A Sensitive Voltammetric Biosensor for *Escherichia Coli* Detection Using an Electroactive Substrate for β-D-Glucuronidase

Karen Zuser, Jörg Ettenauer, Karlheinz Kellner, Thomas Posnicek, Giulia Mazza, and Martin Brandl, *Member, IEEE*

***Abstract*— In this paper, we developed a sensitive and simple electrochemical method for the rapid detection of Escherichia coli in water samples. The general principle of the assay utilizes the enzyme β-D-glucuronidase. This enzyme was induced by adding methyl-β-D-glucuronide sodium salt and its activity promoted the cleavage of 8-hydroxyquinoline glucuronide to the electroactive compound 8-hydroxyquinoline. This cleavage product was further oxidized on the working electrode of a potentiostat using cyclic voltammetry. The obtained current output signal in a specific voltage range (400 to 600 mV) indicated enzyme activity and subsequently was an evidence for E. coli cells in the sample. For our experiment, we designed a low-cost potentiostat and show an evaluation of this instrument. First, the β-D-glucuronidase assay was tested with various concentrations of enzyme solutions before living E. coli cells were investigated. Our presented method allowed a clear and a sensitive identification of 1 colony-forming unit of E. coli without any interference from other investigated bacterial strains. Comprising only few working steps (filtration, incubation, and voltammetric analysis), the method allowed incorporation into an automated prototype that delivered results similar to those obtained from samples treated in laboratory.**

***Index Terms*— 8-hydroxyquinoline glucuronide, voltammetry, Escherichia coli detection, electrochemical oxidation, methyl-β-D-glucuronide sodium salt.**

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I. Introduction

ONE of the most important bacteria in our normal intestinal flora is *Escherichia coli*. The majority of these strains is harmless and helps us digest our daily food. However, some serotypes can cause serious illness and even death. Therefore, the identification and quantification of coliform bacteria, especially *E. coli* as a fecal indicator organism, is essential [1]. Scientists from different fields have focused their research on finding a method that can compete with and possibly replace the classic culture method that is still today the reference technique for the enumeration of these bacteria. The conventional cultivation method takes about 18–72 h for the detection and identification of fecal bacteria [2]–[5]. The selective growth of microorganisms on special agar media and the production of gases, acids and other metabolic products are the crucial factors for their verification. The ability of *E. coli* to produce the enzymes β-D-galactosidase (GAL) and β-D-glucuronidase (GUS) has already been used for specific identification in various culture-based methods. Typically, substrates defined for these enzymes are applied, which led to colored or fluorescent cleavage products after hydrolysis that can be detected [6]–[8]. These two enzymes are the primary targets of enzyme-based, immunological, nucleic acid or other molecular techniques [2]–[9]. A wide variety of different methods for the detection of *E. coli* have been established over the last decades. However, each of the methodologies had to face certain disadvantages: a) Either prolonged detection time (more than one day), or b) poor specificity or c) the legal detection limits for *E. coli* bacteria in water samples could not be reached [10]. Compared to other techniques, electrochemical methods proved to be very fast, sensitive, cost-effective, and very user-friendly [11]–[13]. Hence, many researchers are working with such techniques to identify and detect bacteria and especially pathogens (e.g. *E. coli* O157:H7) [14]–[17].

The goal of this study was to develop an *E. coli* detection method that can be integrated into an automated biosensor system for environmental water monitoring. The final stand-alone instrument should be integrated into the public water supply system as well as in private households with domestic wells. There, the biosensor should carry out water

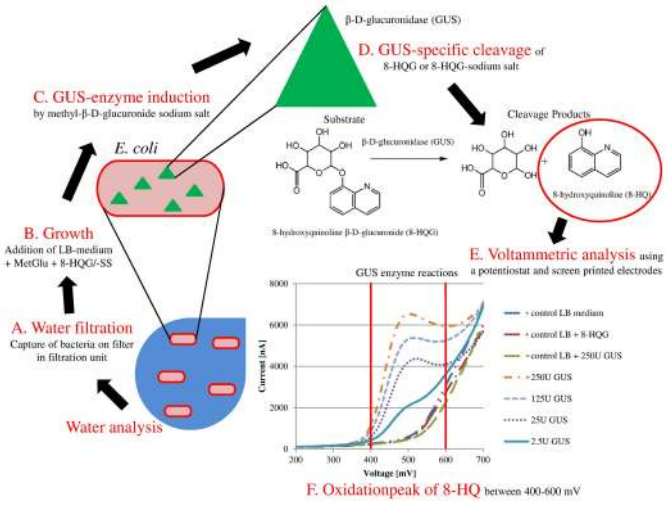

Fig. 1. General procedure of the electrochemical *E. coli* detection method by specific β-D-glucuronidase enzyme activity.

analysis continuously and autonomously, and then transmit the data online to the house owner or those responsible for ensuring and improving the quality of water. Hence, fast counter action can be taken in the event of contaminations. Therefore, the new method had to meet several requirements: a) high sensitivity of the assay to meet the legal requirements for the detection of fecal bacteria [18]–[20], b) short analysis time (less than one day) for continuous and rapid monitoring of water quality, and c) simple procedure with few working steps to allow the test to be incorporated into an automated biosensor system.

Since the β-D-glucuronidase is especially abundant in *E. coli* strains (more than 97% possess GUS) [21]–[23], it is a promising approach to use this enzyme to specifically detect *E. coli* as a fecal indicator. Therefore, we decided to focus on designing an electrochemical method for our biosensor system based on the activity of the enzyme GUS for the specific detection of *Escherichia coli*. We combined this specificity with the high sensitivity of electrochemical measurements and, additionally, included an incubation step to grow the culturable fraction of the *E. coli* bacteria in a sample [24]. Therefore, viable but nonculturable bacteria (VBNC) that are often quantified using molecular methods like PCR remain undetected. We chose 8-hydroxyquinoline-β-D-glucuronide (8-HQG) or 8-hydroxyquinoline-β-D-glucuronide sodium salt (8-HQG-SS), respectively, as substrate due to the electroactive properties of the 8-hydroxyquinoline (8-HQ) cleavage product after GUS mediated hydrolysis (Fig. 1). The substrate used for the GUS enzyme, 8-HQG/-SS, has already been applied in various studies, mainly culture-based, for specific detection of *E. coli* [25]–[27]. Ly *et al.* [28] used mercury-immobilized carbon nanotube paste working electrodes and cost-efficient pencil-rod graphite counter and reference electrodes for cyclic voltammetry (CV) and square wave stripping voltammetry to detect *E. coli*. Kim and Han [29] applied the basic principle of the microbial fuel cell, where a microbial fuel cell was used as an *E. coli* detection unit. Enzymes expressed in *E. coli*–β-D-galactosidase (GAL) and β-D-glucuronidase–were exploited as biological detection elements.

In this study, we report a novel protocol incorporated into a biosensor prototype for the voltammetric detection of *E. coli*. Certain parts of the presented data are pre-published in [30]–[33]. Here, we show in detail a) the design, construction and evaluation of a new, sensitive potentiostat, b) the proof of principle of the proposed methodology with GUS enzyme solutions in different concentrations, c) followed by its verification and optimization with living *E. coli* cells (e.g. incubation temperature, substrate concentrations, specificity and cross-reactivity with other bacteria, detection of low concentrations), d) the application of the assay to filtered water samples spiked with *E. coli* bacteria, and e) water sample analysis with a self-constructed, automatic prototype and comparison of obtained results to samples treated in laboratory.

Our assay is based on the oxidation of *E. coli*-mediated cleavage products on the working electrode of a potentiostat using CV. The increase in current of the output signal in a specific voltage range (400 to 600 mV) is the result of the GUS enzyme activity of the cells, which then indicates the presence of *E. coli* bacteria in the sample. We designed a very reliable experimental set up presented in Fig. 1: For water analysis the first step of this method was the filtration of a water sample and collection of bacteria on a filter membrane (A). By adding Luria broth medium to the filter unit the *E. coli* bacteria started growing (B). The production of the enzyme GUS was induced by adding methyl-β-D-glucuronide sodium salt (MetGlu) to the growth medium. Together with MetGlu, the substrate 8-HQG/-SS was provided in LB for the produced GUS enzyme (B). The synthesized GUS enzyme (C) specifically split off the glucuronide residue from this substrate (D). The resulting electroactive cleavage product 8-HQ could be voltammetrically detected using screen printing electrodes connected to a potentiostat (E). The oxidation on an electrode led to an increase in a specific output current range (F). To compare the different samples, this representative current range was defined where the peak of oxidized cleavage product (8-HQ) occurred, between 400-600 mV respectively. The mean values of the measured current values within this interval were then calculated and displayed graphically. By setting a minimum threshold, we were able to successfully identify *E. coli* bacteria in all tested samples.

II. Materials and Methods

A. Electrochemical Instrument - "EcoStat" Potentiostat

A typical potentiostat consists of a 3-electrode electrochemical measurement system, a difference amplifier (operational amplifier) and a source generator that controls the current or voltage applied to the electrochemical cell. The diagram of a potentiostat circuit is delineated in Fig. 2. The current flow through the electrochemical cell via the counter electrode (CE) and the working electrode (WE) is controlled by the output of the operational amplifier. The cell current can be calculated from the voltage drop via a resistor, Rm. The reference or reference electrode (REF) is used to measure the electrode potential of the working electrode. The potential measurement is carried out with high resistance and, therefore, with very

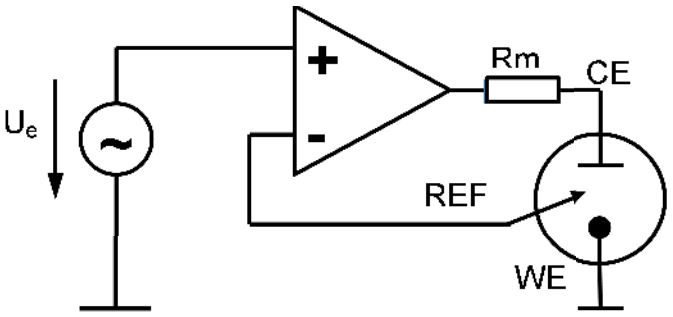

Fig. 2. Diagram of a potentiostat with electrochemical cell.

low currents. In order to keep the ohmic voltage drop low, the reference electrode is placed as close as possible to the working electrode. The normal hydrogen electrode can be used as a reference electrode. However, in most cases, electrodes characterized by a simple structure and a fast adjustment, a potential for equilibrium that is rapidly constant over time and reproducible, are used [34]. Here, cations of the electrode metal form with anions of the electrolyte a sparingly soluble compound. An example is the silver-silver chloride electrode, which typically consists of a silver wire coated with silver chloride immersed in a potassium chloride solution [34], [35]. Nowadays, screen printed electrodes are mainly used for electrochemical measurements, where the electrodes are printed directly on a semi-flexible substrate. The CE and the WE are printed as gold electrodes and the reference electrode as a silver-silver chloride electrode [36]. These types of measuring cells can be produced at low costs and provide stable and reproducible results.

B. Design of "EcoStat"

Most of the potentiostats available on market are costly and bulky devices, which cannot be integrated in a small handheld sensor. Our objective was to detect analytes in environmental samples and quantify them by electrochemical redox reactions on screen printed electrodes. For this purpose we designed and built a small, low cost and highly integrable potentiostat called "EcoStat" for CV measurements. The prefix "Eco" is related to its application for the detection of *Escherichia coli* in drinking water, while the suffix "Stat" is derived from the term "potentiostat" [31].

In a potentiostat the electrode potential of the WE can be adjusted to a constant value relative to a reference. For this purpose, a control circuit compares the measured voltage between WE and REF (actual voltage) with a predetermined desired voltage. The system adjusts the values accordingly by changing the current flowing through the cell. If the electrode potential has to be changed over time (e.g. CV), the desired voltage profiles are determined in advance by a function generator/DAC. Parallel to the potential regulation, the potentiostat measures the current flowing through the electrochemical cell. The control voltage DAC_out is usually supplied by the signal generator/microcontroller. For our applications, where CV is used, a triangular shaped signal (Ue) can be generated for measurements. For high signal accuracy at the reference electrode,

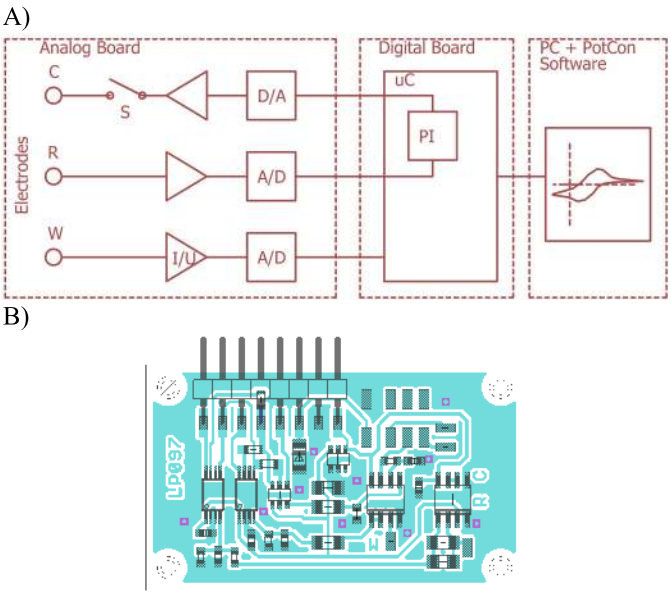

Fig. 3. A) Block diagram of the EcoStat device. B) PCB board [31].

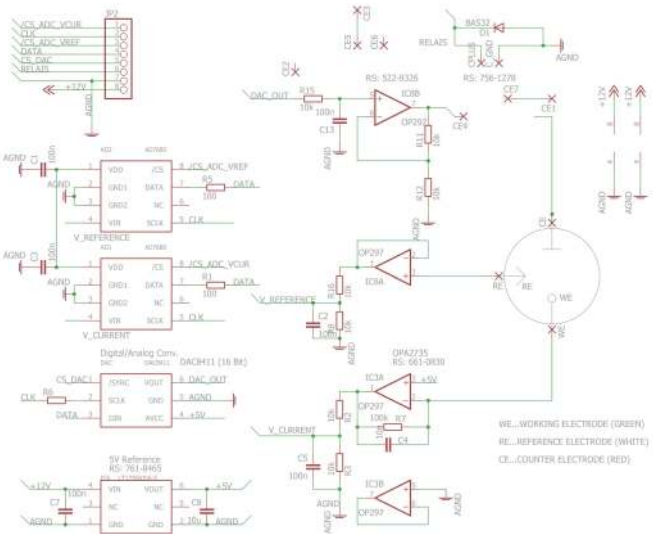

Fig. 4. Circuit diagram of the analog board [31].

a configurable digital proportional-integral (PI) -controller is used and implemented in the microcontroller's software. This is an advantage in signal to noise ratio compared to typically used analog proportional (P)–controllers [37], [38].

The main components of the potentiostat are the precision operational amplifier OP297 (Analog Devices, USA) and the microcontroller ATMEGA328 (Atmel Corp. USA) for signal processing. The signal generation and signal conversion is done by an external high resolution 16 bit digital to analog converter (DAC8411, Texas Instruments, USA) and by two 16 bit analog to digital converters (AD7680, Analog Devices, USA), sketched in Fig. 3 and Fig. 4.

EcoStat's performance and accuracy was evaluated and compared with the "CheapStat" [39] open-source potentiostat and two high-end devices: the VersaStat4 (Princeton Applied Research, USA) and the Reference 600 (Gamry Instruments

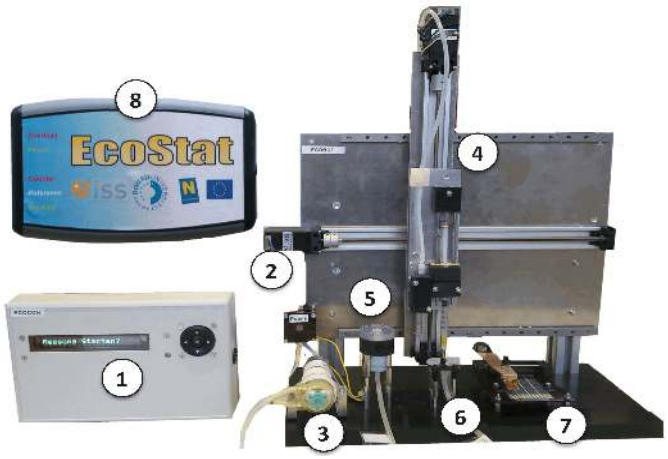

Fig. 5. Modular concept of the biosensor prototype: EcoCon (1), EcoBot (2-7) and EcoStat (8) [33].

Inc., USA). The CheapStat device used for comparative tests was rebuilt by our working group according to the instructions, schematics, layouts and software from the CheapStat homepage. On the commercial instruments, all software based filters have been disabled, while hardware based filters that could not be switched off, can still have some influence on the measurement data. For the evaluation of the four potentiostats, we carried out two technical experiments with two different dummy cells: a) with an impedance of 10 MΩ and b) with 1 kΩ parallel 1 μF [31]. Furthermore, the four instruments were compared by measuring a ferricyanide solution (100 μL of 0.5 mM ferricyanide solution, pH 7.5) with SPE [32]. All data were analyzed graphically in Microsoft Excel to compare the four devices.

C. Electrochemical Measurements: Electrodes and Settings

For the electrochemical analysis, screen-printed electrodes (BE2050824D1; Fig. 6B) [40] were purchased from Gwent Electronic Materials Ltd. (United Kingdom). They are particularly suitable for electrochemical sensors and biosensors associated with enzymes. The each 10×50 mm (W $\times$ L) electrodes in size are arranged in arrays of four sensors printed per substrate (laser scribed alumina, polyester, PVC, Valox FR1). Each of them consisting of a disc-shaped working electrode (2 mm diameter) and a ¾-ring-shaped counter electrode in the ratio 1:4. The rectangular reference electrode has a size of 0.5 $\times$ 1 mm. The screen printed electrodes are made of carbon/graphite paste for working and counter electrodes in combination with Ag/AgCl reference electrodes and can work with small sample volumes (25-100 μL). CV was used for all experiments using a potential ranging from 0 to 800 mV with a step size of 1 mV and a scan speed of 50 mV/s. All measurements were performed at room temperature with a working volume of 100 μL that ensured complete coverage of all three electrode areas. To avoid inconsistencies of results due to electrode fouling and to guarantee the reproducibility, for each measurement a new disposable screen-printed electrode was used. Electrochemical measurements were performed using the Gamry Reference 600+ (Gamry Instruments, USA) as well as the self-designed potentiostat, EcoStat, with the appropriate software, PotCon.

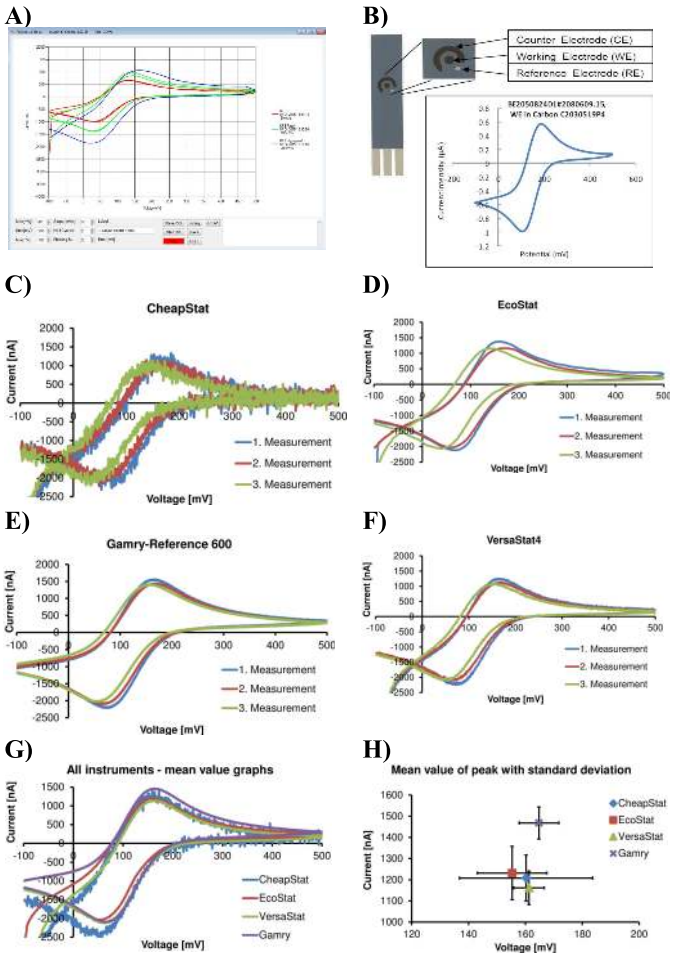

Fig. 6. A) Screen-shot of the PC-User interface "PotCon" with measurements of a 0.5 mM ferricyanide solution (pH 7.5). B) Extract from the datasheet of the SPE with the standard curve of 0.5 mM ferricyanide solution (pH 7.5) [28]. CV graphs of a ferricyanide solution analyzed with the four potentiostats: C) CheapStat, D) EcoStat, E) Gamry Reference 600, and F) VersaStat4. Triplicate measurements are shown for each used instrument. G) Summarized voltammogram of the mean values of the triplicate measurements of every potentiostat. H) Mean values with standard deviations of the peaks of the curves of the triplicate measurements from G) [31], [32].

D. Chemical Reagents and Growth Media

For this study, commercially available β-D-glucuronidase enzyme from *Escherichia coli* (GUS; EC 3.2.1.31), β-D-galactosidase from *Escherichia coli* (GAL, EC 3.2.1.23), methyl β-D-glucuronide sodium salt (MetGlu), 8-hydroxy quinoline glucuronide (8-HQG) and 8-hydroxyquinoline glucuronide sodium salt (8-HQG-SS) were ordered from Sigma-Aldrich (Austria). Luria broth (LB) and LB agar were obtained from Carl Roth (Germany). All media and plastic articles used were sterilized by autoclaving for 21 min at 121 °C.

E. Bacterial Cultivation and Cell Enumeration

For all experiments, *E. coli* strain ATCC 11303 obtained from the American Type Culture Collection, ATCC (USA) was used to test the designed assay. In addition, the following

strains from ATCC were applied as negative controls but also to study the method's cross reactivity, respectively: *Bacillus atrophaeus* (ATCC 9372), *Brevundimonas diminuta* (ATCC 19146), *Citrobacter freundii* (ATCC 8090), *Pseudomonas putida* (ATCC 49128), and *Pseudomonas stutzeri* (ATCC 17588). The *E. coli* strain was grown overnight at 37 °C under stirring at 190 rpm (mrc LM-5902 incubator, United Kingdom) in liquid LB medium. All other strains were incubated according to the supplier's instructions in liquid LB medium at 30 °C or 37 °C, respectively. Bacterial growth of the cultures was monitored using spectrophotometric measurements of the optical density at 600 nm (Ultrospec 3300 pro, GE Healthcare Life Sciences, Austria).

For the detection of very low cell counts, overnight stock cultures were first diluted to an OD of 0.5, and then serial diluted in LB medium (in 10-fold steps). In order to determine the number of viable cells, 100 μL of dilutions (10^{-5} to 10^{-9} dilutions of the stock culture) were plated on solid LB agar plates in triplicate. After overnight incubation at 37 °C, the grown colonies were counted to calculate colony-forming units (CFU) per milliliter. These data were compared to cell counts calculated from measured OD values of the stock cultures.

In each reaction, a positive and at least one negative control were included to verify the success of the experiments and exclude the possibility of false-negative results and contaminations.

F. Electrochemical Verification of Enzymatic 8-HQG Cleavage

As a preliminary test prior to application of the assay to living *E. coli* cells, the ability of the β-D-glucuronidase enzyme to cleave the specific substrate 8-hydroxyquinoline glucuronide was studied [30]. Therefore, different concentrations of enzyme ranging from 2.5, 25 and 125 units were tested. 8-HQG (1 mM) was added to the enzymatic solution (500 μL total volume), briefly vortexed and incubated up to 2 h at 37 °C without agitation in microcentrifuge tubes. The substrate has also been tested with the β-D-galactosidase enzyme (60 U/mL). After 15, 30, 45 and 120 minutes, 100 μL aliquots of each sample were analyzed using the EcoStat potentiostat. Additionally, the following controls were included in the experiment where no enzyme, substrate or both were analyzed. 8-HQ was then oxidized on the working electrode of the SPE. Furthermore, the enzymatic reaction of GUS with 8-HQG and 8-HQG-SS, respectively, as substrates were compared and electrochemically analyzed. To compare the different samples, a representative current range at which the peak of the oxidized cleavage product (8-HQ) occurred, was defined in the range of 400 to 600 mV. The average values of the measured data in this definition range were then calculated and displayed graphically using MS Excel.

G. Voltammetric Detection of E. coli and Temperature Evaluation

After verification of the general principle of the assay by testing a simple enzymatic solution, the developed method was tested using live *E. coli* cells [30]. Therefore, aliquots (450 μL) of overnight cultures of *E. coli* and *C. freundii*, as negative control, were supplemented with 7.9 mM methyl-β-D-glucuronide sodium salt (MetGlu) to induce production of the enzyme GUS. Furthermore, the overnight cultures (OD 0.5) were serial diluted (10-fold steps); *E. coli* and *C. freundii* 10^{-5} dilutions were mixed with MetGlu (7.9 mM) and included in the assay. Substrate 8-HQG (1 mM) was added to all samples to be cleaved by the produced GUS enzyme. The 8-HQG enriched LB medium was included as a negative control. All samples were incubated for 4 h at 37 °C and 44.5 °C, respectively. Every hour, 100 μL aliquots were drawn from the samples for voltammetric measurements. CV analysis was performed as described above. Data analysis and graphical visualization of the current output values were carried out using MS Excel by calculating the mean values for the potential range from 400 to 600 mV.

H. Detection of Low E. coli Concentrations

To detect very low concentrations of *E. coli*, a dilution series of an overnight culture (OD 0.5) was prepared [30]. Dilutions 10^{-7}–10^{-9} were used for the experiment. Each dilution used (in triplicate) was supplemented by the enzyme inducer (MetGlu; 7.9 mM) and the GUS enzyme substrate (8-HQG-SS; 3 mM). After 7 h of incubation (44.5 °C), the first voltammetric measurements were performed to determine the current output of the cleavage product (8-HQ) from the samples. Subsequently, a CV analysis was conducted every hour, up to a total of 10 h of incubation. LB medium supplemented with MetGlu and 8-HQG-SS was included as negative control. *E. coli* with the added enzyme inducer was a second negative control. In addition, the stock solution of *E. coli* mixed with MetGlu and 8-HQG-SS served as a positive control. Voltammetric measurements were done as described previously. The mean output current of the tested dilutions containing oxidized cleavage product after different time points was calculated for the range of 400 to 600 mV using MS Excel. Additionally, mean values were calculated for the triplicates of each dilution for each measurement point with the corresponding standard deviation and displayed graphically using MS Excel.

I. Cross Reactivity With Other Bacterial Strains

In order to validate the designed assay for cross reactivity and false-positive results, various bacterial strains (*Bacillus atrophaeus*, *Brevundimonas diminuta*, *Citrobacter freundii*, *Pseudomonas putida*, and *Pseudomonas stutzeri*) were tested [30]. For this purpose, fresh cultures of each strain were incubated overnight and then diluted to an optical density of 0.5. Thereafter, aliquots of the diluted stock cultures (OD 0.5; 450 μL) were mixed with the enzyme-inducer (MetGlu; 7.9 mM) and the GUS substrate (8-HQG-SS; 3 mM). In addition, mixtures of bacterial strains, in each equal proportion, with and without *E. coli* were prepared. After 7 h of incubation (44.5 °C), the first voltammetric measurements were performed. Subsequently, voltammetric analysis was done every hour (7, 8, 9 and 10 h) up to 10 h of incubation. The same positive and negative controls were included, as mentioned above.

CV analysis and data processing were done as described previously.

J. Water Filtration and Automated Laboratory Prototype

We tried to combine the enzyme assay with the membrane filtration of water samples. As for the 'detection of low *E. coli* concentrations', different dilutions (10^{-7}-10^{-9}, corresponding to 420, 47 and 5 CFU per mL, in triplicates) of an overnight culture (OD 0.5) were added to spike 100 mL water samples. Therefore, Swinnex filter holder with 0.22 μm Isopore polycarbonate membrane filter (both 13 mm; Merck Millipore, Germany) were used to capture the *E. coli* bacteria. By using a peristaltic pump the whole sample was sucked through the filter unit. Afterwards, 200 μL of LB medium supplemented with 7.9 mM MetGlu and 3 mM 8-HQG-SS were added on top of the Isopore membrane in the filtration unit. The in- and outlet of the filter holder were sealed with screw caps and afterwards incubated at 44.5 °C. For performing voltammetric measurements, 100 μL aliquots were withdrawn from top of the filter holder and pipetted onto the SPE. Included positive and negative controls as well as CV and data analysis were performed as stated above.

Subsequently, a laboratory prototype was developed to integrate the methodology described above and every manual working step performed in the laboratory into an automated detection system. An overview of the modular device is shown in Fig. 5 [33]. Briefly, the stage controller EcoCon (1) allows adjustment and regulation of each working step of the whole device. EcoBot represents a robotic apparatus consisting of a pressure sensor (2), a peristaltic water and air pump (3), Z-head with water nozzle and pipette (4), a filter unit with polycarbonate filter and a surrounding heat incubator (5), reservoirs with growth medium and rinsing solution (6), and screen printed electrodes (7). Finally, the USB potentiostat EcoStat is connected with the SPE and performs CV measurements. In order to perform the water analysis, the head with its water nozzle docked to the inlet of a filter holder. The water sample was sucked through the filter with a peristaltic pump and *E. coli* bacteria were collected on the filter membrane. Then, the head pipetted 200 μL of growth medium with MetGlu (7.9 mM) and 8-HQG-SS (3 mM) from a reservoir and transferred it to the filter holder. The filter unit was kept at a temperature of 44.5 °C to enable optimal *E. coli* growth and enzyme production. After incubation, the pipette transferred 100 μL of solution from the filter unit to the working electrode of a SPE. 8-HQ was oxidized by EcoStat using CV to an output signal in the current range of 400-600 mV. After the analysis, an automatic cleaning step was carried out by pumping the rinsing solution through the device. In a general check, the functionality of every step of the developed device was assessed. Furthermore, water samples spiked with *E. coli* cells (40 CFU) were analyzed with this biosensor and the results compared with same samples manually treated in triplicates in the laboratory.

III. Results

A. Ecostat Potentiostat

The developed EcoStat unit is able to transmit recorded and unfiltered measurement data to the PC in real time. In addition, all data can be exported and saved as.png,.csv or in the proprietary .pot format. The PotCon software allows an easy filtering of measurement data (e.g. by a floating mean filter), even on reloaded .pot files as shown in a screenshot image of the graphical user interface in Fig. 6A.

All measurement results with the four potentiostats of a ferricyanide solution are shown in Fig. 6. For CV measurement, the source voltage was triangular in cyclic form ranging from −100 mV to +500 mV with a slope of 50 mV/s. EcoStat allowed us to obtain similar results to the measurements presented in the BE2050824D1 electrode datasheet [40] (Fig. 6B). With our self-made EcoStat we were able to obtain the typical duck shape curve with similar current values compared to the other instruments (Fig. 6C-H). The three measurements showed slight deviations, but compared to the CheapStat instrument (Fig. 6C) a lower noise level was observed. In general, the two commercial instruments (Fig. 6E and 6F) showed the best results with the smallest deviation between the three measurements and very low noise disturbances [32].

An analog P-controller is incorporated in most potentiostats. However, such a controller is a source for high signal noise due to its broadband feedback [37], [39]. To avoid this inconvenience and for better signal accuracy on the electrode, EcoStat uses a configurable, digital PI controller. To further reduce the noise, the EcoStat instrument is strictly separated into an analog and a digital board. In addition, by increasing the resolution of the analog/digital converters from 12 to 16 bits, the measuring range could be extended and the accuracy improved.

The measurements with all potentiostats evaluated on a resistive and capacitive cell are shown in Fig. 7. Using a 10 MΩ dummy cell (CE-RE = RE-WE: 10MΩ), an ascending straight line must be detected (Fig. 7A), while with the second dummy cell (CE-RE: 1 kΩ, RE-WE: -1 μF) a rectangular shape must be created (Fig. 7B). The slope rate was 50 mV/s for all measurements.

CheapStat device delivered a very high noise floor with the 10 MΩ dummy cell. Hence, an accurate and sensitive detection without additional filtering was not possible. On the contrary, EcoStat and the two high-end potentiostats showed precise results with a very low noise level. With the capacitive dummy cell (1 kΩ-1 μF), the CheapStat instrument exposed the highest noise level of all. The very high noise floor of the CheapStat did not even allow a separation between the different current levels. The EcoStat unit also displayed a high noise level, but the different current levels could be clearly separated [31].

In conclusion, the results showed a very good performance of the high-end potentiostats (VersaStat4 and Reference 600). The self-built EcoStat allowed to obtain highly satisfactory measurement data with good accuracy and reduced signal noise compared to the CheapStat open source device. The EcoStat device has some improvements over other cheap and inexpensive devices such as CheapStat: the output signal is more stable due to a digital PI controller, a 16 bit resolution of the converter leads to a wider measuring range and improved accuracy; data acquisition, display and filtering are carried out via a user-friendly and easy-to-use PC interface called PotCon.

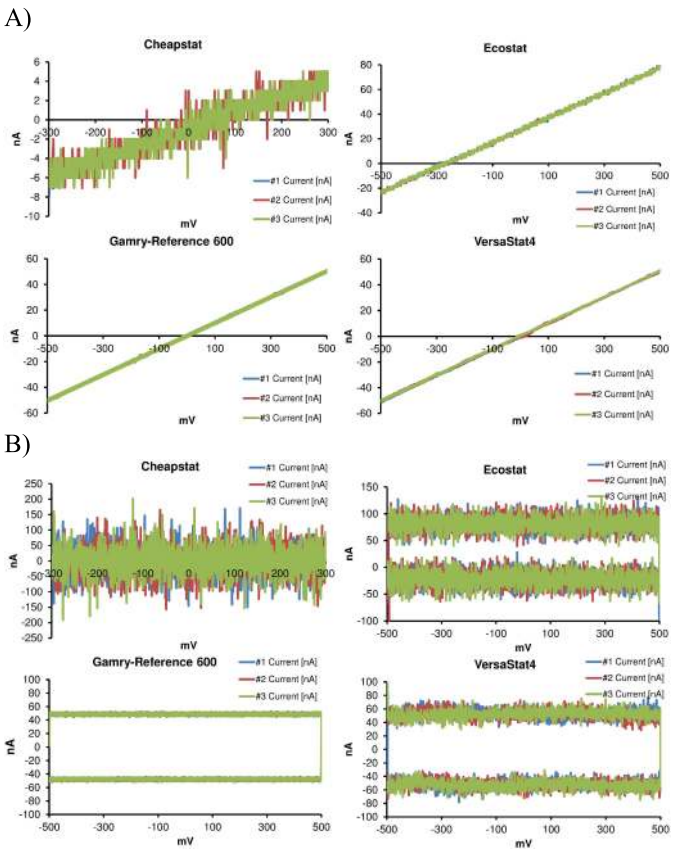

Fig. 7. Results of triplicate measurements of the dummy cells A) CE-RE = RE-WE = 10 MΩ and B) CE-RE = 1 kΩ and RE-WE = −1 μF with the four different potentiostats [31].

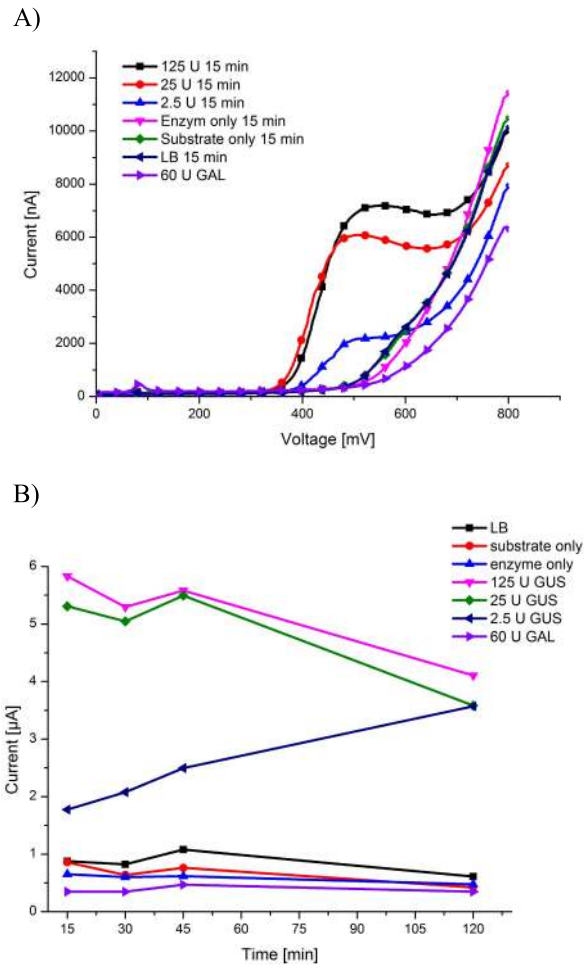

Fig. 8. Enzymatic cleavage of 8-HQG. A) Voltammogram of the oxidized cleavage product (8-HQ) of the substrate 8-HQG through the enzyme β-D-glucuronidase after 15 min. B) Mean output current [μA] of 8-HQ oxidation after 15, 30, 45 and 120 min. Different concentrations of GUS enzyme (2.5, 25 and 125 U) and the non-specific cleavage through 60 U β-D-galactosidase (GAL) were evaluated. Negative controls where no enzyme, substrate, or both (no enzyme and no substrate, only LB medium) were analyzed, respectively [30].

The data is transferred to the PC in real time, can be exported or saved in different file formats and loaded into the software for further processing and filtering of the signal. As EcoStat performed very well compared to other instruments, it is a cost-effective and accurate alternative to carry out electrochemical analyses for environmental applications such as the detection of *E. coli* in water samples.

B. Gus Enzyme Mediated 8-HQG Cleavage

Examination of 8-HQG cleavage by GUS and the further oxidation of 8-HQ on SPE were tested with various concentrations of enzyme substrate mixtures [30]. The general voltammogram of the oxidized 8-HQ showed an s-shaped signal with a peak around 400 mV, increasing to 600 mV and gradually leveling off thereafter (Fig. 8A). The maximum current was obtained in an applied potential range of approximately 480 to 550 mV. After 15 minutes of incubation, a strong signal could be yielded from the 125 U sample (Fig. 8A) with a maximum peak of about 5.8 μA. Voltammetric measurements of the 25 U sample showed the same curve shape with a slightly lower peak (5.3 μA). The sample containing only 2.5 U of GUS enzyme delivered the lowest signal after 15 minutes of incubation with a peak at 1.8 μA.

After 30 minutes incubation, the signal for the 125 U sample showed a slightly lowered maximum peak (5.3 μA), while the enzyme solution 25 U gave a signal similar to that observed after 15 minutes (5.1 μA). The 2.5 U sample yielded a maximum increase of 2.1 μA. After an additional 15 minutes of incubation (45 minutes), the oxidized 8-HQ signal increased for all samples. The 125 U sample showed a maximum output current of approximately 5.5 μA, and the 25 U sample delivered a raised peak of 5.4 μA. From the lowest concentrated sample (2.5 U) an ascending signal was measured with a maximum peak at 2.5 μA. After 2 h of incubation, very similar curves were obtained for all the samples tested. The signal of the 125 U and 25 U samples decreased compared to the current outputs measured after 45 minutes of incubation, with peak maxima of 4.1 μA and 3.6 μA, respectively. The current curve of the 2.5 U sample was converging towards the form of the higher concentrated samples and showed a peak maximum at 3.6 μA. The mixture of β-D-galactosidase with the substrate 8-HQG did not show a signal, and no peak at 400–600 mV could be detected. This sample yielded a baseline signal of about 0.5 μA, which

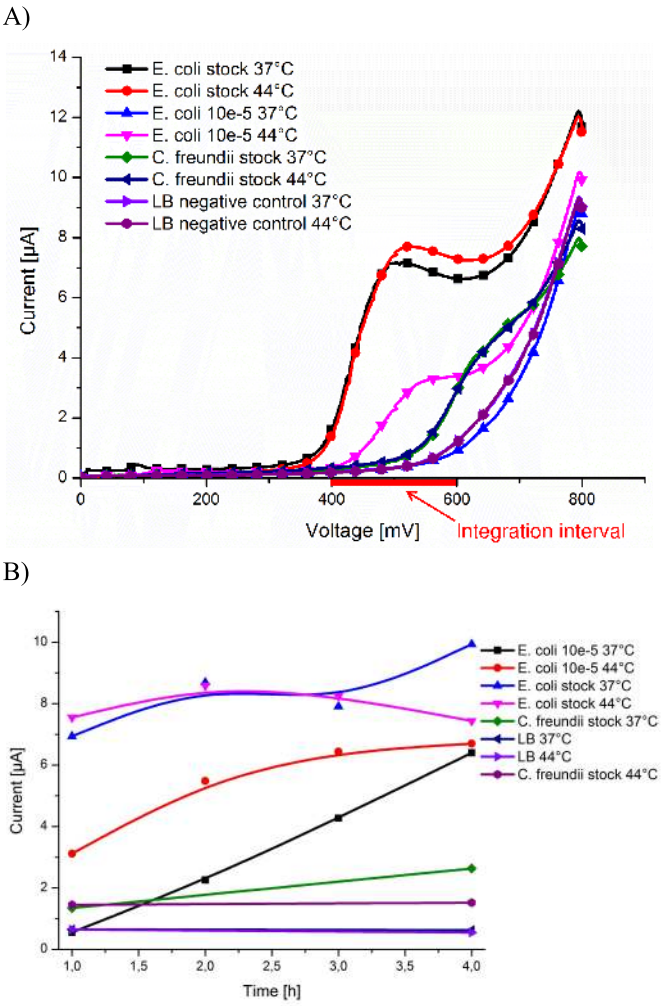

Fig. 9. Bacterial mediated cleavage of 8-HQG. Voltammograms of *E. coli* and *C. freundii* samples (each stock cultures and 10^{-5} dilutions, respectively) after 1 hour (A) and for the entire 4-hour measurement period (B). A) After 1 h, the *E. coli* stock cultures showed typical s-shaped voltammograms with a current output signal between 400 and 600 mV. B) The mean values of the current signal [μA] in the integration interval are displayed for all samples over the entire measuring time range (1-4 h; [30]).

was comparable to the negative control signal. The controls, in which the LB medium alone, the LB medium mixed with the substrate (8-HQG), and LB supplemented with the GUS enzyme showed no current peaks (base current of 0.5-1.0 μA). Additionally, 8-HQG and 8-HQG-SS as substrates for GUS were compared. The results showed that 8-HQG-SS revealed about 1 μA higher signal peaks than 8-HQG. A further evaluation of different substrate concentration of 8-HQG-SS ranging from 1 mM to 9 mM showed that 3 mM was the optimal amount (data not shown). Based on these results, further experiments with this substrate were continued.

C. E. coli Detection and Incubation Temperatures

After the general evaluation of the assay with the enzyme solution, live *E. coli* cells were investigated [30]. Therefore, *E. coli* and *C. freundii*, each at two different concentrations, were incubated with MetGlu as the inducer and 8-HQG as the specific substrate for GUS. After 1 h of incubation, a clear peak, starting at about 400 mV, evolved with the *E. coli* sample (overnight stock culture) incubated at 37 °C (Fig. 9A) with a maximum between 480–530 mV (7.1 μA). The stock culture of *E. coli* incubated at 44.5 °C showed a slightly higher signal, approximately 7.8 μA. The 10^{-5} dilutions of ATCC strain 11303 growing at 44.5 °C showed a peak after 1 h of incubation with a peak top at 3.2 μA, while the incubated dilution at 37 °C showed no increase in current at this time. The LB negative controls, incubated at 37 °C and 44.5 °C, respectively, both did not yield a detectable signal and nor a peak could be observed. A baseline current of 0.5 μA was obtained. From the cultures of *Citrobacter freundii* (overnight stock cultures at 37 °C and 44.5 °C), a signal could be obtained after 1 h—from about 550 mV to 700 mV with an average maximum of 1.5 μA (Fig. 9B). However, the measured output current did not give rise to a distinct peak such as that observed with the *E. coli* samples (Fig. 9A and 9B). After 2 and 3 h incubation (Fig. 9B) the undiluted cultures of *E. coli* showed high peaks, near 8.5 μA after 2 h and 3 h (37 °C sample) and a declining signal after 3 h for the incubated sample at 44.5 °C (approximately 8.0 μA). Signals from the diluted *E. coli* samples increased gradually, showing peak maxima of 5.5 μA (2 h) and 6.4 μA (3 h) for the sample at 44.5 °C, and maxima of 2.3 μA (2 h) and 4.2 μA (3 h) for the diluted sample at 37 °C, respectively (Fig. 9B).

The overnight culture of *E. coli* (37 °C) increased further to a peak height over 10.0 μA, while the bacterial stock signal incubated at 44.5 °C further decreased below 7.5 μA. The diluted sample of *E. coli* (37 °C) converged toward the peak of the diluted sample at 44.5 °C after 4 h of incubation at approximately 6.5 μA. Negative controls of LB medium cultured at 37 °C and 44.5 °C did not show a signal (baseline current at 0.8 μV). Peaks of *C. freundii* samples increased after an additional three hours of incubation. However, the location of the peak was the same as that already observed after 1 h—about 550 to 700 mV. The *C. freundii* sample grown at 37 °C (2.8 μA) yielded a higher signal than those at 44.5 °C (1.8 μA). Due to the high signals generated by the incubation of *E. coli* samples at 44.5 °C, all subsequent experiments were carried out at this temperature. In an additional experiment with equal amounts of bacteria, the concentration of the inducer MetGlu was varied from 3.9 mM to 35.2 mM. Results showed that the GUS production was best stimulated by adding 7.9 mM MetGlu (data not shown).

D. Detection of Low E. coli Concentrations

For the detection of low concentrations of *E. coli*, several samples were prepared by diluting an overnight grown culture [30]. Dilutions 10^{-7}-10^{-9} (corresponding to 313, 10 and 1 CFU per mL) were mixed with the enzyme inducer and the GUS enzyme substrate. After 7 h of incubation, the first voltammetric measurements were carried out. The positive control, an undiluted overnight culture of *E. coli*, delivered from the beginning a very high output current with a signal maximum of more than 6.0 μA (Fig. 10). All triplicates of the 10^{-7} dilution (313 cells) of *E. coli* ATCC 11303 showed an initial current increase at approximately 400 mV, but no

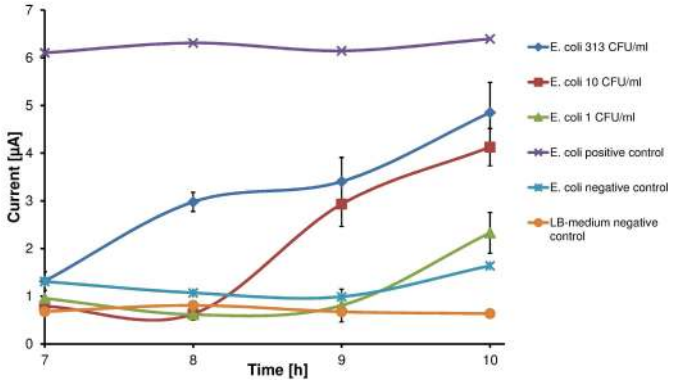

Fig. 10. Detection of low cell counts. Displayed mean values (400-600 mV) and standard deviation of triplicates of measured output current [μA] from different concentrations of *E. coli* (313, 10 and 1 CFU/mL) after 7, 8, 9 and 10 h incubation. The LB medium (with MetGlu and 8-HQG) and *E. coli* (with enzyme inducer) were included as negative controls. The *E. coli* stock culture mixed with MetGlu and 8-HQG served as positive control [30].

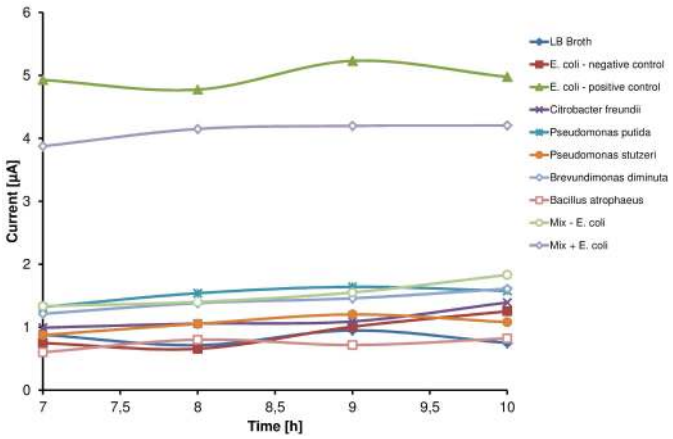

Fig. 11. Evaluation of cross-interactions. Visualized mean values (400-600 mV) of the output current [μA] from *E. coli* (positive control) and other than *E. coli* strains (*Bacillus atrophaeus*, *Brevundimonas diminuta*, *Citrobacter freundii*, *Pseudomonas putida* and *Pseudomonas stutzeri*) after 7, 8, 9 and 10 h. Positive and negative controls are the same as in Fig. 10. The output current values obtained for the non *E. coli* strains were below the signal for 1 CFU/mL of *E. coli* (1.9 μA, Fig. 10; [30]).

distinct signal peak could be detected (Fig. 10). After an additional hour of incubation at 44.5 °C, these samples (10^{-7}) displayed a significant increase in current with mean signals of 2.8 to 3.1 μA. One hour later (9 h incubation), the output from these dilutions increased along with the 10 CFU-samples (10^{-8} dilution) and a visible signal with peak maxima between 2.6 and 3.2 μA was observed. In addition, a current output signal from the most diluted culture (1 CFU/mL; 10^{-9} dilution) began to appear. Finally, after 10 h of incubation, all triplicate *E. coli* specimens with 1 CFU/mL showed a clear measurable signal between 1.9 and 2.8 μA. All negative controls, the LB medium containing substrate and inducer, as well as the culture of *E. coli* supplemented with the inducer, showed no signal between 400 and 600 mV during the measurement period (baseline at 0.8 μA).

E. Cross-Reactivity and False Positive Signals

In order to investigate whether the test design exhibited cross-reactivity with other bacteria and to eliminate the risk of false positive results, five strains other than *E. coli* were examined [30]. The results of the 7-hour voltammetric analysis showed a strong signal for the positive control (4.9 μA), indicating that the GUS enzyme in the bacteria began to cleave the supported substrate 8-HQG to the electroactive compound, 8-HQ (Fig. 11). Such a strong signal could be detected until the end of the measurement time after 10 h for the positive control (4.7–5.2 μA). None of the other strains studied, *Bacillus atrophaeus*, *Brevundimonas diminuta*, *Citrobacter freundii*, *Pseudomonas putida*, and *Pseudomonas stutzeri* emitted an increasing output current signal, and no peaks could be observed for any of the strains tested in the designated 400–600 mV potential range. The average measured current was between 0.7 to 1.6 μA for the entire 10-hour period. As a result, *B. atrophaeus* had the lowest output current, which was similar to the negative control with LB medium (baseline around 0.8 μA). All other tested strains showed higher signals with slightly fluctuating current outputs around 1.0 μA. *B. diminuta* was the only strain that delivered a continuously increasing signal over the entire time range with an increasing output of 1.3 μA after 7 h to 1.7 μA after 10 h, respectively. The measured signal of *C. freundii* also rose from 8 h to 10 h incubation, giving a maximum of 1.3 μA after 10 h. However, all current values detected for the strains studied were lower than the output signal observed for 1 CFU/mL of *E. coli* (1.9 μA). Furthermore, mixtures of the bacteria with and without *E. coli* were tested. The bacterial mixture lacking *E. coli* showed a slightly increasing signal from 1.4 μA after 7 h until 1.8 μA after 10 h incubation. The mixed sample containing *E. coli* bacteria already showed a strong signal after 7 h (3.9 μA). The current output further increased until the end of the measurement period to 4.2 μA.

F. Filtered Water Samples Spiked With E. coli

The results of the above described experiments performed in microcentrifuge tubes indicated that the designed assay could be used to investigate *E. coli* spiked samples. The presented assay was applied for Swinnex filter holders with 0.22 μm polycarbonate filters. The results of the each in triplicate incubated samples are shown in Fig. 12. *E. coli* positive control samples delivered very high signals over the complete measurement time. The obtained mean currents (400–600 mV) of the triplicates oscillated between 3.5 and 4.2 μA between 13 and 17 h incubation. The samples spiked with 420 CFU *E. coli* bacteria already showed a high signal peak (3.7 μA) after 13 h. The lower concentrated samples (47 CFU) delivered a mean current output of 1.9 μA after 13 h. The current signal continuously increased during the following incubation period; 2.7 μA after 15 h to finally 3.4 μA at the end of the measuring time. Water samples spiked with only 5 CFU showed a peak maximum at 1.5 μA after 13 h. The current response further rose over the threshold to 2.2 μA after 15 h and finally to 3.4 μA after 17 h. The LB negative control showed very low current output signals over the whole time period of 17 h (0.9 μA after 13 h till 1.2 μA after 17 h). Similar, a constant mean

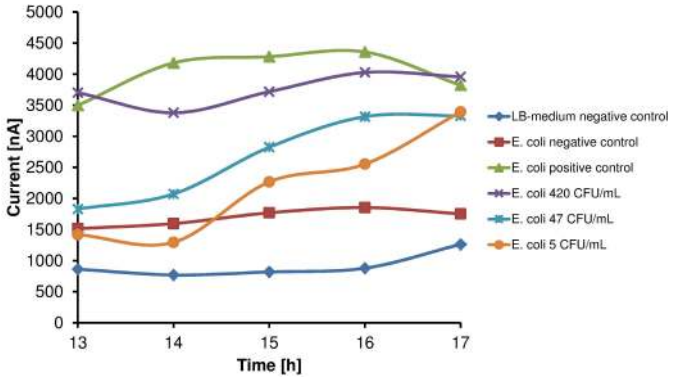

Fig. 12. Detection of low cell counts in filtered samples. Displayed mean values (400-600 mV) and standard deviation of triplicates of measured output current [μA] from different concentrations of *E. coli* (420, 47 and 5 CFU/mL) after 13 to 17 h incubation.

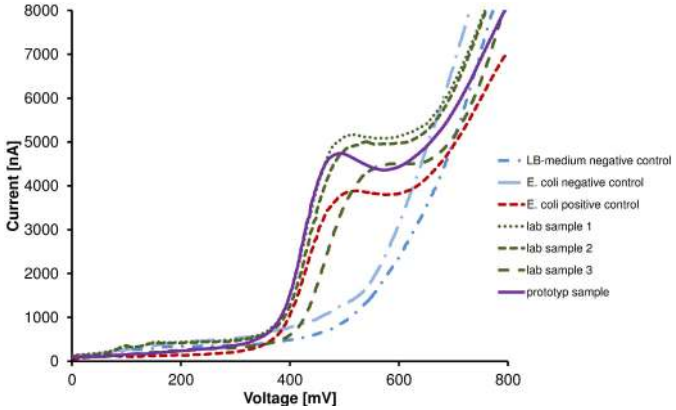

Fig. 13. Measured current output curves obtained from the voltammetric analysis showing an s-shaped signal with an oxidation peak between 400-600 mV for the cleavage product 8-HQ. Approximately 40 CFU per ml were applied for all samples [33].

current signal from the *E. coli* negative control was obtained (1.5 to 1.6 μA).

G. Laboratory Prototype

In the general proof of principle every single step of the instrument was tested for proper execution. The device was tested with 40 CFU of *E. coli* in water samples. Fig. 13 shows the results of the voltammetric measurements of the different samples [33]. The negative controls, LB growth medium supplemented with MetGlu and 8-HQG-SS and *E. coli* with MetGlu, did not provide a peak value in the current output range between 400-600 mV. The signal of the positive control already decreased after 18 h incubation. The filtered samples treated in the laboratory showed peak maxima between 4.5-5.1 μA (green curves in Fig. 13); and the water sample analyzed with the biosensor prototype had a peak maximum of 4.7 μA. A graphical comparison of the mean peak maxima (400-600 mV) is displayed in Fig. 14. The negative controls delivered a signal below the threshold limit of 2 μA that corresponds to 1 CFU. All other spiked water samples showed strong mean current signals between 3-4.4 μA. The signal obtained with the self-built automatic device lay within the standard deviation of the laboratory samples (Fig. 14).

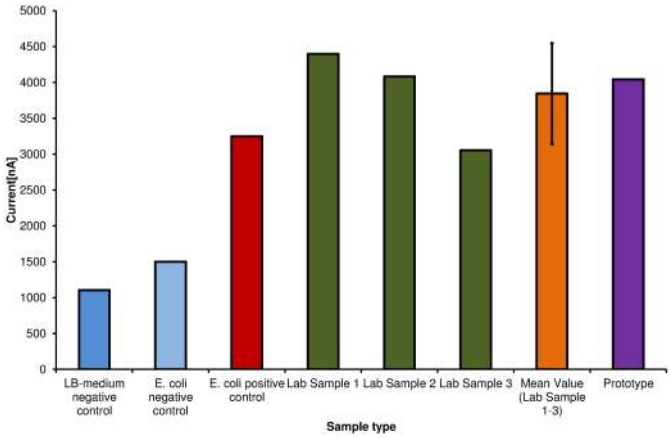

Fig. 14. Mean values of the measured current signal for oxidation voltages between 400-600 mV are shown for all samples. The signal measured with the prototype lay within the standard deviation of the values obtained from the lab samples [33].

These results showed that the designed system enables reliable detection of *E. coli* bacteria. The developed sensor represents a much faster detection method for *E. coli* bacteria than the conventional cultivation technique.

IV. Conclusions

A. Enzymatic Cleavage of 8-HQG/-SS

The voltammetric behavior of 8-hydroxquinoline was studied in great detail by Stević *et al.* [41]. Using CV, the authors investigated the oxidation process using a glassy carbon paste electrode as the working electrode. Their results showed a clear single anodic peak, whose potential changed according to the pH of the supporting electrolyte. In the pH range around 7, a well-defined peak began to appear at 400 mV, sharply increased to 600 mV and then flattened gradually. In our study, we could observe similar findings for the voltammetric detection of the cleaved 8-HQG/-SS substrate using different concentrations of the enzyme solution β-D-glucuronidase (Fig. 8 and Fig. 9). The least concentrated sample (2.5 U) showed a continuous increase during the 120-minute incubation period. Finally, after 2 h, the signal from this sample adjusted to peak maxima of the higher concentrated enzymatic solutions. For the 25 U and 125 U samples, the maximum enzyme activity was already reached after 45 minutes of incubation. No further signal increase could be detected. However, the output current of both samples decreased after 120 minutes, indicating that the supplied 8-HQG substrate became the limiting factor and was depleted. Furthermore, no non-specific cleavage of the 8-HQG substrate through the β-D-galactosidase enzyme has been detected, which is due to the glucuronide residue that cannot be processed by this enzyme (Fig. 8).

B. Voltammetric Detection of E. coli

The application of this simple and rapid method to study living *E. coli* cells has shown that strong signals can be detected. Undiluted overnight cultures yielded high current outputs after

1 h of incubation. The evaluation of two different incubation temperatures, 37 °C and 44.5 °C respectively, showed that the higher incubation temperature was favorable for enzyme production and enzyme activity (Fig. 9). These findings were further confirmed by the results of the measurement of 10^{-5} dilutions of *E. coli* strain ATCC 11303. Here, the sample cultured at 44.5 °C showed a peak after 1 h of incubation, while the dilution of *E. coli* incubated at 37 °C showed no increased current at that time. *Citrobacter freundii* also belongs to the group of coliform bacteria and, like to *E. coli*, has the enzyme GUS. Therefore, from the stock culture used, a signal could be obtained. However, the measured output current did not give rise to a distinct peak such as that observed with the *E. coli* samples (Fig. 9A and 9B). Moreover, for this strain, the different incubation temperatures did not have a strong influence on the measured current. The results showed that the expressed GUS enzyme from *C. freundii* overnight cultures had lower activity than the *E. coli* overnight samples. The different location of the peak may be due to changes in pH during bacterial growth in the LB medium. Growing cells or cells in the stationary growth phase, respectively, caused a change in pH of the medium. A more acidic medium would shift the peak of about 400–600 mV to a higher voltage range, as Stević *et al.* [41] indicate for the study of the oxidative behavior of 8-HQ.

Contrary to *E. coli*, *C. freundii* favored lower incubation temperatures. The sample grown at 37 °C yielded a higher signal than the 44.5 °C sample. These findings promote the specific detection of *E. coli* at higher incubation temperatures (44.5 °C) where the highest signals could be obtained. Our results correlate with those of Kim and Han [29]. In their study, a microbial fuel cell was used to rapidly detect and quantify *E. coli* bacteria. In order to shorten the detection time, the authors studied various temperatures: 24 °C, 32 °C, 36.5 °C and 44.5 °C. The higher temperature allowed faster identification of *E. coli* (10 CFU/mL) and reduced their detection time to less than 11 h. Our results confirm that the best incubation temperature for rapid growth of *E. coli* is 44.5 °C [42]. In addition, expression of the enzyme GUS is twice as high at 44.5 °C compared to 25 °C [42], [43].

C. Detection of Low E. coli Concentrations

In our study, we were able to detect specifically 1 CFU/mL of *E. coli* by its GUS activity within 10 h of incubation. Compared to other studies, where only the enzyme GAL was used to identify *E. coli*, the application of the GUS enzyme significantly increases the specificity of detection. Pérez *et al.* [9] applied an amperometric culture-based method for rapid detection of *E. coli*. The cleavage product of 4-aminophenyl-β-D-galactopyranoside (4-APGal), by GAL-mediated hydrolysis, was oxidized using a flow injection analysis system, which detected 1 CFU/mL after 10 h. A similar strategy has been implemented in other studies [44], [45]. Authors identified 6×10^5 CFU/mL in 2 h, or 1 CFU in 100 mL within 6–8 h, respectively, using bacteriophages for the *E. coli* specific release of the enzyme. The incorporation of bacteriophages could be excluded in our method, which, simplifies the protocol and broadens the detection spectrum in the genus *Escherichia coli* by avoiding the species specificity of bacteriophages. However, the application of a sole GAL-based assay for a distinct detection of *E. coli* might be strongly influenced by the presence of other coliform bacteria with this enzyme, which has not been investigated in depth by the authors. In our study, the non *E. coli* strains tested did not yield a significant signal. The resulting output current values were lower than the signal for 1 CFU/mL *E. coli*, indicating that none of the bacteria tested could use and cleave the 8-HQG to 8-HQ. Nevertheless, other strains will also be evaluated with the described method. In Kim and Han's study [29], the two enzymes, GUS and GAL, were targeted using 4-APGal and 8-HQG, and cleavage products were analyzed by oxidation on the microbial fuel cell. Therefore, the authors were able to obtain a very accurate identification of the fecal indicator organisms, but were unable to reach the detection limits as presented in our study (42 CFU/mL in 560 min compared to 1 CFU/mL in 600 min).

D. Filtered Water Samples and Prototype

The designed assay could also be applied to filtered water samples that were spiked with *E. coli* bacteria. The results showed retardation in detection time of approximately 5 h. This delay might be due to the filtration step. The bacteria in the water sample were sucked onto a 0.22 μm filter membrane by a peristaltic pump. Hence, *E. coli* cells are slightly captured on the membrane and bacterial growth after addition of the growth medium is time-delayed. In comparison, samples incubated in microcentrifuge tubes allow a free movement of the bacteria in the vessels that might enhance the growth. Furthermore, the filter units were incubated without agitation, whereas the microfuge tubes were shaken that also ensures thorough mixing. Rochelet *et al.* [46] developed an amperometric detection method for *E. coli* measuring the GUS activity with disposable carbon sensors. The authors applied p-aminophenyl b-D-glucopyranoside (PAPG) as electrochemical substrate and measured the p-aminophenol release after enzymatic hydrolysis. In their study, after filtration, the membranes were removed from the Swinnex filter holder units and incubated with agitation in polypropylene tubes containing PBS, the substrate PAPG, LB and the nonionic detergent Triton X 100. Using this approach 5×10^4 to 10^8 CFU/membrane were detected within 3 h. Syringe filters were applied for the recovery and pre-concentration of *E. coli* from the water matrix and as mL reactors for lysis and GUS extraction in the study performed by Briciu-Burghina *et al.* [47]. As fluorogenic substrate for the released GUS enzyme 6-chloro-4-methyl-umbelliferylb-D-glucuronide (6-CMUG) was used. Similar to the study of Rochelet *et al.* [46] a step involving bacteria lysis was included. Both authors applied 0.1 to 1% Triton X 100 to lyse or weaken the bacterial wall for a better permeabilization of the supplemented substrates, respectively. Therefore, the ratio of extracellular GUS to intracellular GUS possibly changes and leads to measurably higher enzyme activities [47]. Furthermore, Briciu-Burghina *et al.* [47] investigated the GUS activity after variation of the shaking speed ranging from

0 to 250 rpm. A noticeably high rise in activity was detected from 0 rpm to 50 rpm and 100 rpm, whereas with further increased shaking speeds to 250 rpm a plateau was reached. However, up to 21.8% improvement was observed by the authors. We think that an incorporated sample shaking and a bacteria lysis step could possibly improve our designed method leading to a shortened detection time.

At the present time, standard components have been used in a modular system for the prototype. By using special designs, further optimizations could be realized, e.g.: a) the electrodes for the voltammetric analysis could be integrated into the filter chamber, b) the reservoir for growth media could be mounted directly on the filter, c) the filtration unit with the electrodes and the nutrient reservoirs could be combined in a single disposable component, d) extension of the analyzable sample number, e) realization of multiple CV measurements of the same sample at different time points f) further optimization of the methodology could shorten the detection time (bacteria lysis and sample shaking, [46], [47]), g) miniaturization and construction of a microfluidic device, etc.

The results of our study show that the method developed allows the detection of *E. coli* in a very selective way with high sensitivity. The simple procedure, without using magnetic beads, nanoparticles, modified or functionalized surfaces, antibodies, aptamers, self-assembling layers, etc. like in other studies, and the few working steps allowed a successful integration in an automatic device. In addition, pursuing research has shown very promising results, demonstrating that the designed assay could also be applied to other *E. coli* strains. First positive results were obtained with the *E. coli* strains ATCC 12651, 23226, 15766 and 11775 (data not shown) [48]. Through implementation of the stated improvements our designed assay could displace the so far available biosensor instruments on the market for *E. coli* detection [49]–[54].

Subsequently, in a follow-up project, the developed methodology must be investigated on further laboratory strains as well as on wild-type strains isolated from environmental samples. Through the practical evaluation and further validation of the patented methodology, the designed laboratory prototype can be optimized and miniaturized to facilitate integration into water supply systems for investigation under real life conditions.

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