A Novel Approach to Rapidly and Sensitively Detect and Purify Water Contaminated with *Shigella, E. coli, Salmonella,* and *Cholera*

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Abstract

Waterborne diseases cause 3.4 million deaths annually, concentrated in countries lacking sanitary water. Conventional methods, such as PCR, can be costly, take several days, and have detection limits of up to 1,000 CFUs. Thus, the purpose of this study was to engineer a system that could efficiently detect and purify bacterial presence in a more rapid time frame and a lower detection threshold. Graphene was utilized to create biosensors through the immobilization of enzymes that target analytes released during respiration of *Salmonella, Shigella, Cholera*, and *E. coli*. When these bacteria respire, they produce analytes that, when in contact with the enzymes, produce a varying electric current that can be read by an ammeter to determine the presence of bacteria. The sensors were able to detect the presence of at least 1 CFU of bacteria in 1 L of water instantaneously. A mechanized approach was then taken to purify contaminated water samples through the use of arduino microcontrollers. This system was successful in detecting minute levels of bacteria in a rapid time frame and purifying the water of pathogens.

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III. Key Words

Waterborne diseases, graphene, biosensor, π-π interactions, enzyme immobilization, Arduino, hydroxyl radicals, bacterial contamination

IV. Abbreviations and Acronyms

1-pyrenebutyric acid (PBA), Colony Forming Unit (CFU), *Escherichia coli (E. coli),* CVD (chemical vapor deposition), PCR (polymerase chain reaction), DMSO (dimethyl sulfoxide), hydroxyl radicals (OH$\cdot$),

V. Acknowledgements

VI. Biography

Rachel Chang is a rising senior at Manhasset High School. After Rachel was fortuitously put into her school's science research program by accident, she came to truly love research and hopes to pursue it as a career. Rachel has also been playing violin since she was 4 years old, picking up new instruments along the way. She is the concertmaster of the Metropolitan Youth Orchestra and the school's symphony orchestra, and was fortunate enough to participate in the All-State Symphony Orchestra this past year. Rachel hopes to continue working in the field of Environmental Engineering in order to create novel solutions to environmental problems faced around the world.

Ryan Thorpe will be entering his senior year at Manhasset High School. Ryan first became interested in research in the 8th grade after he visited the science research lab at his school. Since then, Ryan has developed an intense passion for science, and he has researched sleep deprivation, bacterial contamination, and breast cancer development in both a high school and professional lab setting. Outside of science, Ryan is an avid cross country and track runner, musician, and is a founding member of the Manhasset Historical Society. In the future, Ryan wishes to continue his education in the field of biomedical or environmental engineering and hopes to develop sensors as a means of improving medical science and the environment.

Introduction

Waterborne diseases cause 3.4 million deaths annually. Worldwide, there exists 783 million individuals that do not have access to clean, potable water. The consumption and usage of contaminated water is the leading cause of the development of debilitating waterborne diseases such as Cholera, Shigellosis, E. coli, and Salmonella. 319 million of these people do not have access to purification methods in order to eliminate biological contaminants in their water. [4] Yet, despite this severity, the traditional methods used to detect the presence of these bacteria can take 1-2 days, and have a detection limits of up to 1,000 CFUs [3]. As these pathogens have no known cure, a more sensitive, rapid detection device and purification method must be created in order to identify and eliminate microorganism presence in water sources to prevent contamination and the outbreak of pathogenic disease.

A biosensor is a type of analytical device that is capable of detecting a biological component and converting it into an electric signal through an electrode and a transducer. These sensors can detect analytes rapidly, specifically, and sensitively utilizing receptors, nucleic acids, antibodies, or enzymes [7, 8]. In this study, biosensors were constructed using graphene and enzymes in order to detect analytes produced by waterborne pathogens. Graphene is a two-dimensional allotrope of carbon that has a one atom thick hexagonal molecular structure with benzene rings. Due to the unique nature of its structure, electrons are able to flow rapidly throughout the thin material [5]. With this high conductivity and electron mobility, it serves as an ideal electrode for the creation of biosensors. Putzbach's study [14] determined that enzymatic biosensors can be created through the incorporation of nanomaterials. Electrochemical biosensors detect materials through an electric signal that is created by an enzymatic reaction. Putzbach[14] determined that enzymes act as the transducer of the biosensor. Enzymes provide selectivity to the biosensor, which make it the optimal transducer for detection devices. The enzyme layer is then able to catalyze the production of a current that generates a signal.

Enzymes can be used to trigger an electrochemical response from a graphene biosensor to detect the presence of specific analytes released by bacteria during respiration. Utilizing enzymes in order to create electrochemical biosensors has been done in previous studies. For example,

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Labroo [12] constructed a nanosensor in order to detect lactate in human sweat. This was done by transferring a graphene electrode onto a flexible substrate and immobilizing lactate dehydrogenase for lactate onto the electrode. When the lactate came into contact with this enzyme, it was converted into an electrical stimulus that displayed the presence of this biomolecule. [12] Similarly, Ang et. al [2] created an amperometric biosensor in order to measure the glucose content of fruit. Ang's study immobilized glucose oxidase onto a chitosan membrane and attached it onto platinum electrode in order to measure electrical output. This allows the presence of glucose to be monitored on the electrodes.

These methods were utilized in the formation of the biosensors for bacterial analyte detection, as the utilization of oxidase enzymes and graphene electrodes were essential in converting bacterial respiratory products into a detectable current. In this experiment, the enzymes Lactate Oxidase, Glucose Oxidase, Glycerol Oxidase, and Galactose Oxidase were immobilized by utilizing a linker molecule, 1-pyrenebutyric acid (PBA). This acid is a linker molecule that acts as a bridge between the enzyme and the graphene through π-stacking. PBA has an aromatic molecular structure, containing benzene rings that non-covalently stabilize atop the graphene hexagonal rings. These enzymes are immobilized onto the graphene through π-π interactions. π-interactions are a type of non-covalent bond that can exist between π-systems. π-interactions are extremely stable and control a broad spectrum of biological interactions, such as interactions between aromatic molecules and protein structures. [7] π-interactions were analyzed in their applications in enzyme immobilization. π-π interactions occur between 2 aromatic molecules, or 2 molecules that contain hexagonal benzene rings in their molecular structure. The two benzene rings, or the π rings, are able to stack up one another and align themselves to stably bind the molecules together. Due to this property, enzymes can be utilized to engineer enzymatic biosensors to detect various biomolecules. The linker molecule then bonds with the enzyme through an amide bond that occurs between the amine group of the enzyme and the pyrene group of the PBA, a process known as amidation. [10,12] This safely and securely immobilizes the enzymes onto the graphene without damaging either structure. These π-interactions are necessary to allow for the permanent immobilization of enzymes, which act as the transducer of the biosensor for the detection of biomolecules.

Equally as important as the detection of bacteria is the elimination of bacteria from water sources. Hydrogen peroxide and sodium hydroxide can be utilized in order to eliminate organic material in water. When hydrogen peroxide and sodium hydroxide are added to water, the water will experience a raise in pH that contributes to a process known as hydrogen peroxide bleaching, a process by which hydroxyl radicals are formed. These radicals have the potential to degrade lipids and proteins. As bacterial pathogens are made of lipids and proteins, these radicals can eliminate bacterial presence. [11] This would effectively purify water and make it safe for consumption, as the only byproducts of this reaction are carbon dioxide and water.

Engineering Goal

Despite the widespread nature and severity of bacterial contamination in water, there is a lack of sensitive and rapid technology to detect these microorganisms. The purpose and engineering goal of this study was to address the limitations of both the detection and purification of pathogens. This experiment aimed to create various graphene-based biosensors in order to recognize minute levels of specific bacteria in a rapid time frame and fabricate a mechanized purification system to eliminate this bacterial presence.

Materials and Methodology

Phase 1:

A graphene biosensor was created in order to rapidly and sensitively detect bacterial contamination in water by *S. typhi, V. cholerae, E. coli*, and *Shigella* through the isolation of graphene and immobilization of specific enzymes that target the analytes produced by each bacterium.

In this study, *E. coli K12* was used as a model organism for *E. coli* O157:H7*, Enterobacter aerogenes* modelled *Shigella dysenteriae, Sarcina aurantiaca* was used as a model for *Salmonella typhi,* and *Vibrio fischeri* modelled *Vibrio cholerae,* because of the metabolic and respiratory parallels between the organisms. Firstly, both *E. coli* K12 and *E. coli* O157:H7 are gram-negative, rod-shaped, and both perform mixed acid anaerobic and aerobic respiration. It is through respiration that both strains of *E. coli* can generate D-lactate (Forester, 2014). Second, *Sarcina aurantiaca* was used as a model organism for *S. typhimurium* as both organisms have CrsA-monitored respiration pathways by which glucose-6-P is produced [1]. Third, *Vibrio*

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fischeri was used as a model organism for *Vibrio cholerae* because both organisms belong to the *Vibrio* genus, and both organisms produce α-D-Galactose-1,P as a byproduct of glycolysis. Finally, *Enterobacter aerogenes* was used as a model organism for *Shigella dysenteriae* because both organisms produce glycerol during respiration [13].

Graphene Isolation

Graphene was isolated in two manners in order to analyze the efficiency of more cost effective methods.

First, CVD Graphene was purchased on a $Ni/SiO_2/Si$ wafer from Graphene Supermarket. In order to isolate the graphene layers, the nickel and silicon/silicon dioxide layers were removed. To accomplish this, thermal release tape was adhered onto the graphene with applied pressure in order to remove air bubbles and increase the continuity of the graphene layer. It was then exposed to 30 mL of deionized water and ultrasonicated for 180 seconds. The ultrasonicator produces 20 kHz of sound waves that intervene between the layers of the wafer, breaking the intermolecular interactions and removing the silicon/silicon dioxide layers from the graphene wafer. Then, in order to remove the nickel layer on the wafer, nickel etching was performed using a 0.1 M solution of ferric chloride. The ferric

Photo by Author
Fig. 1 Isolated graphene layer on PET film.

chloride was warmed to 55°C on a hot plate underneath a fume hood. The graphene with tape was then placed in the solution and stirred for approximately 10 minutes, removing the nickel layer. The graphene on the tape was then removed from the solution and rinsed with deionized water [12]. These procedures were repeated for the isolation of graphene for the 3 remaining graphene wafers. At this point, only graphene remained on the thermal release tape. In order to transfer it onto a flexible substrate, the thermal release tape was adhered onto a PET film and placed on a hot plate set to 130°C, the release point of the tape, for 10 seconds. [40] When at 130°C, the tape released the graphene molecules onto the plastic substrates. The graphene layer following isolation procedures is seen in Figure 1.
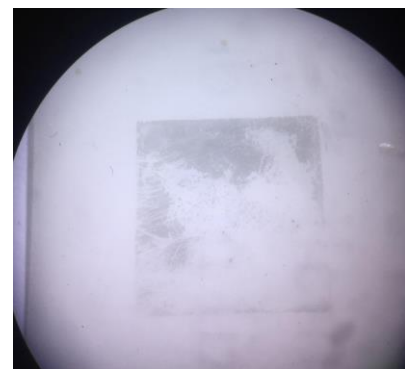
Graphite-Flake Dispersion

Despite CVD graphene's ability to form a highly functioning electrode, a graphite-flake

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dispersion between oil and water can also be utilized in order to reduce overall costs of the biosensor. Graphite from a standard graphite pencil was rubbed against a square of thermal release tape, which was then adhered to another piece of thermal release tape. 15 mL of water was added to a beaker, and 15 mL of oil was pipetted on top of the water. The two substances remained separated due to the differences of polarity of water and oil. These two substances formed an interface caused by the surface tension that arose from the hydrogen bonding of water. The graphite thermal release tape was then placed in the oil and water interface and ultrasonicated for 3 minutes, which would create an emulsification. The thermal release tape was then removed and a single-layer of graphite, or graphene, existed on the plastic tape films. [16]

Graphene Functionalization

The graphene on the plastic films, both from CVD and graphite origin, were submerged into a 5 mM solution of 1-pyrenebutyric acid dissolved in DMSO and left at room temperature. After 2 hours, the substrates were removed and rinsed with distilled water. This bonded the PBA onto the graphene through π-π interactions. [10] Glucose Oxidase, Glycerol 3-Phosphate Oxidase, Galactose Oxidase, and Lactate Oxidase, purchased from Sigma-Aldrich in anhydrous form, were immobilized onto the graphene. In order to immobilize the enzymes onto the graphene, 24 µL of distilled water was added to the anhydrous molecules of the enzyme

Photo by Author
Fig. 2 Immobilized glucose oxidase on graphene with conductive paint.

and the solution was ultrasonicated for 180 seconds. This was repeated for the remaining enzymes. One aliquot of each enzyme was then micro-pipetted onto one of the graphene substrates and left at 7°C overnight to immobilize the enzymes onto the graphene. Conductive paint terminals were then applied onto opposite, parallel sides of the graphene biosensor. The completed biosensor is seen on Figure 2.

Testing

In order to test the functionality of these biosensors, the bacteria *Enterobacter aerogenes, Vibrio fischeri, E. coli* K12, and *Sarcina aurantiaca* were cultured. All bacteria were purchased from Carolina Biological and cultured onto LB agar plates using sterile techniques. An isolation streaking technique was performed in order to make colonies visible on the agar plates for testing.

Photo by Author
Fig. 3 Biosensor connected to eDAQ ammeter via Pt/Ti counterelectrodes

In order to test the sensitivity of the biosensor, differing colony counts were inoculated into various water volumes. 0, 1, 5 and 10 colonies of each bacteria were incorporated into 100 mL and 1 L of distilled water. The control group contained solely distilled water, while the variable groups contained a specific number of bacterial CFUs in one of the water volumes. A 5 µL sample was then micro-pipetted into the center of the designated biosensor for testing bacterial presence with an eDAQ ammeter (EPU357 Conductivity isoPod) through platinum/titanium electrode wires seen in Figure 3. This ammeter measures the current in nanoamps. If bacteria are present in the sample, they are respiring and releasing analytes onto the biosensor that would free electrons and generate a changing electric signal. Based on the values outputted by the ammeter, the presence of bacteria will be determined. [12]

Phase 2:

A mechanized purification unit was created utilizing an advanced hydrogen peroxide reaction. Sodium hydroxide and hydrogen peroxide can be ejected into a water source in order to generate hydroxyl radicals that can eliminate organic material in water. The OH- groups of sodium hydroxide cause hydrogen peroxide to denature into $2OH\cdot$ (Hydroxyl Radicals), due to the raise in pH, at faster rates. When hydroxyl radicals come into contact with organic material, it creates safe byproducts of carbon dioxide and water, forming after the degradation of organic material and the neutralization of the reaction with sodium bicarbonate. [15]
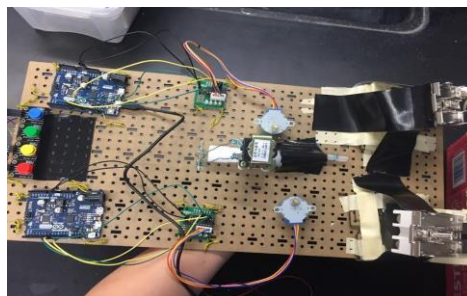

Photo by Author
Fig. 4 Assembled purification unit

A mechanized unit was created using an Arduino microprocessor to regulate the implementation of chemicals into water sources. The Arduino Zero was then connected to a Darrington Board using jumper cables. The pins of the Arduino (D8-D11) were connected to the corresponding number of the Darrington Board (INT1-INT4). This allowed for the Arduino to power the board as well as upload the rotation code onto it. The Arduino and the Darrington Board were then mounted onto a RobotGeek workbench using metal wiring. A stepper motor was attached to the Darrington Board using 5 lead adjoined wires.

A stepper motor code provided with the Arduino module was manipulated to fit the parameters of the purification unit. Once this code was uploaded, a spring was attached to a micropipette via adhesive glue. The micropipette was then elevated off the RobotGeek Workbench using a stack of 4, 3.5x2.7x0.3 cm plastic prisms and adhered using glue. This system would allow for the safe uptake and ejection of purification chemicals into water.

This process was repeated in order to mount two micropipettes onto the workbench. The micropipettes were filled with sodium hydroxide (0.5M, 10μL) and hydrogen peroxide (3% 10μL). The activation of the Arduino units led to the ejection of these chemicals into a water source. After ejection, sodium bicarbonate was added to water in order to disrupt the functioning of the hydroxyl radicals and was filtered out of water with a coffee filter. [11] In order to test the functionality of the device, bacteria were inoculated into a water source, and a sample of the water was placed on the biosensors in order to confirm the presence of bacteria in water. Next, the purification unit was activated, leading to the ejection of chemicals loaded in the micropipettes. Samples of water were then removed from the decontaminated water source in order to confirm that the purification unit had success in eliminating bacterial presence in water. This was accomplished by placing volumes of water onto the biosensor and monitoring the current with the EDAQ ammeter. A flat, unchanging current graph designated that the machine had success in eliminating bacterial presence in water.

Results
Phase 1:

Current Created by Bacterial Presence
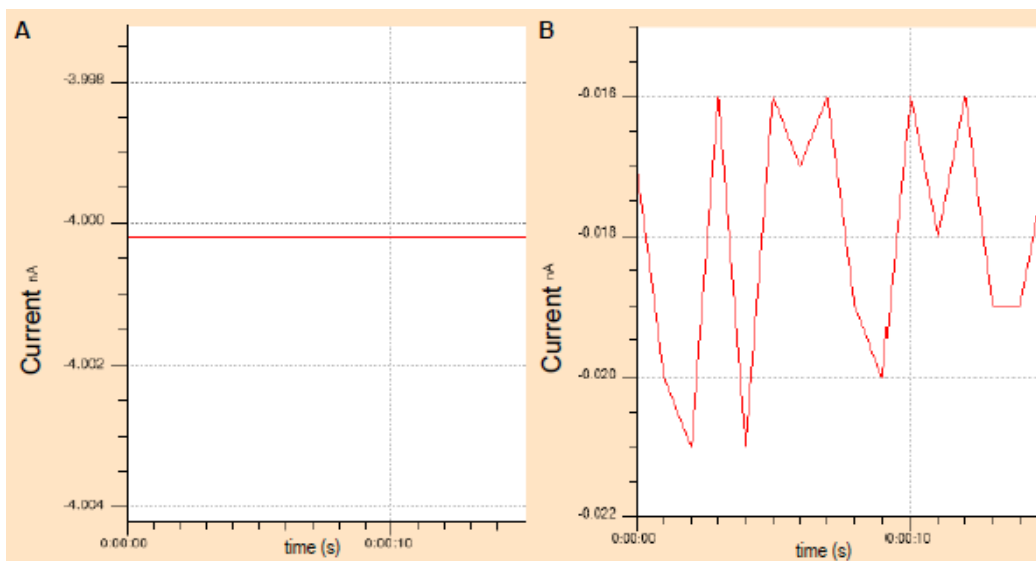
Graph 1. A) The amperage output of a control group of distilled water. B) The output of 1 CFU of *S. aurantiaca* in 100 mL of water. The values of the amperage outputted by the ammeter are used to calculate the range of the electric current.

During testing, each of the biosensors were exposed to a sample of contaminated water for 15 seconds. Samples contained varying volumes of water (100mL and 1000mL) and varying CFU counts (0, 1, 5, and 10 CFU). Graph 1A shows the output of a sample of solely distilled water. As there are no bacteria present in a sample, no analytes are freeing electrons, which creates this unchanging current output. However, when bacteria are present as seen in Graph 1B, the bacteria releases analytes that undergoes a series of chemical reactions to generate a changing current. A changing current output represents that bacteria are present in the sample. The range of the current values that were outputted by the eDAQ ammeter was calculated for each of these trials.

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Average Range for Bacterial Biosensor Output
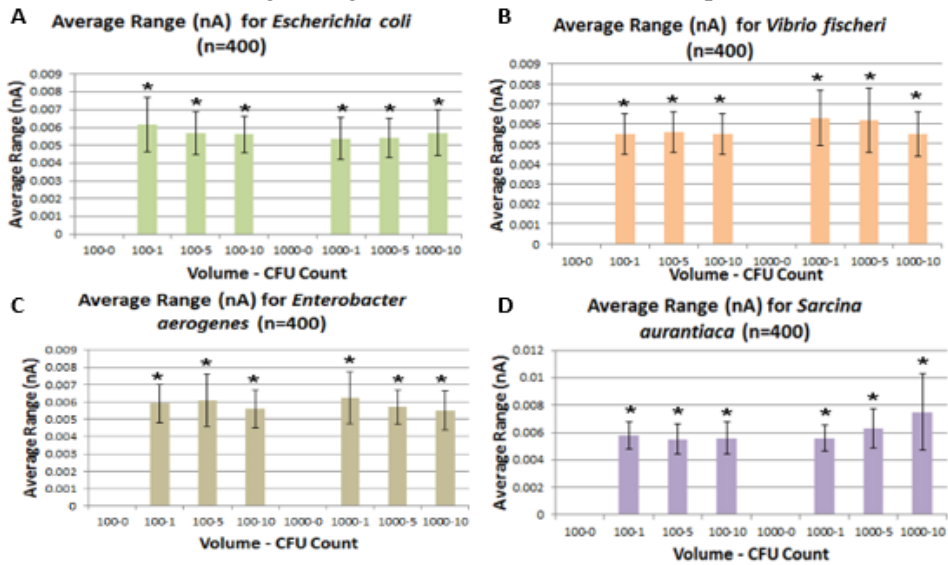
Graphs 2: Displays the average amperage (nA) range of A.) *E. coli* B.) *Vibrio fischeri* C.) *Enterobacter aerogenes* D.) *Sarcina aurantiaca,* for each respective biosensor in varying volumes of water. Asterisk denotes a significance of $p<0.001$ between the control and other variable groups.

The average range of the current that was outputted by the eDAQ (Graph 1) was calculated and seen here. There was no significance found between variable groups, however, there was a p value <0.001 which shows that while these biosensors were not able to directly quantify the number of CFUs present in a sample, it was able to definitively confirm the presence of bacteria.

Phase 2:

Bacterial Presence Before and After Purification
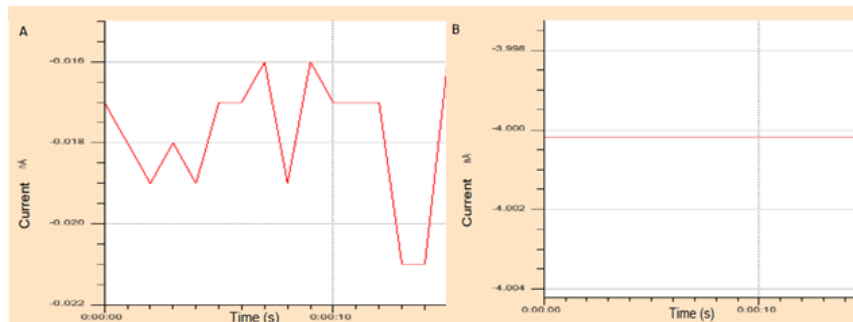
Graph 3. A) Displays the amperage output of 1 CFU of *Sarcina aurantiaca* in 100 mL of water. B) Displays the current outputted by the eDAQ following the activation of the purification unit.

These graphs show that the purification unit had success in eliminating bacterial presence. The changing current in Graph 3A confirms that bacteria are present in the sample, and after the activation of the purification unit, the current flatlines, designating that the bacteria have been removed from the sample.

Discussion

   The purpose of the first phase of this study was to create graphene biosensors that were capable of detecting the presence of prevalent waterborne pathogens such as *S. typhi, V. cholerae, S. dysenteriae*, and *E. coli*, which are the bacteria that cause Salmonella, Cholera, and Shigellosis, and E. coli, respectively. These biosensors successfully detected the presence of these microorganisms at distinctly low levels in varying volumes of water and a rapid time frame. These two devices improve greatly on current methods of bacterial detection and purification. PCR, a traditional method of measuring bacterial contamination in water, requires a thermal cycler which can cost thousands of dollars, constant hands on work for 1-2 days, and prior training before use. PCR and traditional plating methods have detection limits from 100 to 1000 CFUs [3]; meanwhile, the World Health Organization deemed that even 1 CFU of bacteria in 100 mL of water was unsafe. These biosensors are drastically more rapid, sensitive, inexpensive, and user-friendly.

   When a sample is pipetted onto the graphene biosensors in contact with the ammeter, the presence of a changing electric current can be used to signal the presence of bacteria. When analytes that are released during bacterial respiration come into contact with its designated enzyme, it's catalyzed into hydrogen peroxide and pyruvate. The hydrogen peroxide is then oxidized through the applied voltage given by the ammeter, which frees electrons that the graphene harnesses in order to generate an electric current. As seen in Graph 1 above, the EDAQ ammeter graphed the change in nanoamps outputted by the biosensor over 15 seconds. Graph 1a shows that there was no change in current outputted by the biosensor when uncontaminated distilled water was placed on it, as no analytes were present from bacterial respiration to be converted into electrical stimuli. However, graph 1b shows the results of a sample containing 1 CFU of *S. aurantiaca*. There is a fluctuating output, as the analytes come into contact with the enzyme and free electrons. The analytes do not all couple with the enzyme at the same time, therefore, electrons are not all freed at one time. Additionally, bacteria respire at different rates which causes the level of analytes present in a water sample to vary over a time interval.

   50 trials were run for each variable group, with 15 seconds per trial. The range of these trials were calculated and compared on Graph 2. It was found that in groups where no bacteria

contaminated the water, the biosensor outputted a constant value, and therefore the range was equal to zero. However, in water in which there was microorganism contamination, there existed a range of the values that was not equal to zero. This varying current and range of values signals the presence of these specific bacteria.

The data received in this experiment proved that the biosensors were able to detect and signal the presence of bacteria through the immobilization of enzymes onto the graphene substrates. Enzymes can be immobilized onto graphene through π-π interactions, which is a type of noncovalent bond that occurs between two aromatic molecules. Because graphene and 1-pyrenebutyric acid are both aromatic, meaning that they contain hexagonal benzene rings in their molecular structure, the parallel, aromatic rings align themselves with each other [8] when 1-pyrenebutyric acid solution is incubated onto the layer of graphene. This binds the PBA onto the graphene. This acid then acts as a linker molecule to allow for enzyme immobilization, as the enzyme then latches onto the acid through an amide bond that occurs between the amide group of the enzymes and the pyrene group of the pyrene butyric acid. This secures and immobilizes the enzymes onto the graphene molecules [9].

When the analytes that are released during bacterial respiration come into contact with the designated enzyme that is immobilized on the graphene, a series of chemical reactions occurs that signals the presence of bacteria through a changing electric current, as seen in Graph 1b. The analyte is catalyzed into hydrogen peroxide and pyruvate, and the hydrogen peroxide is oxidized. The oxidation of hydrogen peroxide frees electrons that the graphene is able to harness due to its conductive properties to generate an electric signal [9]. For example, *V. fischeri* produces the metabolite α-D-Galactose-1 as a byproduct of respiration. When the metabolite comes into contact with galactose oxidase, the enzyme catalyzes the analyte into hydrogen peroxide and pyruvate. The hydrogen peroxide is then oxidized through the applied voltage of the ammeter, which frees electrons that graphene harnesses in order to generate a current measurable by the ammeter [14]. Because bacteria are respiring at different rates, the concentration of analytes in the sample vary, which causes the current produced by the biosensor to vary as presented by Graph 1b. Therefore, when water was contaminated with the specific bacteria designated to the enzyme, the EDAQ ammeter measured a varying current value that signaled the presence of

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bacteria in water. When bacteria are not present in water, no metabolites are being produced by bacterial respiration. Therefore, no analytes are freeing electrons, which cause the current to flatline as seen in Graph 1a.

After running a One-Way ANOVA followed by a post-hoc scheffe test, there was no statistical significance between variable groups containing various CFU counts. However, a p value of <0.001 was discovered between the control group with solely distilled water and all other variable groups containing bacteria. This shows that while these biosensors did not appear to have the ability to quantify the number of bacterial CFU's present in a sample based on the threshold it was tested at, these biosensors were capable of definitively signaling the presence of bacteria through a changing electrical current. This was found in Labroo's study [12], in which there existed a threshold that the lactate concentration had to surpass in order to create a significantly different electrical output. This suggests that if these biosensors were to be tested with higher levels of bacteria, and thus a higher concentration of analytes, the electrical output would correlate with the number of CFU's of bacteria present in a sample.

The purpose of the second phase of this study was to develop a purification unit that could purify water contaminated with bacteria, creating potable and usable water. Using the EDAQ ammeter and biosensors, it was confirmed that the Arduino-programmed micropipette purification unit had the ability to decontaminate water that contained bacterial pathogens, thus fulfilling the purpose of this study. Current methods for purification are costly, take a long period of time, and poses risk for further contamination as water must be shipped out to external facility to be treated. This purification system allows bacteria to be eliminated from water directly within the water source in a cheap, safe, manner.

When the biosensors confirm the presence of bacteria in water via a varying current, the purification unit can be activated to eliminate the bacteria. The rotation code adapted in this study can be activated on the two Arduino boards. This would allow for stepper motors to rotate, and push forward rails on rail mounts that push forward the plungers of micropipettes, releasing sodium hydroxide and hydrogen peroxide into the water. These two chemicals can lead to the generation of hydroxyl radicals that mark a very effective, but safe method by which water can be purified. Hydroxyl radicals uncouple bonds that exist in organic material, combining with

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hydrogen and carbon atoms to form water and carbon dioxide. The radicals will interact with the organic material into all the bacteria have been degraded, where upon sodium bicarbonate can be added into the water in order stop the functioning of the radicals, and allow for complete elimination of bacteria in water. The biosensors can be used in order to confirm the success of the purification unit in eliminating bacteria, as it can monitor bacterial presence regardless of any other substance that may exist in water as the enzymes immobilized on the biosensors only free electrons when the specific correlate analyte binds with the enzyme. When the bacteria are eliminated, there are no analytes being produced in the water, and therefore the current read by the ammeter remains at a constant value, which shows that the purification unit had success in eliminating bacterial presence. [15]

The purification unit in this study consisted of several features that greatly improve upon current methods of purification. For one, the purification unit had the dimensions of 44cm x 21cm x 0.5cm. This size is much smaller than current municipal water treatment facilities, and therefore, upon implementation in a real-world situation, there exists no real size constraints due to the compact nature of the device. This would allow for the system to be utilized onsite in water sources in order to apply an immediate purification response to water if the biosensors were to confirm bacterial presence in water. Additionally, the purification system construction was much cheaper than that of a water treatment plant. While water treatment plants have the capacity to eliminate other forms of water contamination like nitrates, the unit constructed in this study costs a couple of hundred dollars, whereas most water treatment facilities cost multiple hundreds of millions of dollars. Moreover, the purification unit in this study that was created allowed for cost-effective, on-site bacteria elimination that marked a critical improvement on past methods of water purification. [17]

Using these two systems, bacterial detection and water purification can occur immediately, on-site, and at a lower cost to ensure the sanitation and affordability of clean drinking water.

One area of this study that could be improved upon was the use of water-based conductive paint. Working in a high school laboratory, silver-based paint could not be utilized due to its low flash point. Therefore, water based paint was used as an alternative; however, this

allows only a small volume of a sample to be pipetted onto the biosensor rather than immersing the entire substrate in a water source. If this study were to be replicated in a professional laboratory in which hydrophobic silver-based paint could be utilized, the procedure to manually transfer the sample onto the biosensor could be eliminated. The biosensors could then be simply placed in a water source, such as a well, and able to continually monitor the presence of bacteria. In spite of this limitation, however, it is important to recognize that this system had the capabilities of detecting 1 CFU of the four major bacterial contaminants of water in 1 second, and purifying water contaminated with bacterial in a rapid manner.

Two different electrode configurations were used in this study in order to try to generate biosensors that are more cost effective for those living in poorer regions in the world. The purchased CVD graphene and the graphene harvested from pencil graphite exhibited no observable differences in output. This reflects that utilizing graphite in order to harvest graphene would be a significantly more cost-effective alternative, while retaining the biosensors' sensitivity and rapidity. Thus, countries in the developing world, with a more readily available supply of graphite over graphene, could utilize graphite as an effective electrode alternative for the developing world.

Overall, this system has important implications for the developing world in terms of water contamination monitoring and purification. The detection devices and purification unit has the potential to be implemented into a drinking source in the developing world in order to consistently monitor the presence of *E. coli, Shigella, Salmonella,* and *Cholera* in the water. If the detection device identifies that there exists bacteria in the water, the purification unit can be implemented in order to add the chemical agents necessary to generate hydroxyl radicals in order to purify water contaminated with the bacteria. These results are fundamental to the advancement of water sanitation throughout the world, in the developing and developed world alike. In the developing world, women and children spend upwards of 6 hours each day collecting water [18]. This system can alleviate this issue, allowing women and children to receive an education, by providing a user-friendly, labor-saving sanitation system for drinking water. Similarly, access to safe water in the developing world has the potential to provide almost $32 billion dollars in economic benefits. [4]

These systems can be applied to industrial settings as well. Biosensors can be formatted into a cylindrical structure in order to be placed in a pipe of flowing water. The biosensor can then be covered in electrically conductive carbon black epoxy, which will prevent high volumes of water at high speeds from causing the device to flow away. This device implementation would allow for countries in the developed world to constantly monitor bacterial presence and reduce the threat that waterborne pathogens have on the world.

Conclusion

This detection/purification system was successful in various areas of interest:

1. These sensors were capable of detecting bacterial presence in less than 1 second, which is far more rapid than the current methods of bacterial detection.
2. These biosensors were also able to discern lower levels of bacteria (1 CFU) than these conventional methods, such as PCR and colony counting, are capable of.
3. In addition to these improvements upon the functionality of detection devices, they are also user friendly and can be implemented easily in the developing world.
4. The mechanized purification unit was successful in eliminating bacterial presence in water in a more rapid, cost effective parameter.

In conclusion, this study yielded the creation of sensors that had the ability to detect model organisms for *E. coli*, *Salmonella, Shigella,* and *Cholera.* Working in conjunction with these biosensors, a mechanized purification system could monitor and purify microorganism presence in order to sanitize water sources contaminated with bacterial pathogens. This system could potentially eliminate the threat of waterborne diseases and greatly expand sanitary water resources throughout the world.

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