Detection of *Escherichia Coli* Bacteria in Wastewater by using Graphene as a Sensing Material

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Abstract. Graphene is a family of carbon bonded in hexagonal honeycomb crystalline structure that has many superior properties. It was very suitable to be applied on sensor application due to the superior properties on electrical, physical, and optical. Furthermore, graphene also provide a large detection area since it has 2D structure. In this research, we develop graphene as a nanosensor for detection of *Escherichia coli* (*E. coli*) bacteria. The sample *E. coli* bacteria were cultured from domestic wastewater by using plate culture method and then isolated to get pure single colony. The serial dilution was performed to create different concentration of bacteria. Field emission scanning electron microscope and biochemical test were performed to ensure the sample genuinely target *E. coli* that defined by the physical size and optical properties. Raman spectroscopy measurements were also performed on the graphene films, and it was found that the ratio of G peak and D peak intensity changing do to the presence of *E. coli*. The electrical properties of graphene shows the increasing number of the bacteria 4 to 273 cfu result in decreasing the resistance from 4.371 to 3.903 ohm gradually.

1. Introduction

Microbiological test commonly used to detect pathogenic bacteria, like *Escherichia coli* (*E. coli*), in water conventionally is using culture or biochemical properties test. However, this conventional method generally takes several days to determine whether the water is contaminated by bacteria. Therefore, developing a device to detect harmful bacteria rapidly, and easy to perform is important to be done. Carbon material like graphene has been known to have biocompatibility and exhibit outstanding properties is very promising to be sensing layer.

Graphene, a monolayer of graphite consisting of *sp²*–hybridized carbon atoms has become technologically and scientifically important due to their outstanding properties such as excellent mechanical strength (Young modulus ~1.10 TPa) [1], high carrier mobility at room temperature (up to ~10,000 cm²/V s) [2], and excellent thermal conductivity (5000 W/m K) [3]. Moreover, graphene exhibits unique properties such as high specific surface area (SSA) of 2600 m² g⁻¹ [4], high transparent toward visible light (~2.3% absorption) [5], and bio-compatibility. These properties make...
graphene very promising for future electronic and composite industry. Particularly, it is an excellent candidate to develop low cost sensors. Basu et al. (2012) used graphene for resistive gas/vapour sensors. They said that graphene and its oxides might promise the development of less expensive and more efficient gas sensors in near future [6].

In the last years, research on graphene increase significantly with applications in the fields of nanoelectronics, sensors [7], composite materials [8], biosensing [9], and energy harvesting technology [10]. One of the fields that attracted many attentions of researchers is graphene-based sensor, such as a gas sensor, electrochemical sensor, biosensor, and many more. The 2D structure of graphene can provide a larger detection area, and homogenous surface for uniform and effective functionalization, as compared to 1D nanostructure sensing elements. Moreover, it is more suitable to interface with flat cell membranes. Kalbacova et al. (2010) showed that graphene is able to support cell adhesion and growth, indicating its biocompatibility [11]. The presence of fecal coliform bacteria in aquatic environments indicates that the sanitary has been contaminated with the fecal material of human or animals. The presence of fecal coliform contamination is an indicator that a potential health risk exists for human exposed to this water [12]. Not only can cause diarrhea, but coliform bacteria may also cause severe anemia or kidney failure which can lead to death. Fecal coliform testing is one of the several tests of water quality that requires a very careful set of sterile procedures, as well as expensive equipment and consuming time. *E. coli* bacterium is a rod-shaped bacterium normally live in the intestines of people and animals. Most *E. coli* is harmless and actually is an important part of a healthy human intestinal tract. However, some *E. coli* is pathogenic, meaning they can cause illness, diarrhea, and even death. Therefore, it is important to develop a sensor to detect *E. coli*.

Many sensors to detect harmful bacteria have been developed. Recently bacteria sensor not only detects the presence of the bacteria but also quantify the number. Several techniques have been invented to detect bacteria such as fluorescence detection, Polymer Chain Reaction (PCR) [13], microfluidic [14][15], and electrochemical methods [16]. Fluorescence detection technique and DNA-biosensors can be used to quantify the amount of the bacteria rapidly. However, fluorescence detection needs dye labeling which requires high expense and professional training for operation [17][18], while PCR method has complicated procedures and facilities and consuming time [13]. Microfluidic systems have been widely used for bacterial analysis because it has shown many advantages such as can analyze a sample with smaller reagent volumes in less time and can perform multiple samples processing on a single device [14]. Wang et al. (2013) presented an integrated micro/nanofluidic device that integrates a micromixer and a preconcentrator for the rapid detection of bacteria. The concentration of bacterial sample (*E. coli*) were quantified by measuring the fluorescence intensity at the preconcentrated region. *E. coli* bacteria sample is tagged with fluorescent dye and continuously preconcentrated at the target position by applying the electric field through preconcentrator [14]. However, this method has to be performed in specialized laboratory environments with the assistance of sophisticated equipment. Shaibani et al. (2016) reported a detection of *E. coli* bacteria by an electrochemical method using pH sensitive hydrogel nanofiber-light addressable potentiometric sensor (NF-LAPS). In their experiment, electrospun polyvinyl alcohol (PVA) hydrogel nanofibers act as a sensing layer. The changes on pH of the media indicate the presence of *E. coli* due to *E. coli* cells ferment sugar molecules and increase the acidity of the surrounding [16]. This method only able to detect bacteria instead of quantifies the amount.

The main important element of the biosensor device is sensing layer. Sensing layer will directly in contact with bio component to make effort or change behavior of the sample. Many sensing layers are made of certain materials such as metal oxide [19], polyvinyl alcohol (PVA) hydrogel nanofibers [16] or carbon [9]. Carbon type which is widely used as sensing layer is graphite or graphene because it has a high specific face area and high mobility, low electrical noise and easy to obtain. However, only a few types of research are using graphene as a sensor material for a biosensor especially for bacteria sensor. Basu et al. (2014) applied graphene as *E. coli* sensor on flexible acetate on their research. Cu foil was used as substrate and Au was deposited onto substrate as electrode. An O-ring is added to the surface layer as a bacteria chamber. The sensor device is based on impedance
measurement that is conducted by Electrochemical Analyzer instrument CHI 600c. The results show the impedance of graphene decrease with increasing \textit{E. coli} concentration [9].

In this study we use graphene based-resistive sensor to detect the presence of \textit{E. coli} due to the electrical response changing of graphene sensor. Increasing the number of the bacteria result in increasing the conductivity of the sensor as well as increasing the intensity ratio of $I_D/I_G$ peak obtained from raman spectra.

2. Experimental Method
Graphene films used in this experiment were multilayer CVD graphene on Si/SiO$_2$ substrate from Graphene Supermarket. The thickness of the SiO$_2$ coating is 285 nm and the thickness of the graphene layer is around tens nanometer. A pair of leg electrical connection from Ossila were attached on graphene layer as electrode.

The sample bacteria were collected from domestic wastewater by plate culture method. Microbiology Chromocult Coliform Agar from Merck were used as a medium and the sample were incubated at 37\degree C for 24 hours to grow the bacteria. After 24h incubation, single colony of bacteria were isolated to new agar medium and incubated at 37\degree C for 24 hours. Then, the single colony was transferred to autoclaved liquid media (Nutrient broth from Beckton Dickson) before ready to use.

The electrical response of the sensor was carried out using IV-meter (Lucas Pro4). Atomic force microscope (AFM) and Field emission scanning electron microscope (FESEM) images of the samples were done to obtain the size of the bacteria and to make sure the bacteria well attached on graphene film. The raman spectroscopy measurements were done at room temperature using Horiba HR spectrometer with using laser with 532 nm wavelength.

3. Results and Discussion
Figure 1 shows the AFM image of pristine graphene. The film showed a uniform surface with many grain spread evenly. The roughness of the film was 6.789 nm and the grains are relatively large of 186.96 nm and regular in shape. The surface morphology of film can also be used to kill bacteria. Commonly, the surface of films that have significant roughness or peaks can cause the bacterial cell wall to rupture under its own weight upon contact. The surface of the graphene film did not show a sharp edge of grain and tend to curved, which means does not harm bacteria membrane to adhere on graphene surface. Even more, graphene was also known to have a good biocompatibility.

![Figure 1. AFM images of pristine graphene film.](image.png)

To ensure the sample bacteria we collected before was \textit{E. coli}, biochemical and optical test was performed. Biochemical test was done using Readycult coliform and showed a positive result where the color of the medium changes to blue-green that indicated the presence of \textit{E. coli} as shown in Figure 2. Furthermore, we also performed optical test using FE-SEM to confirm it. Figure 3 shows the FESEM image of \textit{E. coli} bacteria in 5000X and 10.000X magnification. We can see most of the
bacteria form to a colony. The size of the bacteria we collected from our domestic wastewater was about 1.6 μm in length.

![Image](a) ![Image](b)

**Figure 2.** Biochemical test using Readycult (a) before and (b) after introduce to *E. coli* sample

![Image](Figure 3. FE-SEM image of the *E. coli* bacteria)

**Figure 3.** FE-SEM image of the *E. coli* bacteria

Figure 4 shows the I-V characteristic of the graphene before and after exposure to the different concentration of *E. coli* bacteria. Increasing the number of the bacteria results in decreasing the resistivity of the graphene/ increasing the current flow. This is attributed to the negatively charged *E. coli* induces hole in graphene. The linear pattern indicated the metallic nature of the sheets and formation of Ohmic contact between the sheets and electrode [20]. From the I-V graph, the slope can be used to calculate the resistance of the graphene sensor. The resistance decrease from 4.371 to 3.903 ohm gradually with increasing the number of bacteria from 4 to 273 cfu respectively.
The raman characteristics of carbon material like graphene shows a well-known peak of D and G bands ($\sim 1350$ and $\sim 1580$ cm$^{-1}$). These peaks are usually assigned to the graphitized structure and local defects (disorder) particularly located at the edges of graphene and graphite platelets respectively. Figure 5 shows raman spectra of graphene and immersed graphene in different concentration of $E. \text{coli}$ bacteria. The raman spectra shows D peaks are much higher than 2D peaks which is confirmed that the graphene used was a multilayered graphene. This was also confirmed by the position of G band that located 1576.62 cm$^{-1}$, below 1585 cm$^{-1}$ which is usually corresponded to single layer.

Figure 5. Raman spectra of the graphene before and after exposure to the bacteria with different concentration
Figure 6 shows the intensity ratio of D and G peaks (I_D/I_G) of graphene and immersed graphene in different concentration of E. coli bacteria. Smaller I_D/I_G peak intensity ratio can be assigned to lower defects or disorder in the graphitized structure [20]. Generally, Figure 6 showed increasing the number of the bacteria result in increasing the I_D/I_G from 0.145 to 0.297 gradually after contact with bacteria for 10 minutes. The spiky increasing of I_D/I_G ratio occurred between pristine graphene and sample that was introduced to 4 cfu of bacteria.

Figure 6. Peak intensity ratios of I_D/I_G obtained by raman spectra as the function of number of the bacteria

4. Conclusion
In summary, we have already studied the interaction of graphene and different concentration of E. coli. Graphene film showed a smooth surface that very suitable for bacteria to attach without rupturing its membrane. The electrical properties and raman spectra of graphene film when used to detect E. coli bacteria was performed. It was found that increasing the number of the bacteria result in decreasing the resistivity of graphene. This is related to the increased hole doping in graphene due to negatively charged E. coli. Moreover, the raman characteristic exhibit the peak intensity ratio of I_D/I_G was also increase as well.

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