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Genetically Engineered Bacterial Biosensor for Detection of PFOA and PFOS in Water Samples

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Abstract

Several techniques were developed for identifying and quantifying Perfluorinated alkyl substances (PFASs). However, high cost, tedious procedures for separation before analysis and limited sensitivity are the limiting constraints of the existing PFAS sensors. Hence, there is a need to develop simple, low-cost and effective sensors for detecting emerging pollutants called perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). An attempt was made to develop genetically modified bacterial biosensor system for the detection of PFOA and PFOS in water samples. The biosensor response was achieved from 10 ng/L to 10 ppm of PFOA and PFOS compounds with 24 h incubation time. The biosensor was successfully applied for the detection of PFOA or/and PFOS in different water samples and the results agree well with those results obtained by liquid chromatograph-mass spectrometer. Therefore, the developed biosensor system can be utilized for monitoring PFOA and PFOS compounds in the water samples such as pond, Lake, River and wastewater.

Keywords: *Biosensor; Detection method; Emerging Pollutant; PFASs; PFOA and PFOS.*

1. Introduction

PFASs are pervasive contaminants produced by anthropogenic activities in the environment. Due to their extensive use in industrial and commercial products, PFASs are of great interest. It has been hypothesized that residual, unreacted PFASs found in commercial products and insufficiently treated industrial effluent are the primary sources of PFASs compounds in the environment (Sunantha et al., 2022) [1]. Among PFCs, PFOA and PFOS are of considerable interest due to their extensive application in various industries. PFOA has fifteen fluorine atoms and eight carbon atoms with a carboxylic acid group at the end. PFOS has eight arbon atoms and seventeen fluorine atoms with sulfonic acid group (Figure 1). The surface tension-lowering property and the ability to create stable foams have led to widespread use of these compounds in textile, plastic, electronics and upholstery industrial applications (Boone et al. 2019; Brun et al., 2023) [8, 9]. For example, in plastics and rubber industry (emulsifying agent for polymerization and latex stabilizer purposes), petroleum industry (wetting agent for oil well treatment), paint and pigment production industries (leveling agent for floor waxes, adjuvant for waxes to improve oil and water repellency), photographic (anti-static and cleaning purpose), chromium plating (mist suppressant), floor polishes and play an important role in pharmaceutical industries (Prevedouros et al. 2006) [3]. The maximum permitted concentration of PFOA and PFOS in drinking water is 70 ppt, according to the EPA (Hariharan et al., 2023) [2]. However, humans and animals experience adverse effects when their concentration exceeds the permitted limit (US EPA 2016) [3].

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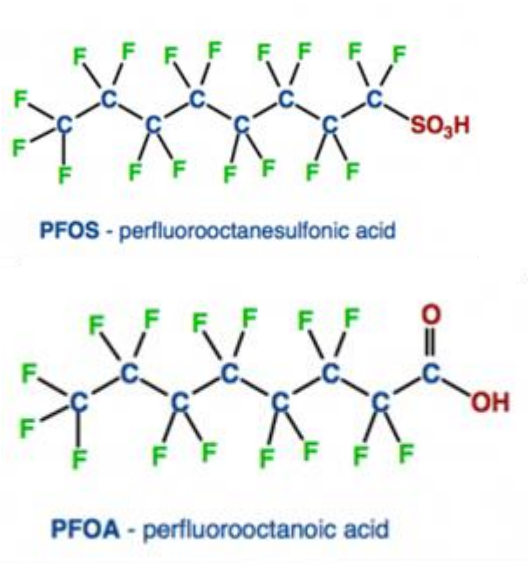

Figure 1 Structure of PFOA and PFOS Compounds

It is necessary to monitor the concentration of these PFOA and PFOS in the environment due to their extensive use. HPLC and LC/MS are currently used techniques that accurately identify and quantify these compounds. Still, they are also very sensitive, expensive, and require sample purification prior to chromatographic analysis. Hence, there is a need to develop low-cost sensors for detecting PFAS in water samples. Several methods, including microbial biosensors, nano-based sensors, optical, and others, have been tried to develop the quick assay. In terms of specificity and selectivity, the biosensor system is the best method for pollution monitoring. Timely sample preparation, high operational expenses, and a lack of on-site applicability are among the disadvantages of analytical procedures. Rapid detection methods are crucial as a result. We developed PFAS sensor would enable rapid detection and ease of use, devoid of purification before analysis for detecting PFAS.

2. Method

The bacterial culture, such as *P. aeruginosa* (PAO1), *P. aeruginosa* (1688) and *Burkholderia* FA1 were selected for our study. In order to select effective bacterial strain for identifying PFOA and PFOS resistant gene, biodegradation study was conducted. The environmental and industrial application of defluorinase enzyme was proposed to break PFOA and PFOS substrate which release fluoride ions (Figure 2). Cell free extract of defluorinase enzyme activity was performed as per the protocol of Kurihara et al. (2003) [7] with slight modification. We followed cloning work according to Sambrook and Russell [4].

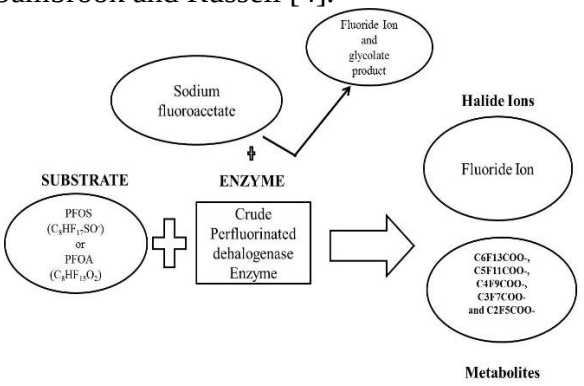

Figure 2 Reaction Mechanism of Defluorinase Enzyme

3. Results and Discussion

3.1. Results

The bacterial culture, such as P. aeruginosa (PAO1), *P. aeruginosa* (1688) and Burkholderia FA1 were grown on MSM with PFOA or PFOS as a carbon source. Among these strains, P. aeruginosa (PAO1) showed better growth than P. aeruginosa (1688) and Burkholderia FA1. The defluorinase activity of P. aeruginosa (PAO1) was studied on PFOA and PFOS substrate and the results showed that the defluorinase enzyme is able breakdown the PFOA and PFOS which yield the F- ions. The defluorinase enzyme was specific to C-F bonds and checked with sodium fluoroacetate. The defluorinase enzyme showed the highest activity towards sodium fluoroacetate than the PFOA and PFOS due to the rigidity of these two compounds. The P. aeruginosa (PAO1) was able to grow on PFOA and PFOS at 10 ppm of concentration and degraded up to 58% of PFOA and 52% of PFOS in 5 days with the release of F- ions in MSM medium. ESI-MS analysis showed that the metabolites formed during the degradation study. Perfluoroheptanoic acid, Perfluorovaleric Acid, Perfluoropionic acid from PFOA and Perfluorohexanoic acid from PFOS were identified as metabolites. P. aeruginosa (PAO1)

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having a defluorinase gene was confirmed through enzyme assay and biodegradation study. Primer designed for identifying pfc-DEF gene gave positive amplification with genomic DNA from P. aeruginosa (PAO1). The defluorinase gene encodes nucleotides and gfp genes were cloned into pRSET vector. The positive recombinant was confirmed and transformed into E. coli BL21 (DE3). The positive clone containing the pfc-DEX: gfp insert was then induced overnight with 1.5 mM IPTG and the sample was run in 12% SDS-PAGE and results indicated expression of pfc-DEX: gfp. The recombinant biosensor was exposed to PFOA and PFOS compounds and the gfp expression were observed under fluorescence microscope. The time dependent induction of the biosensor was determined by incubating the genetically modified E. coli BL21 (DE3) cells in minimal medium with PFOA and PFOS substrate. The biosensor showed good response in 24h incubation (Process of the sensor shown in Figure 3). To observe the dose response of the biosensor to PFOA and PFOS compounds, the modified E. coli (pRSET- pfc-DEF/gfp) and control E. coli BL21 (DE3) strain were induced with different concentration of PFOA and PFOS. For this assay, optimized 24h induction time was chosen; since the green fluorescence signal was achieved during this period was significantly high. A good response of the developed biosensor observed between 300 ng/L to1000 ng/L of

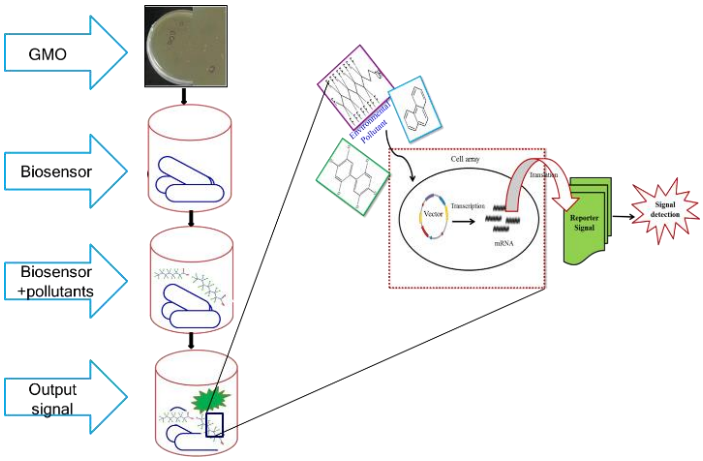

Figure 3 Process of the Developed sensor to against PFOA and PFOS

PFOA or/and PFOS compounds. The biosensor could detect PFOA and PFOS compounds at concentrations ranging from as low as 10 ng/L upto 10 mg/L as evident from the results of fluorescence intensity and cell growth. The specificity of the developed biosensor was treated by using other organic pollutants (polychlorinated compounds, Polyaromatic compounds and pesticides). The specific reactivity was highest with PFOA and PFOS. The cross reactivity was lesser with PCB. There was no response with PAH and pesticides. Different environmental water samples were analysed using this engineered biosensor. The sensor system responded to PFOA or/and PFOS to environmental samples and these results were cross checked in LC/MS for the detection of PFOA and PFOS.

3.2. Discussion

Based on the results of the present study it can be concluded that so far, several specific bacterial sensors were developed for the detection of PCB, PAHs, heavy metal and pesticides. Cheng et al. (2020) [5] have proven using another electrochemical sensor to detect PFOS at lower concentrations. They employed a metal-organic framework as electrodes. Kazemi et al. (2020) [6] developed an electrochemical probe for MIP-based PFAS quantification. By anodic deposition of o-phenylenediamine in the PFOS template molecules, the MIPs were created. PFOS was effectively quantified using the modified electrode with a LOD of 0.025 ng/L, which is less than the EPA health advisory level. The created bacterial biosensor demonstrated limits of detection that were lower than the US EPA's allowed limits with strong selectivity and PFOA and PFOS-specific sensitivity. According to our knowledge, no bacterial biosensor has been constructed for the detection of PFOA and PFOS compounds. This present study demonstrates that engineered bacterial biosensor represents a simple, inexpensive and less laborious alternative to HPLC and LC/MS methods of PFOA and PFOS detection. The advantages of this type of bacterial biosensors may be ease which this can be used on field samples and applied for screening of both PFOA and PFOS compounds in

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field water samples. This developed biosensor was particularly useful when a large number of field samples need to be analyzed.

Conclusion

This present study demonstrates that engineered bacterial biosensor represents a simple, inexpensive and less laborious alternative to HPLC and LC/MS methods of PFOA and PFOS detection. The advantages of this type of bacterial biosensors may be ease which this can be used on field samples and applied for screening of both PFOA and PFOS compounds in field water samples. The future goals of present study involve shortening the response time prove the selectivity and portable properties in the developed bacterial biosensor.

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