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Research Article

Toxicity Biosensor for Sodium Dodecyl Sulfate Using Immobilized Green Fluorescent Protein Expressing *Escherichia coli*

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Green fluorescent protein (GFP) is suitable as a toxicity sensor due to its ability to work alone without cofactors or substrates. Its reaction with toxicants can be determined with fluorometric approaches. GFP mutant gene (C48S/S147C/Q204C/S65T/Q80R) is used because it has higher sensitivity compared to others GFP variants. A novel sodium dodecyl sulfate (SDS) toxicity detection biosensor was built by immobilizing GFP expressing *Escherichia coli* in *k*-Carrageenan matrix. Cytotoxicity effect took place in the toxicity biosensor which leads to the decrease in the fluorescence intensity. The fabricated *E. coli* GFP toxicity biosensor has a wide dynamic range of 4–100 ppm, with LOD of 1.7 ppm. Besides, it possesses short response time (<1 min), high reproducibility (0.76% RSD) and repeatability (0.72% RSD, $R^2 > 0.98$), and long-term stability (46 days). *E. coli* GFP toxicity biosensor has been applied to detect toxicity induced by SDS in tap water, river water, and drinking water. High recovery levels of SDS indicated the applicability of *E. coli* GFP toxicity biosensor in real water samples toxicity evaluation.

1. Introduction

Green fluorescent protein (GFP) was first detected in *Aequorea victoria,* a type of jellyfish, in 1961 [1], but the cloning of the GFP gene only took place 29 years later [2]. GFP possesses several characteristics which make it exceptional; among the characteristics, it does not require cofactors or substrates, is stably expressed as a fusion protein, is relatively nontoxic, and can be readily detected by fluorescence microscopy and other fluorometric techniques [3]. The wild type chromophore is excited with blue light or UV at 396 nm or 475 nm and emits green fluorescence at 508 nm [4]. There are many GFP variants that have been created with shifted absorbance and emission spectra, improved folding, and expression properties. The creation of blue, cyan, yellow, and red GFP variants coupled with new fluorescence imaging approaches has created more potential of GFP in proteins and biosensor studies [3]. *Escherichia coli* GFP is one of the examples where GFP mutant gene (C48S/S147C/Q204C/S65T/Q80R) was introduced and expressed in *E. coli* bacteria.

Water resources are reported to be polluted by pollutants (nanoparticles, pesticides, pharmaceutical, industrial waste, by-products from water treatment plants, etc.) very frequently in these few decades. Surfactants are one of the pollution agents [5] and the presence of surfactants as pollutants is also reported in filtered and treated tap water and drinking water. Surfactants play an important role in decreasing the surface tension of water and allow removal and breakdown of stain or grease particles to take place. Sodium dodecyl sulfate (SDS), also known as sodium lauryl sulfate (SLS), a primary alkyl sulphate with chemical formula $CH_3(CH_2)_{11}OSO_3Na$, is a member of alcohol sulfate family. SDS is one of the anionic surfactants and it takes up a large part in human life, from appearing in the household [6]. SDS will get into the water resources through outfalls of waste

water or through direct application such as agrochemical sprays [7, 8], dispersants, and pesticides [6]. SDS is widely used in biochemical research in cell lysis, in DNA extraction via SDS-PAGE, and as viral biocide [9].

SDS has been reported toxic to aquatic organisms. In fish species, it enhances organ morphologies such as kidney and spleen, alteration of metabolism rate and swimming ability, and changes in growth and death rates [10–12]. SDS shows acute effect to fertilization of ova and sperm, decreasing probability of fertilization in fish [12]. SDS is also reported to be toxic to mammals such as rodents and human [13]. Exposure of SDS to mammals results in physical and biochemical effects: skin irritation, hyperplasia, alteration of serum lipid composition, damage of cells, and decrease in cell proliferation [14–16]. Detection of SDS via chemical analytical methods through capillary electrophoresis, gas chromatography, HPLC, and UV-V has drawbacks such as difficulty to determine LOD with large sample volume, low detection and volatilization rates, production of toxic waste during operational analysis, high cost, and causing damage to sample during detection process [17–19]. The use of dye to bind with SDS in water has been suggested for the quantification of SDS concentration, but there is challenge to produce specific binding of the dye with SDS [20, 21]. Not many SDS-detection biosensors have been reported. While several bacterial biosensors were produced to assist SDS detection, most of them were reported to have long response time (minutes to hours), short dynamic linear range, low reproducibility, and short term of stability [22–24]. In this study, we report a sensitive SDS-detection toxicity biosensor fabricated with immobilization of *E. coli* GFP which was found to have improved properties.

2. Materials and Methods

2.1. Materials. Ampicillin, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), sodium dodecyl sulfate (SDS), and sodium chloride (NaCl) were purchased from Sigma-Aldrich. Nutrient agar and glycerol were purchased from MERCK and HMBC Chemicals, respectively. Yeast extract and tryptone were purchased from Becton Dickinson and Company. All materials used in this study are of the highest purity available. Solutions were prepared in deionized water (Barnstead RO Thermo Scientific).

2.2. Cultivation of Recombinant Bacteria E. coli GFP. *E. coli* GFP colonies were produced by spreading the bacterial stock on nutrient agar and incubated under 37°C for 18 hours. Single colony of bacteria has been inoculated in 4 mL Luria-Bertani medium (LB: 10 g/L tryptone, 5 g/L yeast extract, 10 g/L of NaCl, pH 7.0) with 100 μg/mL ampicillin added. Preculturing of *E. coli* GFP bacteria has been carried out in a rotary thermoshaker and has been set at 250 rpm, 37°C, for 18 hours (until OD_{600} reached 1.3 ABS). 500 μL of yielded recombinant bacteria from the preculture was further cultivated in 50 mL LB medium for 4 hours (optical density 600 nm reached 0.8–0.84 ABS) [25]. Bacteria *E. coli* GFP that has been cultured was centrifuged under 3000 rpm at 25°C for 10 min; the supernatant was discharged. It was then

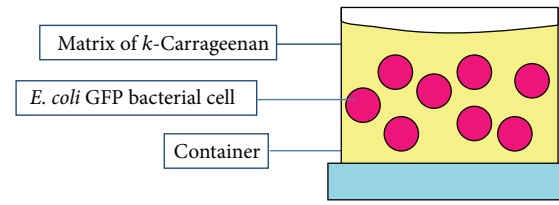

Figure 1: The design of *E. coli* GFP toxicity biosensor.

washed twice with 5 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffer with 171 mM NaCl, pH 7.0, under the same condition [26]. Bacterial cells in pellet form were suspended in 10 mL HEPES buffer and kept in 4°C. The bacteria culture is ready to be used in the following steps.

E. coli GFP bacteria strain was selected because it is more sensitive and fast in giving a response compared with the rest of the GFP mutants. Wild type GFP was not considered because of its weak fluorescence signal. Single colonies were obtained from the nutrient agar after 20 μL of *E. coli* GFP from glycerol stock was spread equally and allowed to grow on the agar. Ampicillin was added into the nutrient agar as well as the LB medium, to ensure plasmid maintenance of the bacteria *E. coli* GFP. The bacteria cultures were washed twice with HEPES buffer to eliminate excessive LB medium and also organic wastes that were produced during the growing and duplication process of the bacteria.

2.3. Fabrication of E. coli GFP Biosensor. Toxicity biosensor is fabricated by immobilizing *E. coli* GFP bacterial cells in *k*-Carrageenan matrix. Immobilization process was carried out on a hot plate with temperature set to 40–45°C. A round container (d = 8 mm) was used as a mould for the immobilization to take place. Figure 1 illustrates the design of *E. coli* GFP toxicity biosensor. Optimized amount of *E. coli* GFP cells (warmed in 35–40°C water bath) was mixed with optimized concentration of *k*-Carrageenan agar (kept in liquid form under 40–45°C heating). Temperature for biosensor preparation was set in between 35 and 45°C, which is a suitable temperature range for active survival of bacteria. Mixture was stirred well to make sure there was no formation of gas bubbles in the matrix and to produce a smooth surface. Homogenous mixture was kept in 4°C overnight to allow thorough solidification to take place.

2.3.1. Immobilization Matrix Optimization. Six concentrations of *k*-Carrageenan matrix were prepared: 1.0%, 1.2%, 1.4%, 1.6%, 1.8%, and 2.0%. All of them were prepared by mixing the specific amount of *k*-Carrageenan powder with deionized water in which the temperature was fixed at 75°C, which is the temperature needed for *k*-Carrageenan powder to dissolve homogeneously in the solvent; higher temperature will denature the *k*-Carrageenan structure. Continuous stirring was set to allow homogenous mixture to form. A constant amount of *E. coli* GFP was then immobilized in 100 μL of *k*-Carrageenan matrix, for all six concentration measures. Each concentration was prepared in 3 repetition sets. The mixtures were kept in 4°C overnight. Toxicity biosensors with different

concentrations of *k*-Carrageenan matrix were then interfaced with fluorescence spectrometer (discussed in detail in Section 2.4). The parameter of biosensor which gives the highest fluorescence reading will be chosen as the optimum one, and that parameter will be set as constant in the following optimization steps.

2.3.2. E. coli GFP Cell Density Optimization. Five different densities of *E. coli* GFP bacterial cells were prepared: 5.84, 11.68, 14.6, 17.52, 23.36, and 29.2 μg/mL. All of them were obtained by dilution and concentration of bacterial culture in LB medium (obtained via process mentioned in Section 2.1). The specific amount of *E. coli* GFP was then immobilized in 100 μL of *k*-Carrageenan matrix, with the optimized concentration measures in Section 2.3.1. Each amount of *E. coli* GFP was immobilized in four similar repetition sets. The mixtures were kept in 4°C freezer overnight. The as-prepared biosensors were then interfaced with fluorescence spectrometer (details discussed in Section 2.3); the optimized cell density was determined from set of biosensors which gave the highest fluorescence reading.

2.4. Fluorescence Response Measurement. Toxicity biosensors which consist of *E. coli* GFP cells immobilized in the *k*-Carrageenan matrix were tested with fluorescence optical fibre probe which acted as a transducer. Fluorescence signals of each toxicity biosensor have been measured with an optical fibre probe which was placed 10 mm above the biosensor, where the probe was connected to a Perkin Elmer fluorescence spectrometer. The excitation wavelength was fixed at 395 nm and emission wavelength was scanned from 400 to 500 nm. Bandwidths of excitation and emission were both set at 5 nm. The reading of fluorescence signals has been measured and read at 436 ± 2 nm. Optical fibre probe was set 10 mm above the biosensor samples each time, to ensure that no additional manipulated variable was added into the experiment. Excitation and emission bandwidths were fixed due to the same reason. 5 nm wide was the best gap to keep away the noises created during the experiment and at the same time was sufficient enough to capture returning fluorescence signals.

2.5. Performance Evaluation of Toxicity Biosensor E. coli GFP

2.5.1. Stability of E. coli GFP Toxicity Biosensor. The operational definition of stability of *E. coli* GFP toxicity biosensor is the time frame when the biosensor gives out persistent fluorescence signal not less than 75% as compared to initial measurement. Fluorescence signal is the referral unit for biosensor stability. 10 sets of biosensor with optimized immobilization parameters have been prepared, sealed with parafilm, and stored in 4°C. A drop of 10 μL of pH 7.0 HEPES buffer was dropped onto the biosensor each time after measurement to maintain the moisture of the matrix before it was resealed with parafilm and returned into 4°C freezer. Fluorescence emissions of the toxicity biosensor were measured daily or within interval until a sharp drop in the fluorescence signal of toxicity biosensor observed.

2.5.2. Repeatability and Reproducibility Studies. The repeatability value of the *E. coli* GFP toxicity biosensor was determined by calculating the percentage of relative standard deviation (%RSD) of the fluorescence intensity of ten sets of toxicity biosensor which were all prepared with optimized parameters and exactly the same technique, while the reproducibility value of the *E. coli* GFP toxicity biosensor was determined by calculating the %RSD of the response of the toxicity biosensors, which were prepared from three different batches, with five replicates each, towards a series of SDS concentrations (20, 40, 60, and 100 ppm). The exposure time was 5 min.

2.5.3. Determination of IC50 of SDS towards E. coli GFP Toxicity Biosensor. Half maximal inhibitory concentration (IC50) is a measure to determine the toxicity level of a chemical towards living organisms. The IC50 value of SDS towards the *E. coli* GFP cells immobilized onto the toxicity biosensor is determined by exposing the biosensor to 12 concentration parameters of SDS in the range of 1–100 ppm. 50 μL of SDS from each concentration was exposed to the toxicity biosensor. Approximately three replicates were prepared for each parameter. Toxicity biosensors were interfaced with fluorescence optical fibre probe, and readings were recorded before the exposure to surfactant SDS (F_o) and 5 min after exposure (F_t). Percentage of relative fluorescence unit (%RFU) for each SDS concentration was determined using the formula as follows:

$$\%\text{RFU} = \frac{F_o - F_t}{F_o} \times 100\%. \quad (1)$$

The value of %RFU indicates the inhibitory level of SDS towards *E. coli* GFP toxicity biosensor. A graph of inhibitory percentage versus SDS concentration is plotted. The IC50 value is determined from the graph.

2.5.4. Evaluation of E. coli GFP Toxicity Biosensor towards SDS Detection. The calibration curve of *E. coli* GFP toxicity biosensor was determined by exposing the biosensor towards 50 μL SDS with 29 concentrations ranging from 0.1 to 1000 ppm. The changes in fluorescence before and 5 min after exposure of the toxicity biosensor were determined. Five replicates were prepared for each concentration. %RFU for each measure was calculated. The linear range and the lowest detection limit of the toxicity biosensor were established from the calibration curve.

2.5.5. Real Sample Exposure and Recovery Studies. Water samples were collected from three different sources from areas around the National University of Malaysia (UKM): (a) tap water collected from the Chemical Sensor and Biosensor Lab, UKM; (b) river water collected from Langat River, Cheras; and (c) filtered drinking water collected from Kajang town. Water sample (b) was filtered with a Whatman qualitative paper number 6 and number 2 to remove suspended particles that will distort the readings of fluorescence intensity. SDS solutions with five concentration measures (10, 20, 50, 80, and 100 ppm) were prepared in the real water samples. *E. coli* GFP toxicity biosensors were exposed to SDS in different water backgrounds. Induction response with the addition

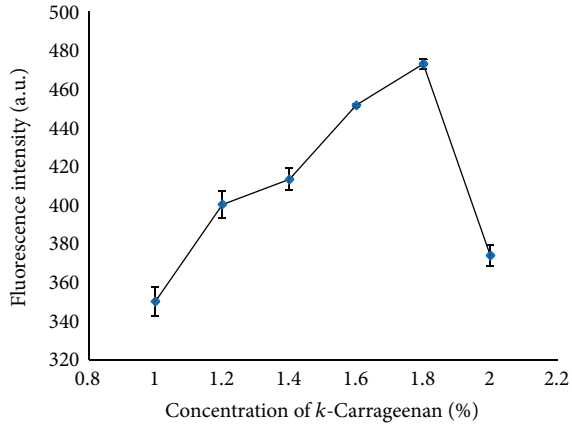

Figure 2: The fluorescence emission spectra of the bacteria *E. coli* GFP cells immobilized in *k*-Carrageenan matrix ($n = 4$).

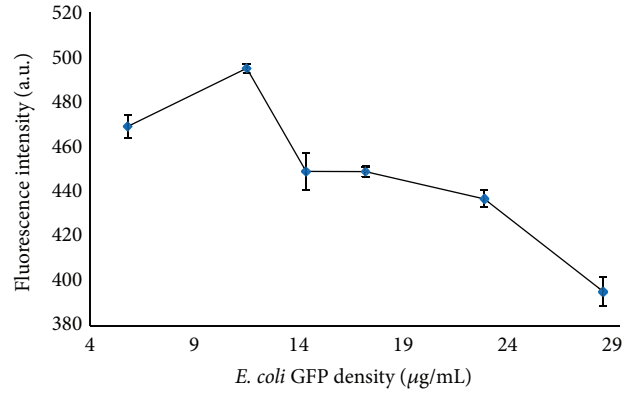

Figure 3: The fluorescence emission spectra of the bacteria *E. coli* GFP cells with varying cell densities (5.84, 11.68, 14.6, 17.52, 23.36, and 29.2 μg/mL) immobilized in 1.8% *k*-Carrageenan matrix ($n = 4$).

of surfactant SDS has been carried out. The experiment was done with five-sample repetition. The ability of *E. coli* GFP toxicity biosensor to work under real water background has been studied.

3. Results and Discussion

3.1. The Optimization of E. coli GFP Toxicity Biosensor. In the fabrication of *E. coli* GFP toxicity biosensor, two major parameters have been studied: the concentration of the immobilization matrix and the *E. coli* GFP cell density to be immobilized. Matrix *k*-Carrageenan has been selected for the ease of its preparation procedure, its gelling temperature falls in the range that is tolerable by *E. coli* GFP cells, it is not toxic to the bacterial cells, and its clear nature allows optical measurement to be done. *k*-Carrageenan is hydrophilic and will be surrounded by water molecules which brought to the process of gelation [28]. The presence of Na^+ cations from the cell suspension buffer enhances the formation of double helix domain of *k*-Carrageenan as proposed by the Domain Model which gives a more rigid structure to the toxicity biosensor [29–31].

Figure 2 shows the effect of the concentration of immobilization matrix to the fluorescence intensity of the toxicity biosensor. It can be understood that the gel viscosity and rigidity increase with the gel concentration, but the optimized gel concentration appeared to be 1.8% instead of 2.0%. Continuous increment in the fluorescence readings was observed for *E. coli* GFP immobilized in 1.0–1.8% *k*-Carrageenan matrix. 1.0% of *k*-Carrageenan appeared to be too watery and was unable to fix the bacteria stationarily, causing bacteria cells to sediment overnight due to gravitation force. The stacking of bacteria cells at the bottom of the biosensor mould blocked the emission of fluorescence from being detected accordingly by the fluorescence spectrometer, giving out low readings. Same explanations applied to biosensors fabricated in 1.2–1.6% of *k*-Carrageenan, which showed improving immobilization ability while, for *k*-Carrageenan 2.0%, it was too thick to be manipulated in the immobilization approach. The gelation took place within a few seconds when gel-cell mixture was prepared. Even distribution of the bacterial cells could not be achieved before the gel solidified, leading to stacking of bacteria. 1.8% is the optimum concentration of *k*-Carrageenan, which fixes the bacteria cells firmly while allowing thorough stirring of mixture.

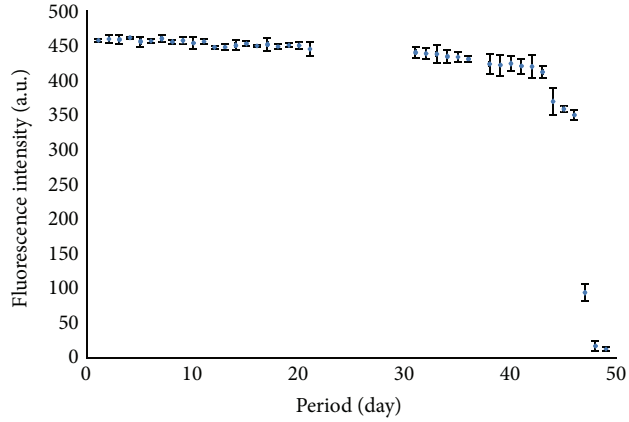

Figure 4: Long-term stability of *E. coli* GFP toxicity biosensor.

Figure 3 shows the effects of immobilized *E. coli* GFP cell density on the fluorescence emission of the toxicity biosensor. Similar stacking theory applied in the optimization of cell density of *E. coli* GFP graph, where the decrease in the fluorescence readings, after 11.68 μg/mL, was due to overloading of bacteria. Increasing bacteria quantity beyond the immobilization limit of the *k*-Carrageenan matrix will lead to overcrowded bacteria cells in a constant amount of matrix. This situation ends with stacking of bacteria cells. Fluorescence light emitted by the bacterial cells at the bottom of the container will not be able to be detected by the fluorescence spectrometer. This situation brought the reverse results, where the more bacteria were being immobilized, the less fluorescence readings were being recorded.

3.2. E. coli GFP Toxicity Biosensor Performance. The *E. coli* GFP toxicity biosensor is stable for a period of 46 days (Figure 4). The biosensor signal remained in the range of

Table 1: Response of *E. coli* GFP toxicity biosensors prepared from three different culture batches towards SDS (20–100 ppm) exposure, $n = 5$.

Set biosensor	Sensitivity (%/ppm)	R^2 value
1	61.39 ± 0.32	0.9877
2	62.19 ± 0.76	0.9875
3	62.12 ± 0.62	0.9833

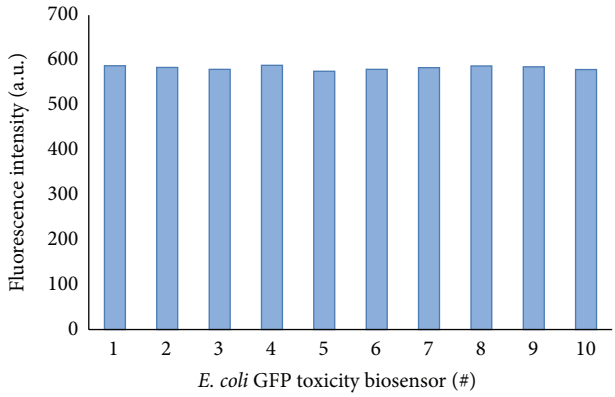

Figure 5: Stability of fluorescence signal of *E. coli* GFP toxicity biosensors which were prepared under constant conditions.

442.04 ± 13.94 for the first 43 days. The fluorescence signal dropped 79.36% from Day 1 and decreased to 2.80% starting Day 47. The decrement of fluorescence signal is caused by increased cell death due to depletion of nutrient and toxicity of respiratory residue.

A series of ten *E. coli* GFP toxicity biosensors have been fabricated and the fluorescence intensity of each biosensor was detected and compared (Figure 5). Fluorescence signals obtained fall in the range of 582.50 ± 4.45, giving a value of 0.76% for %RSD.

Repetition of SDS exposure (20–100 ppm, $n = 5$) for *E. coli* GFP toxicity biosensor prepared from three different batches of bacteria culture (labeled sets 1–3) shows similar level of sensitivity (Table 1). All three sets of *E. coli* GFP toxicity biosensor gave an average sensitivity of 61.90 ± 0.44%/ppm, with R^2 value > 0.98. The toxicity biosensor response towards SDS gave %RSD of 0.72%.

3.3. Response of E. coli GFP Toxicity Biosensor towards SDS Detection. The fluorescence intensity of the *E. coli* GFP toxicity biosensor decreased when it was exposed to SDS. Different concentrations of SDS induced different levels of decrement in the biosensor response. SDS causes cell toxicity to *E. coli* GFP which led to decline of fluorescence signal. Figure 6 shows the overall responses of the *E. coli* GFP toxicity biosensor to 10 ppm SDS exposure. Before exposure to SDS solution, the biosensor gave out a total fluorescence intensity of 976.56. We can understand that, at this stage, the "always on" *E. coli* GFP biosensor expressed the GFP proteins to its fullest, while after a few seconds when 10 ppm SDS solution was added, the fluorescence intensity dropped to 882.58. The SDS toxicity started to take place and that gave effect on

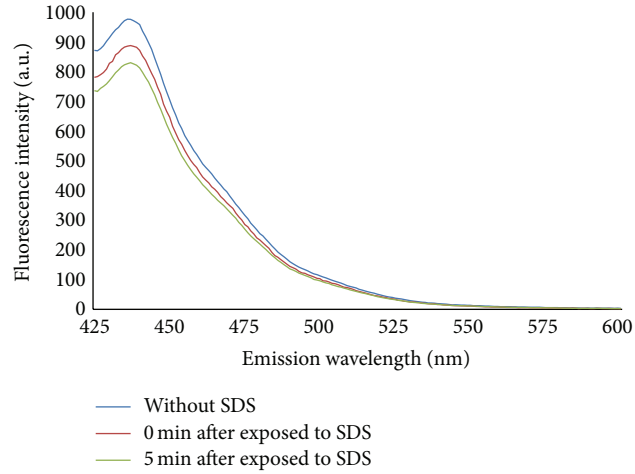

Figure 6: Fluorescence emission spectra of the bacteria *E. coli* GFP toxicity biosensor before and after the exposure to 10 ppm SDS.

the fluorescence signals. Within 5 min after the exposure, more inhibitory reactions between the surfactant and GFP have taken place, which results in further reduction in the fluorescence intensity, to 830.35.

Many derivatives of GFPs have been used in the fabrication of whole-cell biosensors for environmental pollution monitoring purposes [32–36]. In bacteria *E. coli* GFP, the active site that was used in toxic detection and binding is the cysteine groups that were introduced into the beta barrel structure. The redox state of the cysteine determines the fluorescent properties of *E. coli* GFP toxicity biosensor. There are three modes of action that took place which contribute to the observed response: (i) redox reaction of thiol group of the GFP; (ii) denaturation of GFP protein which leads to loss of protein function; and (iii) disturbance of cell capsule's surface protein which leads to cell death [26, 37, 38].

Binding of SDS to the thiol group of the GFP enhances redox reaction that gave effect on the fluorescence signals. The oxidation state of cysteine plays an important role in protein structure and formation. In its thiol form, cysteine is the most reactive amino acid and is often used for adding fluorescent groups. In oxidized forms, cysteine forms disulfide bonds, which are the primary covalent cross-links found in proteins that stabilize the native conformation of a protein. Cysteine is uniquely suited to sensing a range of redox signals as the thiol side-chain (–SH) can be oxidized to several different reversible redox states such as disulphide (R–S–S–R'); sulphenic acid (R–SOH); and *S*-nitrosothiol (R–SNO) [39].

SDS is known to cause denaturation of protein by binding to folded protein; its charged counterion will disturb the balance of the intrinsic charges of the protein and eventually unfold the protein with its negative charge [37]. Dilution of protein takes place when GFP consisting of 4-hydroxybenzylidene imidazolinone, which is commonly known as Y66 chromophore, is exposed to SDS. Y66 chromophore is responsible for the fluorescence emission of the

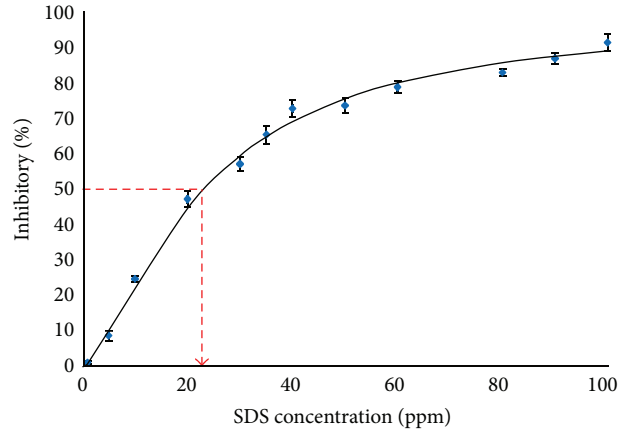

Figure 7: Dose-response curve of *E. coli* GFP toxicity biosensor towards SDS exposure. Red arrow with dotted line indicates the position of IC50 value of SDS ($n = 3$).

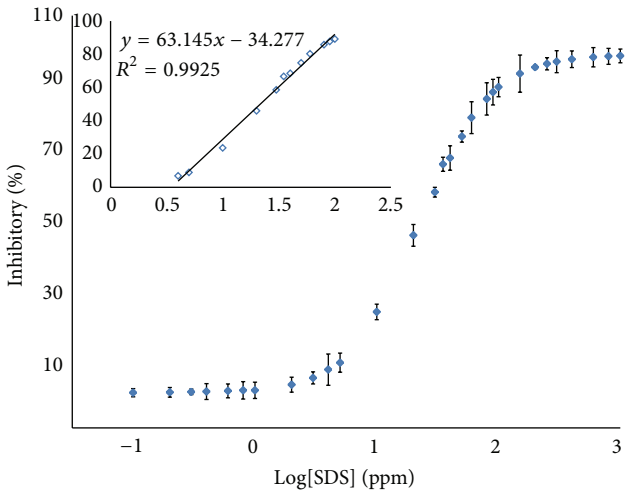

Figure 8: The calibration curve of *E. coli* GFP toxicity biosensor towards SDS exposure. Exposure time was set at 5 min, $n = 5$. Insert shows the dynamic linear range.

E. coli GFP toxicity biosensor [40]. Changing of protein confirmation inhibits the emission of the fluorescence.

Besides the unfolding of GFP, SDS is also believed to bind with proteins embedded on the phospholipid bilayer of the *E. coli* GFP cell capsule. During the exposure of SDS to the *E. coli* GFP toxicity biosensor, the surfactant decreases surface tension of the phospholipid bilayer. Binding of SDS changes the confirmation of embedded capsule proteins, which leads to the breakdown of phospholipid bilayer. Dispersion of phospholipid bilayers in cell membrane by SDS is also possible. Cell membranes play role in holding the bacteria contents (nucleus, proteins, cytoplasm, etc.) together. When the SDS works in decreasing the surface tension of the membrane, the cell membrane will deteriorate and dissemble [38]. *E. coli* GFP bacterial cells decompose when the cell membranes were gone, and hence no more fluorescence signal is being produced.

Table 2: Recovery data of *E. coli* GFP toxicity biosensor for the detection of SDS in tap water, $n = 5$.

Real SDS concentration (ppm)	Detected SDS concentration (ppm)	Percentage of recovery (%)
10	9.58 ± 0.08	95.83
20	20.70 ± 0.28	103.52
50	55.81 ± 0.90	111.63
80	81.23 ± 1.01	101.54
100	91.36 ± 1.24	91.36

Table 3: Recovery data of *E. coli* GFP toxicity biosensor for the detection of SDS in Langat River water, $n = 5$.

Real SDS concentration (ppm)	Detected SDS concentration (ppm)	Percentage of recovery (%)
10	9.30 ± 0.22	93.02
20	20.24 ± 0.33	101.19
50	53.62 ± 0.91	107.25
80	80.62 ± 0.89	100.77
100	91.42 ± 0.60	91.42

Table 4: Recovery data of *E. coli* GFP toxicity biosensor for the detection of SDS in filtered drinking water, $n = 5$.

Real SDS concentration (ppm)	Detected SDS concentration (ppm)	Percentage of recovery (%)
10	9.48 ± 0.33	94.76
20	20.61 ± 0.47	103.07
50	55.50 ± 0.94	111.01
80	80.06 ± 0.69	100.07
100	91.25 ± 1.11	91.25

3.4. Median Inhibitory Concentration of SDS. The response of *E. coli* GFP toxicity biosensor towards SDS exposure shows a sigmoid dose-response curve (Figure 7). Percentage of SDS toxicity inhibition increases when the concentration of SDS increases. The decrease in fluorescence signal of *E. coli* GFP toxicity biosensor after SDS exposure is an effect of cytotoxicity. IC50 of SDS towards *E. coli* GFP toxicity biosensor is determined as 22.75 ppm. This indicates that half of the total responsive activity of the toxicity biosensor will be induced when it is exposed to that dosage of SDS. SDS induces activity of *E. coli* GFP toxicity biosensor at an exponential rate at lower concentrations, while the response is close to plateau at higher levels due to maximum toxicity effect.

3.5. E. coli GFP Toxicity Biosensor towards SDS Detection. *E. coli* GFP toxicity biosensor has been applied to detect SDS in a wide range of 0.1–1000 ppm. The SDS inhibition response has been studied (Figure 8). Inhibitory effect of SDS towards *E. coli* GFP toxicity biosensor can be observed clearly from concentration of 2.0 ppm and onwards. The sigmoidal calibration curve indicates that *E. coli* GFP toxicity biosensor has a wide detection range, with dynamic linear range falling

Table 5: Comparison between *E. coli* GFP toxicity biosensor and reported SDS immobilized whole-cell bacteria biosensors.

Parameter	This study	[22]	[23]	[27]
Bacteria	*E. coli* GFP	*Pseudomonas rathonis*	*Pseudomonas* sp.	*Comamonas testosteroni* TI
Immobilization matrix	*k*-Carrageenan	Gel agar	Gel agar	Gel agar
Dynamic linear range (ppm)	4.0–100	1.0–200	0.04–0.90	—
Lowest detection limit (ppm)	1.7	~0.25–0.75	2.48×10^{-4}	0.25–0.5
IC50 (ppm)	22.75	—	0.16 ± 0.02	—
Response time (min)	<1.0	1.7–2.5	1.7–2.5	12–15
Reproducibility (%)	0.76	3.13	—	4.50
Repeatability (%)	0.72	—	—	—
Biosensor stability (days)	46	2-3	3–5	10

at 4–100 ppm. SDS with concentrations of 300–1000 ppm induces high level of inhibition which leads to a plateau at ~100%. SDS with concentration falling in that range gives high toxicity effect to *E. coli* GFP cells resulting in massive cell death, which gives rise to a quick drop in the fluorescence signal. LOD of *E. coli* GFP toxicity biosensor for SDS detection has been determined to be 1.7 ppm. SDS at lower concentration might take longer (>5 min) to cause toxicity, or the toxicity level is too low and the damage is resistible by *E. coli* GFP cells.

3.6. Recovery Performance of E. coli GFP Toxicity Biosensor. *E. coli* GFP toxicity biosensor was exposed to a series of SDS (concentration: 10–100 ppm) prepared in three different real water sample backgrounds. Recovery data for the biosensor performance in each water background were shown in Tables 2–4. *E. coli* GFP toxicity biosensor showed a recovery percentage of 91.36–111.63% when working under tap water background (Table 2). For *E. coli*, GFP toxicity biosensor which worked under the real river water background gave a recovery level of 91.42–107.25% (Table 3), while, for water background of drinking water, *E. coli* GFP toxicity biosensor is able to detect SDS for a recovery percentage of 91.25–111.01% (Table 4). The biosensor response slightly varies from a controlled experiment conducted in deionized water background (data not shown) due to interaction of SDS and *E. coli* GFP with free nontarget radicles present in the real water samples. Nevertheless, high level of recovery performance enables *E. coli* GFP toxicity biosensor to be applied in real environment.

Optimized *E. coli* GFP toxicity biosensor performance is compared with reported immobilized whole-cell bacteria biosensors that were fabricated to apply in SDS detection (Table 5). In our knowledge, there was no SDS-detection bacterial biosensor fabricated using *k*-Carrageenan as a matrix for cell immobilization being reported. Our toxicity biosensor has a comparatively wide dynamic linear range. Although the LOD of *E. coli* GFP toxicity biosensor is not as low as others, it has an IC50 value which is higher as compared to the one reported by Taranova et al. [23]. *E. coli* GFP toxicity biosensor is able to respond to SDS detection in seconds, while the reported biosensors would need minutes. The fabricated *E. coli* GFP toxicity biosensor possesses high reproducibility and repeatability as compared to the rest. Furthermore, our biosensor is stable for a longer time among comparison.

4. Conclusions

A sensitive and optimized *E. coli* GFP toxicity biosensor has been fabricated, with minimum response time as short as 5–10 s. Sodium dodecyl sulfate shows an inhibition reaction on the fluorescent ability of the bacteria, which was due to alteration of GFP protein and capsule surface embedded functional protein upon SDS binding and lipid dispersion criteria of SDS surfactant towards the phospholipid bilayer bacterial cell membrane. *E. coli* GFP toxicity biosensor is able to work in real water backgrounds to detect toxicity induced by SDS in tap water, river water, and drinking water. This study gives an alternative for SDS toxicity detection in water resources, which is comparatively economic, portable, and easy to prepare.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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