al., 2018), but none of the constructs sense the PFOA and PFOS using bacteria. The present work is mainly focuses on developing a bacterial biosensor for identification of PFOA and POFS in real field water samples. In the present study a new plasmid has been constructed, and is used as a sensing material for PFOA and PFOS. The specificity and sensitivity of the developed biosensor was also evaluated. The system was tested in real water samples and validation of the sensor response was obtained using chromatographic techniques. Fig. S2 represents the schematic diagram of genetically modified bacterial biosensor.

2. Materials and methods

2.1. Microbial culture

P. aeruginosa (PAO1) (MTCC No: 3541) and *P. aeruginosa* (1688) (MTCC No: 1688) were obtained from the Microbial Type Culture Collection, Chandigarh, India. *Burkholderia sp.* FA1 was generously provided by Dr. Tatsuo Kurihara (Institute for Chemical Research, Kyoto University, Japan). The standards of PFOA (CAS: 335-67-1, purity: 96%) and PFOS (CAS: 1763-23-1, purity: 98%) and other chemicals were obtained from Sigma-Aldrich, Bangalore, India. The bacterial cultures were prepared in MSM which was slightly modified from previously described medium (Kurihara et al., 2003) and contained 0.717 g of sodium dihydrogen phosphate (CAS: 7558-80-7, purity: 98%), 0.272 g of

Table 1
Physiochemical properties of PFOA and PFOS.

Property	PFOA	PFOS
Molecular formula	$C_8HF_{15}O_2$	$C_8HF_{17}SO_3^-$
Molecular weight (g/moL)	414.1	500
Melting point (°C)	45 to 54	No date
Boiling point (°C)	189 to192	258–260
Vapor pressure (mm Hg at 25 °C)	0.525	0.002
Organic carbon partition coefficient (Koc)	2.06	2.57
Solubility in pure water (g/L at 25 °C)	3.4	0.57
Pka	2.5	<1.0
Critical micelle concentration(mmol/L)	8.5–10	2.0
Henry's law constant (atm-m^3/mol)	–	3.05 × 10–9
Air water partition coefficient	Not available	$<2 \times 10^{-6}$
Structure		

potassium dihydrogen phosphate (CAS: 7778-77-0, purity: 99%), 1 g of potassium nitrate (CAS: 7757-79-1, purity: 99%), 0.2 g of magnesium sulphate (CAS: 7487-88-9, purity 99%), 0.05 g of calcium chloride (CAS: 10043-52-4, purity: 99%) and made upto 1000 mL distilled water. The pH was adjusted to 7.4 ± 0.2 and the medium was sterilized by autoclaving (121 °C for 15 min). Batch culture of *P. aeruginosa* (PAO1), *P. aeruginosa* (1688) and *Burkholderia* FA1 were grown in 250 mL conical flasks each with 50 mL of MSM containing 5×10^5 cfu/mL (1 milli liter) cells, and 10 mg/L of PFOA and PFOS was added as carbon source and kept for shaking at 150 rpm. The cultures were prepared in duplicates and the bacterial growth was monitored by viable plate count method by taking the samples at an interval of 24 h for 5 days. Stock solutions of PFOA and PFOS were prepared and stored at 4 °C.

2.2. Fluoride analysis

Fluoride (F^-) ion is the major break down product during the degradation of PFOA and PFOS. This F^- ion was assessed using Ion chromatography (Metrohm Basic IC plus 883) with conductivity detector. The samples were filtered through membrane filter (0.22 μm) to remove bacterial cells before being analyzed. The known concentration of sodium fluoride was used as a reference solution. The 3.2 mM sodium carbonate (CAS: 497-19-8, purity: 98%), 1 mM sodium bicarbonate (CAS: 144-55-8, purity: 98%) and 10% acetonitrile (CAS: 75-05-8, HPLC grade, 99%) were used as the mobile phase at a flow rate of 0.60 mL/min. The retention time of F^- ion was recorded at 5.6 min.

2.3. Preparation of cell free extract and enzyme assay

The release of F^- ions during the degradation of PFOA and PFOS is mainly because to the defluorinase enzymatic activity of the bacterial cells. So the defluorinase enzyme activity was analyzed as per the procedure referred from Kurihara et al. (2003). Harvested bacterial cells were suspended in 50 mM potassium phosphate (pH 7.0) buffer containing 2-mercaptoethanol (CAS: 60-24-2, purity: ≥99.9) and the suspension was sonicated until clear solution is obtained. The samples were centrifuged at 12000 rpm for 10 min to remove the cell debris. The reaction mixture of 100 μL containing 2 μmol substrate, 10 μmol of tris sulphate (CAS; 6992-38-7, purity: 99%, pH -9.5) and crude enzyme. The reaction mixture was incubated at 30 °C for 15 min and subsequently the reaction was terminated by the adding 1 M of sulfuric acid. The absorbance value of the reaction mixture was found out at the wavelength of 570 nm using UV–Vis spectrophotometer.

2.4. Analysis of PFOA and PFOS biodegradation

In order to select effective bacterial strain for identifying PFOA and PFOS resistant gene, the study on metabolites data and the quantitative proof validating the biodegradation of PFOA and PFOS was conducted. Biodegradation of PFOA and PFOS by *P. aeruginosa* (PAO1) were checked in MSM with PFOA and PFOS as a carbon source. PFOA and PFOS from MSM broth were extracted by liquid–liquid extraction method as per the procedure given in Arulazhagan and Vasudevan (2009). The extracts were condensed to 1 mL using a rotary evaporator (Buchi, Germany). The degradation rate of PFOA and PFOS were determined through High Performance Liquid Chromatography (K501, Knauer, Germany) equipped with a C18 column and a UV–VIS detector connected to WINCHROME software. The column oven was set at 40 °C. The methanol (CAS: 67-56-1, purity, ≥99.9)/20 mM sodium dihydrogen phosphate were used as the mobile phase at a flow rate of 1.0 mL/min. Standard solutions of PFOA and PFOS were used as reference standard. The degradation rate of PFOA and PFOS were identified based on the peak area. The metabolites formed during the biodegradation of PFOA and PFOS were analyzing the supernatant solution by ESI-MS. Individual compounds were determined by matching the molecular weight of the metabolites by comparing with NIST (National Institute of standards and technology, Mass spectral library).

2.5. Construction of genetically modified biosensor

Genomic DNA was isolated from *P. aeruginosa* (PAO1) strain using Qiagen DNA kit (Qiagen Hilden, Germany) according to their manufacturer instruction. The isolated DNA was quantified and amplified with gene specific primers given in Table 2. *EcoRI* and *KpnI* restriction sites were incorporated at both ends of the sequence. Primers were designed based on the available sequence of defluorinase gene. The polymerase enzymes for cloning were *Pfu* DNA polymerase (Fermentas, USA); Deoxynucleoside triphosphates (dNTPs) and restriction endonucleases were from New England Biolabs (NEB, UK). Oligonucleotide primers were obtained from Sigma-Aldrich, Banglore (India). Polymerase chain reaction (PCR) was carried in thermal cycler (Applied biosystem, Germany) and PCR reaction was performed in a total volume of 25 μl containing 30 ng of DNA, 2.5 μl of 10× PCR buffer containing 15 mM $MgCl_2$, 0.5 μl of 10 mM dNTPs mix, 20 p mole of each primer, 1.25 units of *pfu* DNA polymerase and sterile nuclease free water to a final volume. The reaction condition was performed with initial denaturation at 94 °C for 5 min, 38 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min and final extension 72 °C for 7 min in a thermal cycler. Amplified PCR products were analyzed in 1.2% agarose gel electrophoresis and the product were purified by Qiagen Gel extraction kit (Qiagen, Hilden, Germany). The purified product was sequenced in Eurofins, Banglore (India). The resulting fragments were cloned into pRSET vector (Invitrogen, USA) based on the standard molecular methods (Sambrook and Russell, 2001). The PCR product was sequenced and the sequences were compared with Basic Local Alignment Sequence Tool (BLAST, National Center for Biotechnology Information). The *gfp* gene (700 bp) was cloned into positive clone containing *pfc*-DEF gene using *PstI* and *XhoI* restriction enzymes. The final 4.4 kb construct was transformed into BL21 expression host. The transformed cells were selected on Luria Bertani (LB) agar plates containing ampicillin. Single colonies from the plates were inoculated in LB broth and incubated at 37 °C for overnight from which plasmid DNA was isolated and confirmed the presence of desired gene. The crude protein samples with marker were loaded in 12.5% in SDS-PAGE and analyzed by the method given by Laemmli (1970).

2.6. Fluorescence analysis

The *gfp* expression was analyzed using inverted fluorescence microscopy (Nikon Eclipse TE300). The fluorescence emission was observed under a blue filter in a range of 420–495 nm and UV filter in a range of 340–380 nm in 40× magnifications. The fluorescence intensity was measured through spectrofluorometer (Shimadzu, Japan). The excitation and the emission wavelength were set as 490/10 nm and 510/10 nm.

2.7. Response time and detection limit of the biosensor

The biosensor was exposed to PFOA and PFOS compounds and incubated upto 96 h. The response time were measured from 0 h, 4 h, 6 h, 8 h, 24 h, 48 h, 72 h and 96 h. Detection limit of the biosensor was examined by measuring fluorescence intensity with various concentrations of PFOA and PFOS (10 ng/L, 100 ng/L, 200 ng/L, 300 ng/L, 400 ng/L, 500 ng/L and 1000 ng/L).

2.8. Selectivity study

The selectivity study of the developed bacterial-biosensor was evaluated using different types of persistent organic pollutants (POPs) such as PFOA, PFOS, sodium fluoroacetate, Tri-Chlorophenol, Penta-

Table 2
Primer sequences of *Pfc*-DEF and *gfp* gene.

Primer	Primer Sequences	Length (base)	Annealing temperature
Pfc-DEX (F)	CCGGGGTACCAGATGTTTGAAGGATTCGAGCGACG	35	58 °C
Pfc-DEX (R)	AAGGGAATTCCATCATGATTCTCTTCGCTCGGTCTG	36	58 °C
gfp - (F)	ATTCTCGAGCAATGACCGCACTAACAGAAGGAGCT	36	57 °C
gfp - (R)	CGACCTGCAGTTGGTAAGTATCCAAATCCACGGCC	35	57 °C

Chlorophenol, Fluorene, Naphthalene, Phenanthrene, Anthracene, α-endosulfan, β- endosulfan, Chlorpyrifos and Cypermethrin.

2.9. Cross reactivity study

Cross – reactivity was tested with different concentrations and combination of POPs such as chlorinated, PAH, pesticides PFOA and PFOS. The combination of POPs with PFOA and PFOS are shown in Table 3.

2.10. Application of developed biosensor in environmental water samples

The as-developed bacterial biosensor was evaluated for the detection ability of PFOA and PFOS in environmental water samples (sampling sites are shown in Figs. S3, S4 and S5). Noyyal River is a stream with presumable contamination of halogenated organic compounds (Ramaswamy et al., 2011; Shanmugam et al., 2014), upstream of Cauvery river in Erode District, the Cooum River in Chennai containing highly polluted sewage and industrial wastewater was selected for the study. Modified bacteria were inoculated into 100 mL environmental samples and kept in shaker for 24 h. Subsequently, the samples were analyzed for fluorescence intensity using spectrofluorometer.

3. Results and discussion

3.1. Identification of defluorinase gene

The bacterial cultures were grown in MSM supplemented with PFOA and PFOS as a carbon source. Among these strains, *P. aeruginosa* (PAO1) showed better growth than *P. aeruginosa* (1688) and *Burkholderia* FA1 as shown in Fig. 1. Similarly, the Basal mineral salts medium supplemented with PFOA or PFOS also showed an increased growth and its associated release of F^- ions in the strain *P. aeruginosa* (PAO1) when compared to *P. aeruginosa* (1688) and *Burkholderia* FA1. The *P. aeruginosa* (PAO1) strain shows effective degradation of 10 mg/L of PFOA and PFOS and released 2.1 and 1.6 mg/L of F^- ions after 48 h, respectively. F^- ion peaks were not observed in control samples (Fig. S6). Therefore, *P. aeruginosa* (PAO1) strain has been selected for further investigation. The defluorinase enzyme activity which is specific to fluorinated compounds was checked with PFOA and PFOS substrate. The defluorinase enzyme showed the highest activity towards PFOA than the PFOS.

The PFOA and PFOS were degraded in the presence of *P. aeruginosa* (PAO1) without adding any additional carbon source. Particularly, 58% of PFOA and 52% of PFOS were degraded after 96 h (Fig. S7). The metabolites formed during the biodegradation of PFOA and PFOS were analyzed from the supernatant solution by ESI-MS. Individual compounds were determined by matching the molecular weight of the metabolites with the comparison of NIST. The mass spectra were revealed that the PFOA and PFOS were degraded and its associated intermediates are given in Fig. S8. Four peaks corresponding to the metabolite compounds of PFOA and PFOS namely perfluoroheptanoic acid (PFHpA, $C_7HF_{13}O_2$, *m/z*-360), perfluorovaleric Acid (PFPeA, C_4F_9COOH, *m/z*-266), pentafluoropropionic acid (PFPA, C_3F_7COOH, *m/z*-165) from PFOA and perfluorohexanoic acid (PFHxA, $C_5F_{11}COOH$, *m/z*-316) from PFOS were detected.

3.2. Construction of genetically modified bacterial biosensor

The presence of defluorinase enzyme in *P. aeruginosa* (PAO1) was confirmed through biodegradation study. Based on these positive results, the primer was designed from the available sequence. A *pfc*-DEF gene was amplified by using gene specific oligonucleotides primer. The purified *pfc*-DEF gene was sequenced and submitted to NCBI with following accession number: MF459008. The desired gene (800 bp) and *gfp* fragment was cloned into pRSET vector (Fig. S9). The positive clones were selected for further analysis through restriction digestion to confirm the presence of desired insert. The final positive clone was

Table 3
Cross reactivity of biosensor with other organic pollutants.

Study	Control	Concentration (ng/L)		
PFOA + PFOS + Tri Chlorophenol	Only tri chlorophenol	100	500	1000
PFOA + PFOS + Penta Chlorophenol	Only penta chlorophenol	100	500	1000
PFOA + PFOS + Tri + Penta Chlorophenol	Only tri and penta chlorophenol	100	500	1000
PFOA + PFOS + Chlorpyrifos	Only chlorpyrifos	100	500	1000
PFOA + PFOS + α- Endosulfan	Only α- endosulfan	100	500	1000
PFOA + PFOS + β- Endosulfan	Only β- endosulfan	100	500	1000
PFOA + PFOS + Cypermethrin	Only Cypermethrin	100	500	1000
PFOA + PFOS + Chlorpyrifos + α- Endosulfan + β- Endosulfan + Cypermethrin	Only chlorpyrifos+α- endosulfan + β- endosulfan+ cypermethrin	100	500	1000
PFOA + PFOS + Fluorene	Only fluorene	100	500	1000
PFOA + PFOS + Naphthalene	Only naphthalene	100	500	1000
PFOA + PFOS + Phenanthrene	Only phenanthrene	100	500	1000
PFOA + PFOS + Anthracene	Only anthracene	100	500	1000

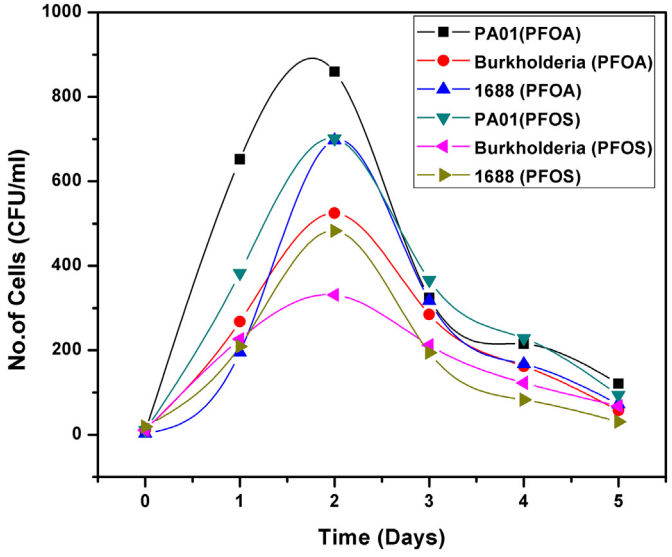


Fig. 1. Growth of *P. aeruginosa* (PAO1), *P. aeruginosa* (1688) and *Burkholderia* FA1 on PFOA and PFOS.

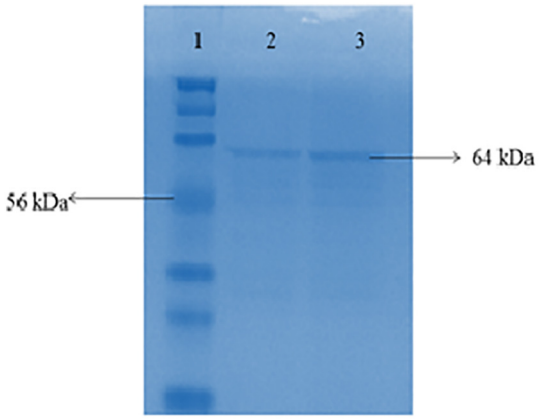


Fig. 2. Expression of IPTG induced recombinant protein in 12.5 % SDS PAGE (lane: Protein marker, lane 2 & 3: induced samples).

selected for expression study. The positive clone was induced overnight with 1.5 mM IPTG and the sample was run in 12.5% SDS-PAGE and results indicated expression of *pfc*-DEF/*gfp* recombinant protein (Fig. 2).

3.3. Evaluation of bacterial biosensor in lab scale

The biosensor was exposed to PFOA and PFOS compounds and the *gfp* expression was analyzed by fluorescence microscope (Fig. 3). No fluorescence emission was detected in the wild *E. coli* BL21 (DE3) strain and pRSET vector and MSM with PFOA and PFOS substrate. The time dependent induction of the biosensor was determined by incubating the genetically modified strain in MSM with PFOA or/and PFOS substrates. On evaluating the fluorescence emission up to 96 h, there is a steady increase in fluorescence emission is observed in 8 h and there is a significant increase in fluorescence emission is observed in 24 h. This enhanced fluorescence emission is attained during the stationary phase of the bacterial cells (Fig. S10). The fluorescence intensity which is measured at different time intervals is shown in Fig. S11. The present study confirms that 24 h incubation time is required to achieve maximum fluorescence intensity for the detection of PFOA and PFOS. This could be due to a mechanism of bacterial biosensor system against the rigidity of PFOA and PFOS compounds. A minimum exposure time required for transcription of fused protein for producing fluorescence signal. The bacterial cells showed the higher fluorescence intensity in PFOA when compared to PFOS compound. The biosensor response was achieved from 10 to 1000 ng/L of PFOA and PFOS with 24 h incubation time. The minimum detection limit of the biosensor achieved 10 ng/L. The fluorescence response was comparatively less till 300 ng/L and the response is increased in 400, 500 and 1000 ng/L of PFOA and PFOS compounds. Specificity is an important factor to evaluate the performance of bacterial sensor and we have analyzed the specificity by using different POPs. The modified biosensor responded to PCB (tri and penta chlorinated), but there were a less viable cells and showed no response to α, β- endosulfan, chlorpyrifos, cypermethrin, fluorene, anthracene, benzo[*a*]pyrene and phenanthrene. Tri and penta chlorinated compounds were found to interfere with the monitoring of PFOA and PFOS compounds when found together (number of viable cells and fluorescence intensity was very less when compared). The defluorinase enzyme was specific to halogenated compounds and fluoroacetate substrate was the best substrate for defluorinase enzyme. Many researchers have proposed that defluorinase enzyme is specific to fluorinated compounds than the other halogenated compounds (chloroacetate, bromoacetate and idoacetate) (Jitsumori et al., 2009; Kurihara et al., 2003; Liu et al., 1998). The selectivity results demonstrate that the biosensor cells show a good fluorescence upon exposure to PFOA or/and PFOS compounds. Based on these results, we tested the cross reactivity of the biosensor with different of organic pollutants and results are shown in Fig. 4. PCB (without PFOA and PFOS) showed low fluorescence response, whereas PAH and pesticides did not interfere with the sensor response even at 100 ng/L concentration.

3.4. Application of developed biosensor in environmental water samples

After the preliminary investigations at the lab-scale, the biosensor was further evaluated for its detection ability in field water samples. The water samples collected from the locations mentioned in the Section 2.10 were initially screened for the water quality characteristics and the results are presented in Table 4. Water from the Noyyal River (Kasipalayam) and Korattur Lake exhibited alkaline condition and

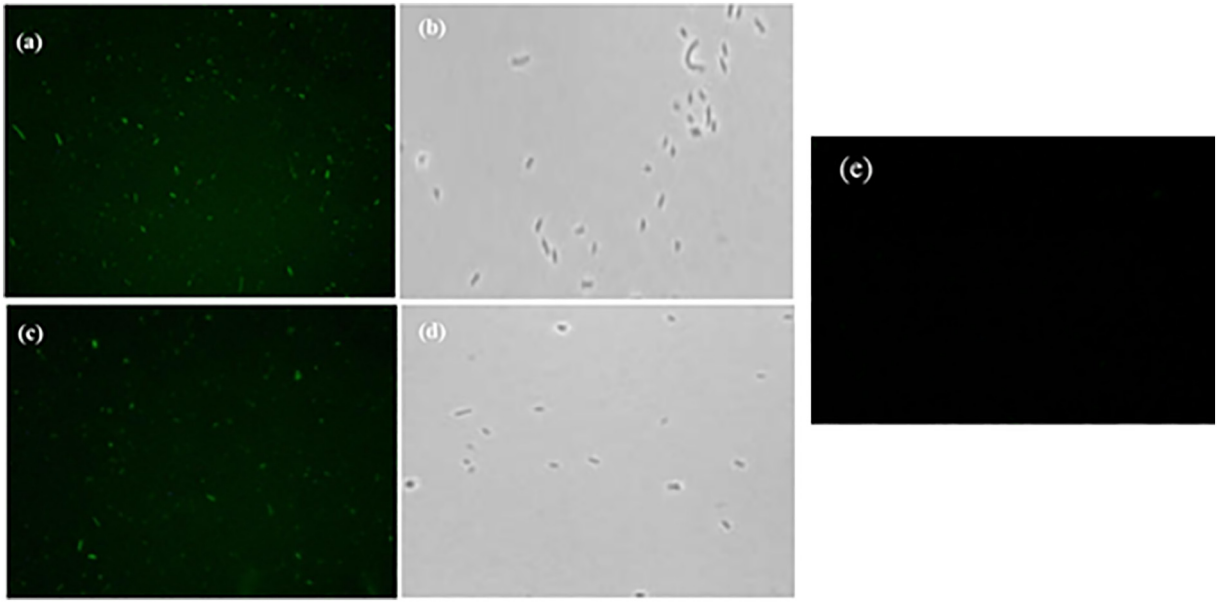
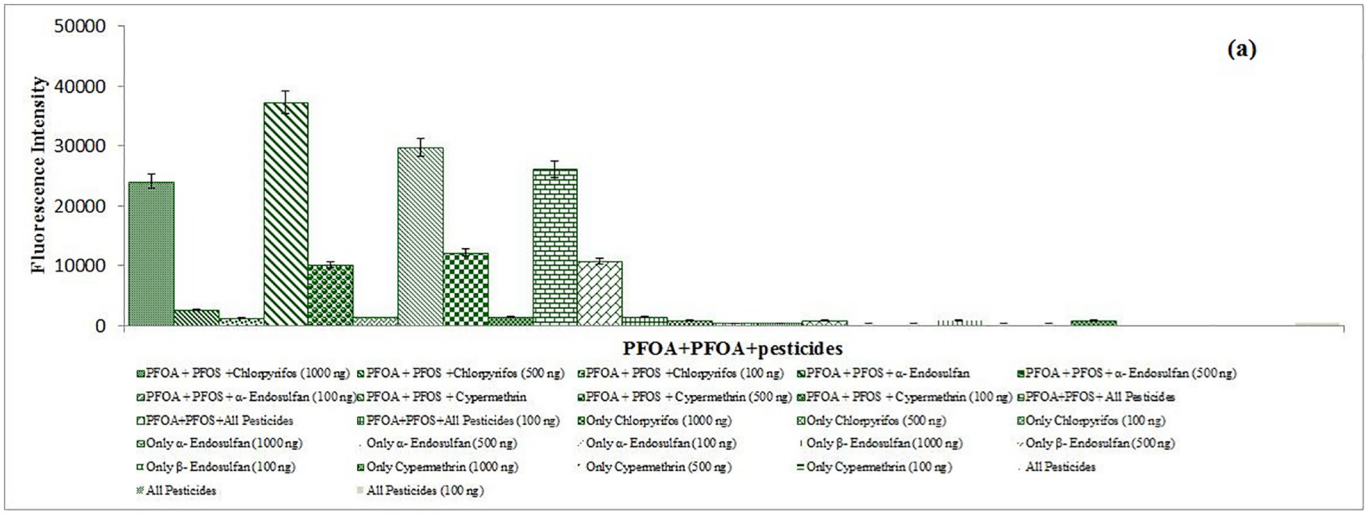
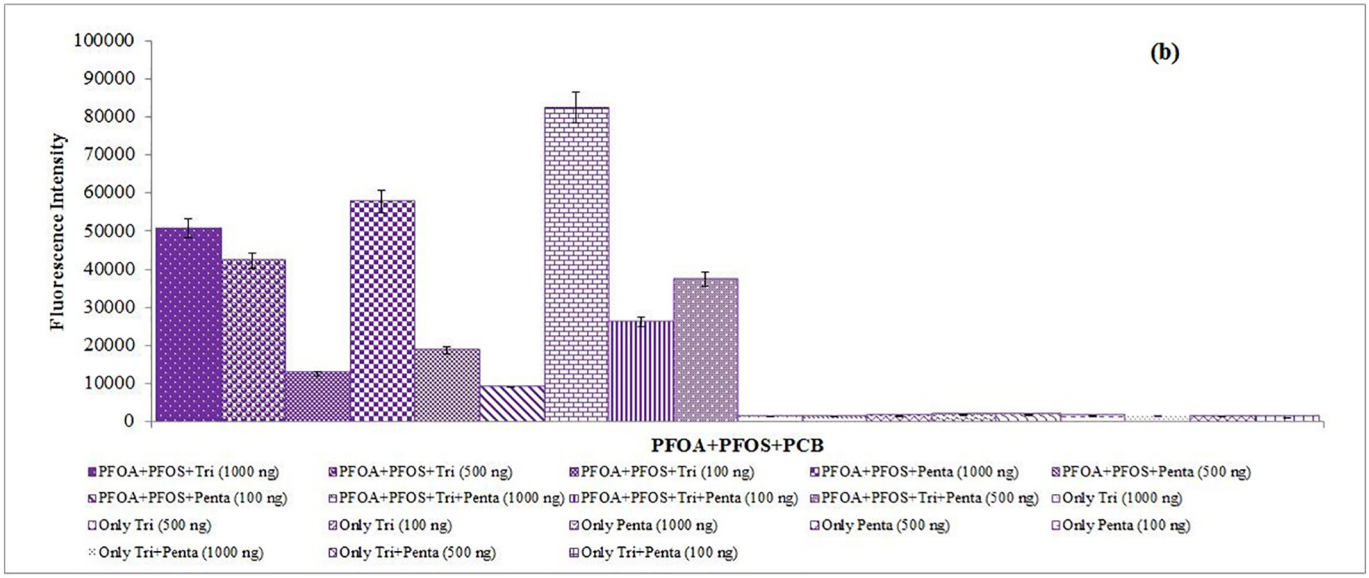


Fig. 3. Color imaging of cells harboring pRSET exposed PFOA (a & b) and PFOS (c & d) inducing expression of *gfp* (Left- fluorescence microscope, Right – visible microscope). Control sample under fluorescence microscope without PFOA and PFOS in MSM (e), as imaged by an inverted fluorescence microscope (Nikon Eclipse TE300).





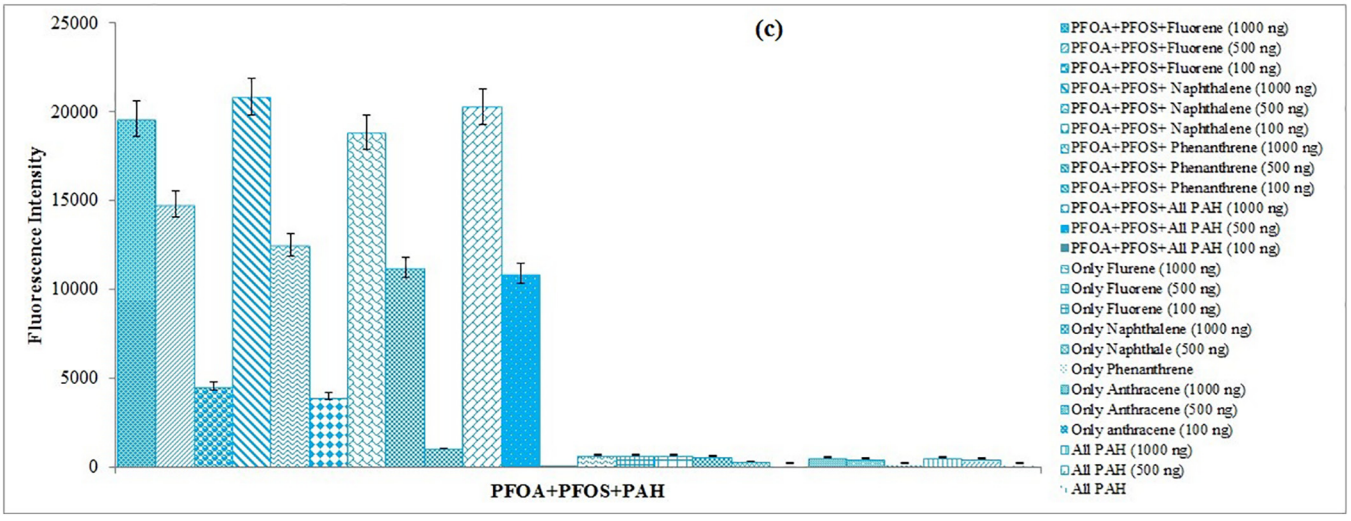


Fig. 4. The cross reactivity was determined by measuring the different the concentration of fluorinated compounds and combination of pesticides (a), PCBs (b), PAH (c) (with PFOA/PFOS and without FOA/PFOS) after 24 h induction in a cell harboring pRSET strain. The fluorescence intensity measured with spectrofluorometer is defined as the culture fluorescence divided by cell density at 490/510 nm.

Table 4
Characteristics and fluorescence intensity of water samples from the three different districts of Tamil Nadu.

Sampling sites	Response	Fluorescence intensity	Characteristics of surface water					
			pH	EC (mS/cm)	TDS (mg/L)	Salinity (ppt)	BOD (mg/L)	COD (mg/L)
Noyyal River (SIDCO industrial site)	×	54	7.8	5.01	3300	2800	23.64	120
Noyyal River (Ponnapuram)	≈	4027	7.9	5.69	3800	3100	26.56	112
Noyyal River (Kasipalayam)	√	3892	8.2	4.94	3300	2700	37.68	66
Noyyal River (Anaipalayam)	√	3021	7.6	5.48	3750	3100	14.79	96
Noyyal River (Old bus stand)	≈	2310	7.0	7.41	4900	4100	93.09	86
Noyyal River (Valam Road)	×	433	7.8	4.97	3300	2800	98.13	78
Cauvery River (Pallipalayam)	×	289	7.0	553	416	BDL	11.82	34
Cauvery River (Kumarapalayam)	×	1680	7.2	498	383	BDL	8.8	44
Bhavani River (Bhavani)	×	544	7.4	476	354	BDL	2.94	44
Cooum River (Nepier bridge)	×	459	7.4	26.80	17,956	15,500	15.00	39
Cooum River (Medical college)	√	4765	7.6	3.42	22,925	1800	73.80	340
Cooum River (Koyambedu)	×	2487	7.1	2.75	1843	1500	59.10	75

Sampling sites	Response	Fluorescence intensity	Characteristics of surface water					
			pH	EC	TDS	Salinity	BOD	COD
Cooum River (Amanjikarai)	≈	3975	7.3	3.13	2097	1600	30.00	84
Porur Lake (Porur)	×	106	7.3	561 μS/cm	375	BDL	3.3	52
Korattur Lake (Korattur)	×	389	8.4	4.82	3.3 ppt	2.9	31.02	158
Ambattur Lake (Ambattur)	≈	2673	7.8	2.61	951	BDL	41.43	97
Velachery Lake (Velachery)	×	790	7.7	1.64	1.2 ppt	1.6 ppt	27.00	81
Puzhal Lake (Red hills)	×	185	7.4	588 μS/cm	402	BDL	8.94	32
STP (Raw WW) (Indira Nagar)	≈	3964	7.6	1.8	4750	BDL	254.16	800
Temple pond water (Marudheeswar Lake)	×	675	7.4	956 μS/cm	907	BDL	29.5	92

Cauvery River showed neutral pH. Higher concentration of TDS and salinity (Very high) were observed in Cooum River from Napier Bridge. The presence of PFOA and PFOS compounds in the water samples were also evaluated by LC/MS. MS analysis revealed the presence of PFCs compounds in Tirupur and Cooum River, Tamil Nadu (Fig. S12). Among the various environmental samples, Cauvery River did not induce significant florescence. In contrast, the biosensor showed substantial fluorescence after exposure to samples of Tirupur and Cooum River (Table 4). But significant induction was seen in Ambatur and Korattur Lake water samples. This confirms that the as-developed bacterial biosensor is working in real field conditions. In comparison with already existing PFOA and PFOS detection sensor, our system found to be on lower detection limit (70 ng/L) of the health advisory for PFOA and PFOS in drinking water by the U.S. EPA. The detection limits of our biosensor are higher than those of electrochemical detection (30 μg/L; Ranaweera et al., 2019 and Electrochemo sensor (0.04 nM, Karimian et al., 2018)) and lower than those of Chemical sensor (0.13 ppb, Cennamo et al., 2018) chromatography (ppt level, LC/MS, U.S. EPA, 2019).

4. Conclusion

We have designed the genetically engineered bacterial biosensor system to analysis of the PFOA and PFOS compounds. The bacterial biosensor system is specific and which able to sense at the very low concentrations (10–1000 ng/L) of PFOA and PFOS. As per our knowledge, this is the first approach that has been used for sensing low concentration of perfluorinated contaminant. The modified bacterial biosensor has showed good analytical performance as for as sensitivity, detection limit and selectivity. The performances of the developed biosensor system were compared with LC/MS analytical techniques. The obtained results indicated that the genetically modified biosensor can be used for screening of PFOA and PFOS compounds, at low concentration of real environmental samples (wastewater, river water and surface water), allowing the fast detection of a PFOA and PFOS contamination. The future goals of present study involve shortening the response time; improve the selectivity and portable properties in the developed bacterial biosensor. We will adapt the biosensor system to micro centrifugal microfluidics platform to improve the biosensor property. The miniaturization bio-sensing system can decrease the testing time and enhance the high throughput screening of samples.

CRediT authorship contribution statement

Dr. G. Sunantha - Planning of work, sampling, lab work, data curation, writing and editing.
Dr. N. Vasudevan - Planning of work, Supervision, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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