Enzyme and Microbial Technology 83 (2016) 22–28

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

A biosensor platform for rapid detection of *E. coli* in drinking water

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ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form
21 November 2015
Accepted 21 November 2015
Available online 26 November 2015

Keywords:
Water quality monitoring
E. coli
β-D-Glucuronidase
Drinking water
Rapid bacterial detection

ABSTRACT

There remains a need for rapid, specific and sensitive assays for the detection of bacterial indicators for water quality monitoring. In this study, a strategy for rapid detection of *Escherichia coli* in drinking water has been developed. This strategy is based on the use of the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG), which is hydrolyzed rapidly by the action of *E. coli* β-D-glucuronidase (GUD) enzyme to yield a fluorogenic 4-methylumbelliferone (4-MU) product that can be quantified and related to the number of *E. coli* cells present in water samples. In this study, the detection time required for the biosensor response ranged between 20 and 120 min, depending on the number of bacteria in the sample. This approach does not need extensive sample processing with a rapid detection capability. The specificity of the MUG substrate was examined in both, pure cultures of non-target bacterial genera such as *Klebsiella*, *Salmonella*, *Enterobacter* and *Bacillus*. Non-target substrates that included 4-methylumbelliferyl-β-D-galactopyranoside (MUGal) and L-leucine β-naphthylamide aminopeptidase (LLβ-N) were also investigated to identify nonspecific patterns of enzymatic activities in *E. coli*. GUD activity was found to be specific for *E. coli* and no further enzymatic activity was detected by other species. In addition, fluorescence assays were performed for the detection of *E. coli* to generate standard curves; and the sensitivity of the GUD enzymatic response was measured and repeatedly determined to be less than 10 *E. coli* cells in a reaction vial. The applicability of the method was tested by performing multiple fluorescence assays under pure and mixed bacterial flora in environmental samples. The results of this study showed that the fluorescence signals generated in samples using specific substrate molecules can be utilized to develop a bio-sensing platform for the detection of *E. coli* in drinking water. Furthermore, this system can be applied independently or in conjunction with other methods as a part of an array of biochemical assays in order to reliably detect *E. coli* in water.

1. Introduction

β-D-Glucuronidase (GUD) activity is characteristic of most strains of *Escherichia coli* and, hence, has been widely used to monitor the presence of this indicator organism in environmental samples, particularly water [1–3]. Approximately 97% of *E. coli* strains have GUD activity and almost all other coliform bacteria lack this enzyme [2]. Studies have shown that *E. coli* can preserves an active metabolism without being able to grow on culture media [4,5]. However, GUD-based assays for the detection of *E. coli* include the important fraction of viable but non-culturable (VBNC) organisms that would be missed by culture-based methods [6,7]. 4-Methylumbelliferyl-β-D-glucuronide (MUG) is a specific substrate for determining the presence of *E. coli* in a given water sample. The hydrolysis of MUG releases fluorescent 4-methylumbelliferone (4-MU) and the intensity of the measured fluorescent signal is proportional to the amount of enzymatic activities, showing a correlation to the number of *E. coli* present in the sample [6].

Many chromogenic and fluorogenic substrates exist for the specific detection of bacterial enzymatic activities and various commercial tests based on these substrates are available. To detect the presence of GUD in *E. coli*, the following chromogenic substrates have been previously used: indoxyl-β-D-glucuronide (IBDG) [8], phenolphthalein-mono-β-D-glucuronide complex, and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Glu) [9]. Although there are several fluorescence-based glycoside enzyme substrates available, substrates based on 4-MU have been more extensively used in diagnostic microbiology for the detection of bacterial enzymes [10–14]. In a recent review, the term "rapid" has been defined as <4 h for the methodologies for the detection of bacteria in recreational waters [15]. Rapid assays to estimate the GUD activity of *E. coli* have been performed without any cultivation steps where direct measurements of GUD activity were successfully applied to river, sea and waste water samples [6,16–18]. The current rapid

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http://dx.doi.org/10.1016/j.enzmictec.2015.11.007
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Fig. 1. Custom Designed Fluorescence Biosensor BDS1000.

procedures are laboratory-based and require bench-top fluorometers for the measurement of fluorescence resulting from the enzyme–substrate reaction. The biosensor used in this study not only provides the results about the viability of the *E. coli* in the water sample in <4 h but also can be customized in handheld or a bench top fluorometer format.

Isopropyl β-D-thiogalactoside (IPTG) is known to be a noncompetitive inducer, i.e., non-hydrolysable substrate by GAL [19] but the possible effect of MetGlu on GLUase activity has not been tested previously. GAL and GUD are well known to be inducible enzymes [19,20]. IPTG has been used commonly in cloning procedures that require induction of β-galactosidase activity, but recently have been used for induction of β-glucuronidase activity [21].

In our previous study, we have developed a method for detecting bacterial enzymatic-biochemical signatures and have shown the usefulness of a custom designed opto-electronic biosensor platform for the detection of *E. coli* and other bacterial cells in biofilm samples [22]. In this study, assays for rapid detection of *E. coli* in water were developed by using the compound MUG, which is hydrolyzed by the specific GUD enzyme to yield the quantifiable 4-MU fluorogenic product. At the Arizona State University (ASU) Environmental Microbiology laboratory, the biosensor instrumentation was assembled and customized for detecting the response of bacterial enzymatic machinery to the added specific fluorogenic substrates in order to rapidly determine bacterial water quality. In order to optimize the assay, fluorescent reagents were optimized to determine the detection limit and the working concentration range of the fluorescence assays. The present study introduces a biosensor that is designed to directly measure GUD activities and rapidly detect *E. coli* in water samples. The results obtained were substantiated by culture-based assays, indicating comparable data.

2. Material and methods

All experiments were conducted under laboratory conditions using aseptic techniques. First, all assays were optimized using pure chemical reagents and standards. Standard curves were generated to evaluate each assay working range of concentrations and detection limits. In the second step of optimization, increasing concentrations of pure *E. coli* cultures (ATCC 25922) obtained from American Type Culture Collection (Manassas, VA), were used as a model for bacterial biochemical and cultural assays. For confirmation, culture-based assays were performed in duplicate to determine Colony Forming Units (CFU) per reaction before and after the assays using selective agar media in order to determine bacterial viability. To rapidly quantify *E. coli*, the activity of GUD was measured using the soluble fluorescent substrate MUG and the resulting 4-MU fluorescence signals generated by the customized BDS1000 fluorescence detector (Fig. 1). The results have been also compared and evaluated with the performance of the reference instrument Aqualog benchtop fluorometer (Horiba, Kyoto, Japan), the only simultaneous absorbance and fluorescence system for water quality analysis, which measures both absorbance spectra and fluorescence excitation-emission matrices.

The assay was performed by adding 3 mL of a representative water sample to a mixture of 0.1 mL of the MUG and 0.9 mL of 0.1 M *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] buffer (HEPES), (VWR, Chester, PA) at pH 8.0 in a 4.0 mL reaction vial (disposable cuvette purchased from Fisher Scientific, Pittsburg, PA). The reaction vial was then placed in the custom designed Biosensor BDS1000 in the Environmental Microbiology laboratory at ASU. Each set of assays consisted of a negative control of 3.0 mL of 0.1× phosphate buffered saline (PBS) containing 0.9 mL of 0.1 M HEPES and 0.1 mL of 8.52 mM MUG. Assays were performed in triplicate by simultaneously processing three aliquots of *E. coli* suspension in three separate reaction vials and examined using the biosensor. The enzymatic activity data were collected for less than 120 min and until a desired linearity was achieved (R^2 = 0.90 or higher), the fluorescence signals were subtracted from the blank signals and reported as the final results. Raw data obtained from the biosensor were analyzed for correlation between different parameters in order to confirm the functionality of the biosensor.

2.1. Custom Designed Fluorescence Biosensor BDS1000

The biosensor instrumentation was assembled and customized in the Environmental Microbiology laboratory at ASU for detecting the response of bacterial enzymatic machinery to the added specific fluorogenic substrates. The optical and spectrometer components for the biosensor were obtained from Ocean Optics (Model# HR 2000, Ocean Optics, Dunedin, FL). The Light-Emitting Diode (LED) based light source provided filtered excitation light at specific wavelengths to allow single excitation, single emission detection of a specific fluorophore in each enzymatic assay. The excitation light spectrum appeared to have a ±10 nm range around the peak maxima. This allowed all fluorescence assays to be carried out at a single excitation wavelength (350 ± 10 nm). The fluorescence signals were collected as Relative Fluorescence Unit (RFU) at single excitation, dual emission wavelength (SEDEW) settings. The measurements were taken by directly placing the vials in the biosensor.

2.2. Stock culture preparation

A pure culture of *E. coli* (ATCC 25922) was grown in Tryptic Soy Broth (TSB) (Becton, Dickinson, Sparks, MD). Log phase bacterial stocks were prepared by incubating the bacterial suspension at 37 °C in a C24 shaker-incubator (New Brunswick Scientific, Edison, NJ) at 150 rpm. The log phase bacterial cultures were stored at 4 °C for at least 24 h before their use for the assays. Bacterial stocks were diluted in 0.1× PBS in a range of 10–10^8 CFU/mL.

2.3. Fluorometric assay reagents

2.3.1. 4-Methylumbelliferone (4-MU) standard preparation

For the GUD assays, the stock solution of the substrate was prepared by placing 0.030 g MUG (Sigma Chemical Co., St. Louis, MO) in a sterile 15 mL centrifuge tube containing 5.0 mL of pure ethanol. After all the crystals were completely dissolved, an amount of 5.0 mL of sterile distilled water (DI water) was added to the homogenized solution. The tube was capped and labeled as 8.52 mM stock MUG substrate solution. The solution was protected from light and stored at 4 °C. For every test, 0.1 mL of this substrate was used.

2.3.2. Preparation of 0.1 HEPES buffer, pH 8.0

HEPES buffer was prepared by dissolving 23.83 g of HEPES in 500 mL of autoclaved DI water. The pH of HEPES solution was adjusted to 8.0 using sodium hydroxide solution. The final volume was adjusted to 500 mL with DI water and sterilized using 0.2 μm filters. For each assay, 0.9 mL of this buffer was used. The solution was protected from light, and kept at room temperature. The HEPES buffer solution was freshly prepared before performing the assays.

2.4. Culture-based assays

At the start and completion of each assay, *E. coli* concentration was measured by the Membrane Filtration (MF) technique using a 0.45 μm membrane (Millipore SAS, Billerica, MA) and Brilliance (Oxoid LTD, Basingstoke, England) or mEndo (Becton, Dickinson and company) media followed by incubation at 37 °C for 24 h. This step was performed to achieve CFUs before and after the assay and to ensure bacterial viability and culturability.

2.5. Development of standard curves using GUS reporter kit

The calibration curves for MUG were generated by using a GUD reporter kit which was purchased from Marker Gene Technologies, Inc. (Eugene, OR), and Aqualog fluorometer were employed. A series of MUG concentrations were prepared by taking different volumes of the GUS assay buffer, GUD extraction buffer, and blank solution (extraction buffer) were utilized for this assay (Table 1). This step was necessary in order to attain the optimum MUG concentration needed to the spiked number of reacting *E. coli* cells.

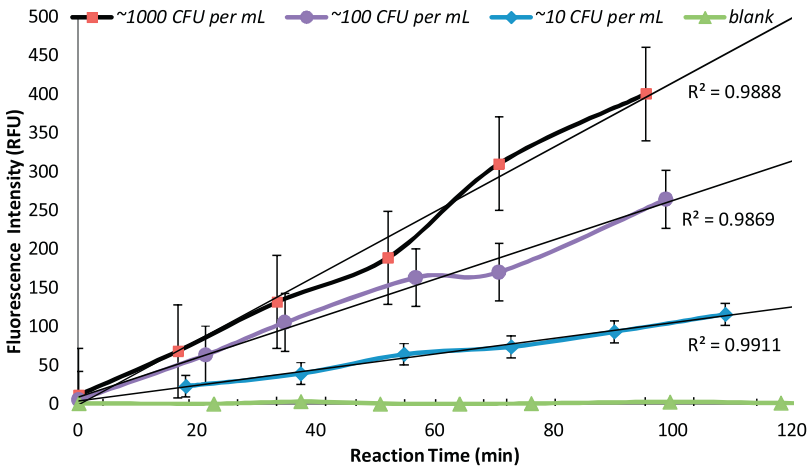

Fig. 2. Time series hydrolyses of MUG by *E. coli* at different concentrations.

A black 96-well plate and Synergy H1Hybrid Multi-Mode Microplate Reader (BioTek, Winooski, VT) was also employed to create the calibration curve for assessing the 96-well format for this assay. Four wells were allocated for each concentration of MUG. The 96-well plate was incubated at ~38 °C for 10 min, and then placed in the plate reader at room temperature. The fluorescence intensity values were averaged and compared to the blank.

2.6. Specificity of MUG assays for the detection of E. coli

2.6.1. Impact of non-target substrates on the detection of E. coli

The impact of 4-methylumbelliferyl-β-D-galactopyranoside (MUGal) and L-leucine β-naphthylamide (LLβ-N) substrates were investigated to measure the activities of galactosidase and aminopeptidase enzymes in *E. coli*.

a) Methylumbelliferyl-β-D-galactopyranoside

In the galactosidase assays, substrate stock was prepared by completely dissolving 15 μmol of MUGal (Sigma Chemical Co.) in 0.2 mL of Dimethyl Sulfoxide (DMSO), Cat. No. 4948V18H15, (Mallinckrodt Baker Inc., Paris, KY). Then the solution was diluted to 10 mL with 0.5× PBS at pH 7.3 and prepared according to a previously reported method [23]. The solution was protected from light and stored at 4 °C. For every test, an amount of 100 μL of this MUGal stock was used.

b) L-Leucine β-naphthylamide (LLβ-N)

Stock solution (40 mM) LLβ-N substrate (Sigma-L1635) was prepared in analytical grade ethanol with pH adjusted to 7.5 using HEPES buffer. For the aminopeptidase enzymatic assays, 0.100 g LLβ-N was added in 9.75 mL of pure ethanol in a sterile 15 mL centrifuge tube and mixed at room temperature until dissolved. The tube was capped and labeled as 40 mM LLβ-N Stock substrate solution. The solution was protected from light and stored at 4 °C.

2.6.2. Impact of pure and mixed cultures of non-target bacteria on the detection of E. coli

The specificity of the MUG based biosensor platform assay was examined by using pure and mixed cultures of non-target bacterial genera. All bacterial cells were obtained from ATCC. *Enterobacter* (ATCC 13048), *Bacillus* (ATCC 14579), *Klebsiella* (BAA-1705), and *Salmonella* (ATCC 19585), cultures were grown in TSB. A volume of 1.0 mL of each strain was taken from pure stocks and suspended in 9.0 mL of the corresponding broth media. The bacterial suspensions were incubated in a shaker-incubator (New Brunswick Scientific C24, Edison, NJ), (150 rpm at 37 °C) to achieve a log phase bacterial culture which ranged between 3 and 8 h, depending on the bacteria used.

2.7. Sensitivity (detection limit) of rapid enzymatic assays

The applicability of the biosensor method was tested using both tap and environmental waters.

2.7.1. Tap water

In order to investigate the sensitivity of the assay, 1000 mL of the city of Tempe, AZ, tap water was collected and 1.0 mL of 1% w/v solution of sodium thiosulfate was added to dechlorinate the water sample prior to the assay. After complete mixing, 200 mL from the water sample was taken and approximately 100 *E. coli* cells were added to the sample. The mixture was vortexed and divided into two

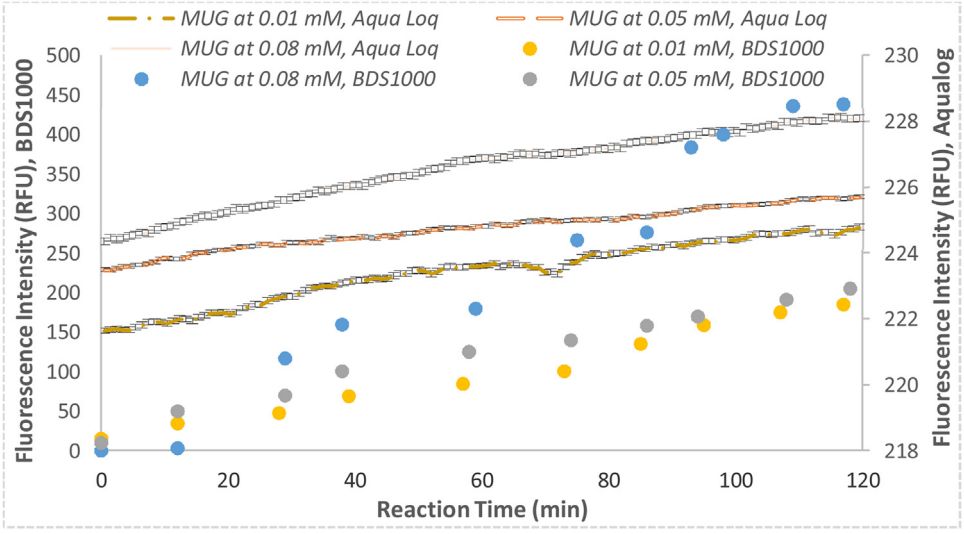

Fig. 3. Comparison of the MUG calibration curves obtained by BDS1000 and Aqualog fluorometer.
Note: the graphs show representative data for three independent experiments; the fluorescence intensity units are arbitrary; note the different scales of the two instruments.

Table 1
Assay conditions for generating MUG calibration curve.

[MUG]	GUD assay buffer (0.1 mM MUG)	GUS extraction buffer	Enzyme or blank solution
0.08 mM	80 μL	10 μL	10 μL
0.06 mM	60 μL	30 μL	10 μL
0.04 mM	40 μL	50 μL	10 μL
0.02 mM	20 μL	70 μL	10 μL
0.01 mM	10 μL	80 μL	10 μL

100 mL aliquots. Bacterial concentration in one of the aliquots was measured by MF as described previously. The second 100 mL aliquot was transferred to a 250 mL centrifuge tubes and concentrated by centrifugation at 1500 × *g* for 15 min. Using a pasteur pipette, the supernatant was carefully aspirated to the 3.0 mL mark above the pellet. Extra care was taken to avoid aspirating *E. coli* cells during this step. The centrifuge tube was vortexed vigorously until the pellet was completely resuspended, and then, a pipette was used to collect any residual volume that dripped down to the bottom of the tube to ensure that all the sample volume was recovered. After transferring the entire sample from the centrifuge tube to a reaction vial, MUG and HEPES were added to the sample.

2.7.2. Lake water

The environmental samples are referred to untreated source waters collected from two locations, Tempe Town Lake, AZ and Consolidated Canals in Mesa, AZ. Applicability of the biosensor for the detection of *E. coli* in environmental samples were tested using Tempe Town Lake samples and IPTG effect on GUD activities were evaluated using water collected from Mesa consolidated canals. The environmental water samples were tested for the presence of *E. coli* by the MF technique as previously described. Different aliquots of the sample were concentrated to 3 mL volume as previously explained. For each subsequent sample concentrate, including blank or spiked samples, fluorescence intensity measurements were performed.

2.8. IPTG effect on induction of GUD activities

The impact of different concentrations of Isopropyl β-D-1-thiogalactopyranoside (IPTG) on GUD activities was investigated in this study. According to Liu et al., the induction condition for the optimum production of the β-glucuronidase gene (AtGUS) protein is at ~0.5 μM IPTG [21], hence, two different concentrations of IPTG have been used for this evaluation.

2.8.1. 0.5 μM and 1 μM IPTG stock solution

A stock solution of 20 or 40 mM of IPTG (Sigma-16758-1G) was prepared in sterile nonopure deionized water. An amount of either 23.83 or 47.66 mg IPTG was weighed and dissolved in 5 mL sterile nanopure deionized water in a sterile 15 mL centrifuge tube. The content was mixed at room temperature until dissolved. The tubes were labeled as 20 or 40 mM IPTG stock substrate solution and stored protected from light at −20 °C. For every test, an amount of 100 μL solutions was added to the samples.

The IPTG effect on the GUD activities was examined using tap and environmental water samples. The environmental water samples were taken from consolidated canals in Mesa, AZ, transported to the laboratory on ice for processing, and tested for *E. coli* presence within 8 h and were analyzed by the MF technique. To each 3.0 mL water sample approximately 100 *E. coli* cells were added and then fluorescence measurements were taken as previously described.

3. Results and discussion

The feasibility of the direct assays for the sensitive biochemical detection of *E. coli* was evaluated using serially diluted cultivated bacteria. The results obtained for *E. coli* concentrations at approximately 10, 100, and 1000 CFU/mL in the reaction vial is presented in Fig. 2. The direct MUG assays analyzed with BDS1000 showed a desirable linearity of the fluorescent signals with increasing numbers of *E. coli* (Fig. 2). As concentrations of bacteria increased, more MUG molecule were broken per unit of time and higher fluorescence intensities were measured. The fluorescence intensity (RFU, arbitrary units) was directly related to the number of bacteria and MUG concentrations. In addition, the reaction time needed to detect *E. coli* was directly proportional to the bacterial cell numbers (Fig. 2). Using BDS1000, all optimized assays resulted in positive linear response of fluorescence signals in the range of bacterial concentrations of 10–10^8 *E. coli* per milliliter. This is comparable with the sensitivity of the previously reported hand-held confocal fluorescence detector FLUO SENS SD (ESE GmbH, Stockach, Germany), which showed increasing trends of fluorescent signals with increasing numbers of *E. coli* in a range of 10–10^8 CFU/mL in the water samples [24]. However, the hand-held sensor-based assay required incubation of samples with the substrate for 30 min prior to the assay [24].

Bacterial biochemical activities for all the assays increased with time and a higher concentration of the bacterial cells (Fig. 2). For each bacterial concentration, the fluorescent signal increased as bacteria continued to hydrolyze MUG molecules over time (Fig. 2). This increase in activity might be explained by the adaptation and survival of *E. coli* to the environmental conditions such as water quality parameters, reaction buffers and substrate consumptions in the reaction vial. The detection time required for the biosensor response versus the culture methods ranges from 20 to 120 min and 24 to 48 h, respectively. The sensitivity of the method was such that it enabled rapid detection well within the 4 h which is the period defined as rapid [15]. No increasing trend in the relative progression of GUD activities was noted in blank (Fig. 2).

Since overnight cultures of *E. coli* cells were stored at 4 °C, the cells were at stationary phase prior to use. The high levels of activity observed in some cultures may indicate that their starved metabolic state led to an increase in bacterial enzymatic activities hydrolyzing the fluorogenic substrate rapidly. As previously reported that full development of enzymatic activities start at lag phase and is required for the enzyme expression [2].

3.1. Calibration curves and comparison study

A comparison study was conducted between BDS1000 as a new device and Aqualog fluorometer as a reference instrument. The calibration curves were used to determine the sensitivity of the

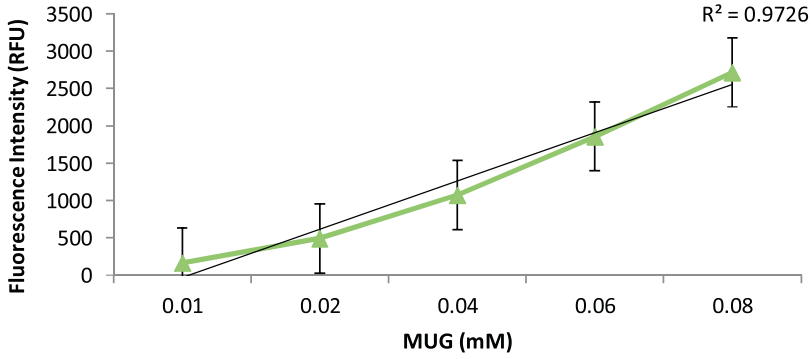

Fig. 4. MUG calibration curve obtained by the microplate reader.

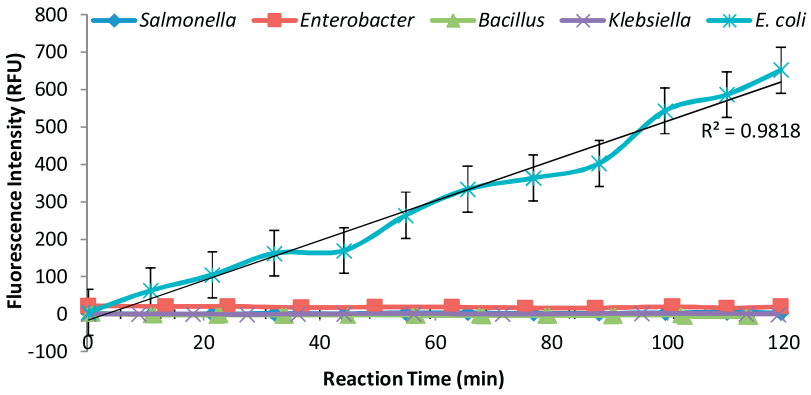

Fig. 5. Specificity of MUG assay on pure cultures of non-target bacterial genera.

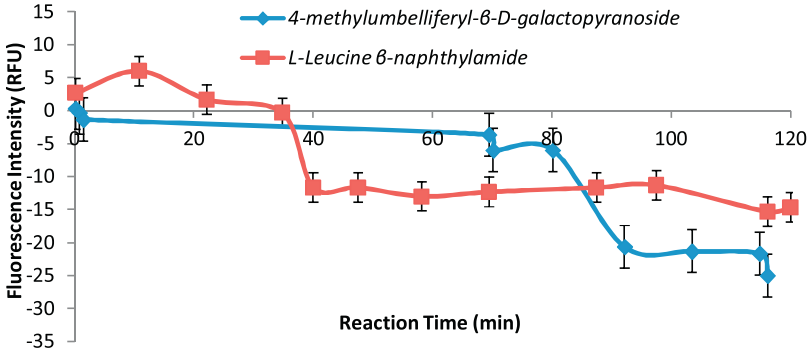

Fig. 6. Impact of non-target substrates on *E. coli* enzymatic activities.

enzymatic assays using the reference instrument (Fig. 3). GUD assay fluorescence signals produced a linear response with a correlation coefficient higher than 0.90 (Fig. 3). The lower detection limit was 0.01 mM of MUG as determined by three standard deviations of the blank; however, the results for MUG at 0.01, 0.05 and 0.08 mM are plotted in Fig. 3. The optimum MUG response was found repeatedly at a concentration of 0.08 mM using approximately 10–1000 *E. coli* CFU/mL. Therefore, MUG at concentration of 0.08 mM was selected as the working substrate solution (Fig. 4). The assay range and sensitivity of the enzyme using the BDS1000 were similar to the results obtained when measuring the same samples with the Aqualog benchtop fluorometer. This optimum concentration of 0.08 mM was confirmed using Synergy H1Hybrid Multi-Mode Microplate Reader. The linearity obtained for this calibration curve ranged from 0.01 mM to 0.08 mM at approximately 10,000 CFU/mL (Fig. 4).

3.2. *Specificity of MUG assays for the detection of non-target bacteria and the assessment of the enzymatic activity of* E. coli *on non-target substrates*

In this study, the specificity of the MUG assay was examined by using pure and mixed cultures of *E. coli* and non-target bacterial genera such as *Klebsiella*, *Salmonella*, *Enterobacter*, and *Bacillus* (Fig. 5). The fecal coliform (FC) group mainly consisted of *E. coli* and *Klebsiella* [25]. No GUD activities were observed for the non-target bacteria and this finding is in agreement with that of the previous research that "species of *Klebsiella* do not normally express GLUase activity" [8]. Furthermore, studies have been performed on the GUD activities of the Enterobacteriaceae and *E. coli* and confirmed that GUD activity was mostly limited to *E. coli* [26]. In the Enterobacteriaceae genus, only 20–29% of the tested *Salmonella* isolates showed GUD positive activities [11,26–28]. However, no GUD activities were measured for *Salmonella* culture in this study.

In addition, the enzymatic activity of *E. coli* was assessed using non-target substrates, MUGal and LLβ-N, in pure culture experiments [29,30]. The enzyme GAL catalyzes the breakdown of lactose into galactose and glucose has been used mostly for enumerating the coliform group within the Enterobacteriaceae family. Chromogenic substrates such as MUGal were used to detect the presence of GAL produced by coliforms [31]. The results showed that no galactosidic or proteolytic enzyme activities were detected with non-target substrates (Fig. 6). For the non-target bacterial cells, the assays were performed using 0.08 mM of MUG spiked with 100 CFU/mL of each of bacterial genera.

3.3. *Determination of the assay sensitivity in different environmental water samples*

The applicability of the method was tested using environmental and tap water samples. No fluorescence was measured by the enzymatic assays (Fig. 7), since no *E. coli* was present as tested using mEndo plates in the samples. However, other types of bacterial colonies were detected on TSA plates. When both samples were spiked by *E. coli* cells from the same bacterial stock, the tap water sample showed higher fluorescence generation than the environmental water sample. The lower fluorescence signal in the environmental samples could be due to either the inhibition of enzymatic activity by other types of bacteria or other contamination. Studies have shown that in the enzymatic assay, microorganisms other than *E. coli*, such as algae or plants, may contribute to GUD activities; however, their possible interference on enzymatic determination depends on their concentrations [32]. The enzymatic activity was greater when they were present in high numbers or in conditions of low contamination, while the interference became negligible in heavily polluted conditions [32,33].

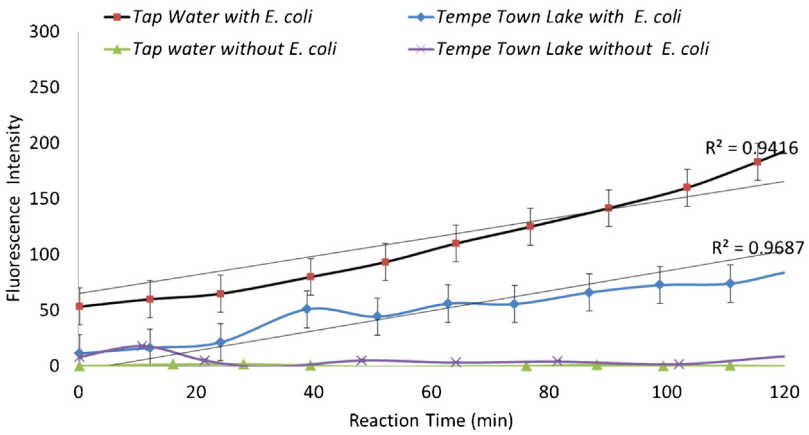

Fig. 7. Applicability of the biosensor for the detection of *E. coli* in environmental samples.

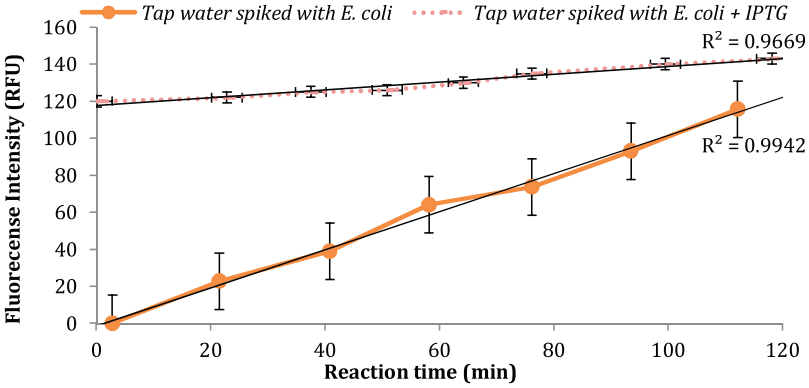

Fig. 8. IPTG Effect on GUD activities in tap water.
Note: all the data points are the average of three replicates of each sample. Spiked *E. coli* concertation was at 100 CFU and IPTG concentration was at 0.5 μM.

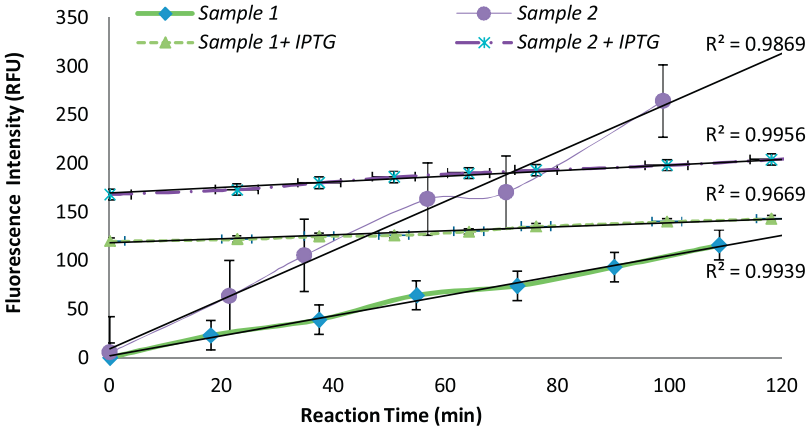

Fig. 9. IPTG effect on GUD activities in environmental samples. Note: all the data points are the average of three replicates of each tested sample.

However, in our study, interference by these compounds would be detected in the blank reading, and thus subtracted from the sample's fluorescence measurements. In addition, the presence of copper at a concentration of 1 ppm in Tempe Town Lake could contribute to the enzymatic reaction inhibition of *E. coli*. Nevertheless, the blank values found in the samples studied were very low, suggesting that no significant interference from non-GUD sources in the water samples existed. The fluorescence measurements carried out on serial dilutions of *E. coli* cultures have shown a sensitivity threshold of less than 10 *E. coli* cells per reaction vial concentrated from 100 mL of water samples. Assays were performed using 0.08 mM of MUG and spiked water samples containing 100 CFU/mL of *E. coli* cells. A distinct signal above background was obtained even at the minimum detection limit, demonstrating the high sensitivity of the BDS1000 that was comparable to the sensitivity of the hand-held fluorescence detector, where the detection limit was less than 10 CFU/mL using river water samples [24]. Moreover, this detection limit and the rapid response of the biosensor should be considered satisfactory to meet the requirement of most monitoring standards for environmental water samples.

3.4. IPTG Effect on tap water and environmental samples

There was no obvious change in the level of GUD activity after the addition of inducer IPTG neither in tap water nor in environmental water samples (Figs. 8 and 9). Conversely, the starting point of GUD activities was higher with the addition of IPTG. The results for adding 0.5 μM IPTG and 1 μM IPTG to the samples were compared and a higher enzymatic activity was observed using 0.5 μM (data not shown), which confirmed previous study results which states that the induction condition for the optimum production of the AtGUS protein was approximately 0.5 μM IPTG [21].

4. Conclusions

In the present study, a rapid procedure has been developed by incorporating biochemical reactions in a fluorescence based biosensor detector. Rapid assays for the detection of *E. coli* were developed by using MUG, which is hydrolyzed by the specific *E. coli* GUD enzyme yielding a quantifiable fluorogenic product that is directly proportional to the number of *E. coli* cells present in water samples. The system is based on monitoring the response of bacterial enzymatic machinery to the added specific fluorogenic substrates.

The data obtained in this study demonstrate that the customized biosensor BDS1000 can function as a useful tool to directly (without processing or concentration steps) analyze the presence of *E. coli* in drinking water samples. Biosensors that are capable of simple and rapid detection of *E. coli* will enable utilities to respond to water quality issues in a timely manner. To the best of our knowledge, enzymatic and physiological processes of *E. coli* have not been investigated to develop biosensors for rapid detection of *E. coli* in water samples. The biosensor in this study can be used independently or in conjunction with other methods as a part of an array of biochemical assays in order to reliably detect *E. coli* in water. In addition, the specific substrate molecules used in the design of this biosensor can be utilized as a platform to monitor bacteria and water quality.

Acknowledgments

Financial support for this work was provided by a grant from the NSF Water & Environmental Technology Center at Arizona State University, Tempe, AZ. The authors would like to acknowledge Olivia Brancati contribution in sample measurements.

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