FABRICATION OF GRAPHENE-BASED RESISTIVE SENSOR FOR ESCHERICIA COLI BACTERIA SENSING

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This thesis work is dedicated to my family, my teachers, and my friends.

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ABSTRACT

The presence of fecal coliform bacteria like *Escherichia coli* (*E. coli*) in aquatic environments indicates that the water has been contaminated with the fecal material of human or animals. The risk of health threatening to humans become high when *E. coli* bacteria are exposed to water, not only causes diarrhea but also lead to death if not treated immediately. Many existing sensors to detect *E. coli* bacteria are not portable and expensive, while the conventional methods are time consuming and need the sterile procedure. Therefore, it is important to develop a sensor to detect *E. coli* rapidly, portable and user friendly. In this work, bacteria sensor based on resistivity was developed using graphene nanostructure as a sensing layer, since carbon material like graphene has been known to have a biocompatibility and excellent electrical property. The *E. coli* bacteria used in this experiment were collected from domestic wastewater and commercial of *E. coli* strain no. ATCCC 25922. A biochemical test was performed to detect the presence of *E. coli* in wastewater. The physical characterization of the bacteria was carried out by using FESEM and it was found that the *E. coli* has size ranging from 1.4-1.6 μm in length. The wettability properties of graphene confirmed that graphene surface is hydrophobic with contact angle of 108.9^{o} which is suitable for bacterial detection. Raman spectroscopy measurements shows that the ratio of G peak and D peak intensity increase due to increase in the number of *E. coli*. Moreover, the electrical property of graphene shows increasing the number of the bacteria from 4 to 273 colony forming-unit (cfu) result decreasing the resistance from 4.371 to 3.903 ohm gradually. Finally, the sensor was successfully designed using SolidWorks and fabricated by integrating graphene film as a sensing layer with Arduino micro-controller. The validation of the sensor was performed by comparing the data obtained by sensor device and plate culture and has been found to have an error rate of ±12.3%.

ABSTRAK

Kehadiran bakteria coliform fecal seperti *Escherichia coli* (*E. coli*) dalam persekitaran akuatik menunjukkan bahawa sumber air telah tercemar dengan hasil kumbahan manusia atau haiwan. Risiko ancaman kepada kesihatan manusia adalah tinggi apabila bakteria *E. coli* terdedah kepada air, ia bukan sahaja boleh menyebabkan cirit-birit, tetapi juga boleh mengakibatkan kematian jika tidak dirawat dengan segera. Banyak sensor yang sedia ada untuk mengesan bakteria *E. coli* adalah mahal dan tidak mudah alih, sementara menggunakan kaedah konvensional memakan masa yang lama dan memerlukan prosedur yang steril. Oleh itu, membangunkan sensor untuk mengesan *E. coli* dengan cepat, mudah alih, dan mesra pengguna adalah penting. Dalam penyelidikan ini, sensor bakteria berdasarkan resistiviti telah dibangunkan menggunakan bahan grafin sebagai lapisan penginderaan, kerana bahan karbon seperti grafin diketahui mempunyai sifat keserasian-bio dan mempamerkan sifat-sifat yang cemerlang. Bakteria *E. coli* yang digunakan dalam eksperimen ini dikumpulkan daripada loji air kumbahan dan komersil *E. coli* dengan no. strain ATCCC 25922. Ujian biokimia dilakukan untuk mengesan kehadiran *E. coli* dalam air kumbahan. Pencirian fizikal bakteria telah dilakukan menggunakan FESEM dan didapati bahawa *E. coli* mempunyai panjang antara 1.4-1.6 µm. Sifat kebolehbasahan grafin pula mengesahkan bahawa permukaan grafin adalah hidrofobia dengan sudut sentuh 108.9^{o} bermaksud ianya sesuai untuk pengesanan bakteria. Pengukuran spektroskopi Raman mendapati bahawa nisbah keamatan puncak G dan D meningkat disebabkan oleh peningkatan jumlah *E. coli.* Selain itu, sifat elektrik grafin menunjukkan peningkatan jumlah bakteria dari 4 hingga 273 cfu (unit pembentuk koloni) akan mengakibatkan penurunan rintangan dari 4.371 hingga 3.903 ohm secara beransur-ansur. Akhir sekali, sensor berjaya direka menggunakan SolidWorks dan direka bentuk dengan menggabungkan filem grafin sebagai lapisan penginderaan dengan pengawal-mikro Arduino. Kesahan sensor dilakukan dengan membandingkan data yang diperolehi oleh peranti sensor dan kaedah konvensional dan didapati mempunyai ralat sekitar ±12.3%.

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LIST OF SYMBOLS AND ABBREVIATIONS

$\pi\alpha$	-	Absorption coefficient
σ	-	Tensile stress
ε	-	Engineering extensional strain
λ	-	Wavelength
e.g.	-	for example
1D	-	One dimension
3D	-	Three dimensions
A	-	Absorption
AC	-	Alternating current
AFM	-	Atomic force microscope
Ag	-	Silver
Al	-	Aluminium
Ar	-	Argon
Au	-	Gold
C	-	Carbon
CCD	-	Charge-coupled device
Ccfu	-	Number of colonies bacteria
CE	-	Counter electrode
CFU	-	Colony forming unit
Cl	-	Chlorine
CNTs	-	Carbon nanotubes
CMG	-	Chemically modified graphene
Cu	-	Copper
CVD	-	Chemical vapour deposition
DAEC	-	Diffusely adherent *E. coli*

DC	-	Direct current
df	-	Dilution factor
DI	-	Deionized water
E	-	Modulus young
EAAC	-	Entero-aggregative *E. coli*
EIEC	-	Entero-invasive *E. coli*
ELISA	-	Enzyme linked immunosorbent assay
EPEC	-	Entero-pathogenic *E. coli*
ETEC	-	Entero-toxigenic *E. coli*
E. coli	-	*Escherichia coli*
F	-	Force
FESEM	-	Field emission scanning electron microscope
FLG	-	Few layer graphene
GC	-	Glassy carbon
GNRs	-	Graphene nanoribbons
GO	-	Graphene Oxide
Gt	-	Graphite
GtO	-	Graphite oxide
GUD	-	Glucuronidase
HSQ	-	Hydrogen silsesquioxane
In	-	Indium
Ir	-	Iridium
JMP	-	Joint monitoring programme
LCD	-	Liquid crystal display
LED	-	Light-emitting diode
L_o	-	Original length of the object
ΔL	-	amount by which the length of the object changes
MWCNTs	-	Multi-walled carbon nanotubes
MUG	-	Methylumbelliferyl-β-D-glucuronide
n	-	Refractive index

N	-	Number of bacteria
NH_3	-	Ammonia
Ni	-	Nickel
NPs	-	Nanoparticles
Pb	-	Lead
PCB	-	Printed circuit board
PCR	-	Polymerase chain reaction
Pd	-	Palladium
PL	-	Photo luminescence
PPA	-	Polyphthalamide
Pspd	-	position-sensitive photo diode
Pt	-	Platinum
PVA	-	Polyvinyl alcohol
QCM	-	Quartz crystal microbalance
RE	-	Reference electrode
rGO	-	reduced graphene oxide
SiC	-	Silicon carbide
SiO_2	-	Silicon dioxide
STEC	-	*Shiga toxin*-producing *E. coli*
SSA	-	Specific surface area
T	-	Transmittance
TCFs	-	Transparent conductive films
TFTC	-	Too few to count
TNTC	-	Too numerous to count
USB	-	Universal serial bus
UV	-	Ultra violet
Vis	-	Visible
Vt	-	Volume transferred to plate
WE	-	Working electrode
Zn	-	Zinc

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

This chapter describes the general information related to this research. In this research, a graphene-based resistive sensor for *Escherichia coli* bacteria sensing were fabricated. Chapter 1 includes the background of study, problem statement, hypothesis, objective, and scope of this research.

1.1 Background of study

Graphene, a monolayer of graphite consisting of sp^2 –hybridized carbon atoms bonded in hexagonal honeycomb crystalline structure 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 $cm^2/V\ s$) [2], and excellent thermal conductivity (5000 W/m K) [3]. Moreover, graphene exhibits unique properties such as high specific surface area (SSA) of 2600 $m^2\ g^{-1}$ [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.

In the last few years, research on graphene significantly increased in many applications such as nanoelectronics [6], sensors [7], composite materials [8], biosensing [9], and energy harvesting technology [10]. One of the fields that has attracted the attention of many researchers is graphene-based sensor, such as a gas sensor, electrochemical sensor, biosensor, and many more. This is due to 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. Particularly, it is an excellent candidate to develop low cost sensors. Basu *et* *al.* 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 [11]. Moreover, graphene is also more suitable to interface with flat cell membranes just like Kalbacova *et al.* research's which shows the ability of graphene to support cell adhesion and growth, indicating its biocompatibility [12].

Among the others sensor, graphene-based biosensor still to be the most interest area to develop, especially for bacteria sensor. This is due to the fact that there are still many victims either sick or died caused by bacteria diseases or infection. According to Joint Monitoring Programme (JMP), around 289,000 children under five die every year from diarrheal diseases. That's almost 800 children per day, or 1 child every 2minutes. 88% of these deaths are attributable to unsafe water supply, inadequate sanitation, and poor hygiene [13]. Every year, more people die from unsafe water than from all forms of violence, including war [14].

The presence of faecal coliform bacteria in aquatic environments indicates that the sanitary has been contaminated with the faecal material of human or animals. The presence of faecal coliform in water is an indicator that the water was contaminated and a potential health risk exists for human exposed to this water [15]. Not only can cause diarrhea, but coliform bacteria may also cause severe anemia or kidney failure which can lead to death. Faecal 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. *Escherichia coli* (*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, diarrheal, and even death. Therefore, it is important to develop a sensor to detect *E. coli*.

Many sensors to detect harmful bacteria have been developed in the last few years. Recently, bacteria sensor not only detects the presence of the bacteria but also quantify the number as well. Several techniques have been invented to detect bacteria such as fluorescence detection, Polymer Chain Reaction (PCR) [16], microfluidic [17][18], and electrochemical methods [19]. Fluorescence detection technique and DNA-biosensors can be used to quantify the amount of the bacteria rapidly. However, fluorescence detection needs dye labelling which requires high expense and professional training for operation [20][21], while PCR method has complicated procedures and facilities and consuming time [16]. Microfluidic systems have been widely used for bacterial analysis because it has shown many advantages such as can analyse a sample with smaller reagent volumes in less time and can perform multiple samples processing on a single device [17]. Wang *et al.* present 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*) 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 [17]. However, this method has to be performed in specialized laboratory environments with the assistance of sophisticated equipment. Shaibani *et al.* report detection *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 [19]. This method only able to detect bacteria instead of quantifies the amount.

The main important part of the biosensor device is sensing layer. The sensing layer will directly in contact with bio component to make effort or change behaviour of the sample. Many sensing layers are made of certain materials such as metal/oxide [22], polyvinyl alcohol (PVA) hydrogel nanofibers [19] or carbon [9]. Metal/oxide over high endurance towards extreme environments. However, not all metals are suitable for bacterial detection. Certain metals can kill bacteria exactly instead of detect, such as silver, brass, and copper [23]. While, PVA offers an excellent biocompatibility, but still the using of PVA as a sensing layer is require a complex setup. The most widely used carbon as a 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, the using graphene as a sensor material for a biosensor especially for bacteria sensor is still limited. Basu *et al.* apply graphene as *E. coli* sensor on flexible acetate on their research. Cu foil is used as substrate and Au is 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 *E. coli* concentration [9]. Moreover, graphene can be applied in the fabrication of ultra-thin coatings for inhibition of the bacterial growth on surfaces, even in the environments which are highly suitable for proliferation of the bacteria [24].

1.2 Problem statement

Microbiological test commonly used to detect pathogenic bacteria, like *Escherichia 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 or not. Therefore, developing a sensor device to detect harmful bacteria rapidly, easy to perform, and portable has to be done. Based on the previous research, carbon material like graphene has been known to have biocompatibility and exhibit outstanding properties which will be used as a sensing layer. The problem is how to fabricate graphene-sensor with a good sensitivity of slightly change of biological element.

1.3 Hypothesis

Based on previous research, the resistivity of the graphene-based sensor will decrease due to the presence of *E. coli* bacteria and continuously decrease proportional to the increasing of the concentrations of *E. coli*.

1.4 Objective

The objectives of this research project are:

1. To design *E. coli* bacteria sensor device using SolidWorks software.
2. To fabricate a graphene-based sensor device for *E. coli* bacteria detection.
3. To verify and validate the accuracy of graphene-based *E. coli* sensor.

1.5 Scopes

The scopes in this research are:

1. Graphene thin film used in this experiment is a commercial graphene.

2. The electrode used in this research is electrical connection leg from Ossila
3. The electrical parameter used in this research is resistivity.
4. *Escherichia coli* bacteria used in this experiment ware collected from domestic wastewater and strain no. ATCC 25922.

CHAPTER 2

LITERATURE REVIEW

This chapter describe the early information and theories related to graphene as sensing layer for bacteria detection which then becomes the basis of this project. Moreover, the theories about bacteria itself and methods to detect are also obviously discussed in this chapter.

2.1 Graphene

Graphene is simply one atomic layer of graphite, mineral that naturally occurs in metamorphic rock in different continents of the world. Graphene is still counting as family of carbon which has six electrons surrounding its nucleus. Moreover, carbon was also the sixth abundant element found on earth. Carbon has the unique capacity of forming various organic compounds and also possesses unusual polymer-forming ability that can bond with many elements in many different ways. Figure 2.1 shows the simple schematic of graphene and graphite.

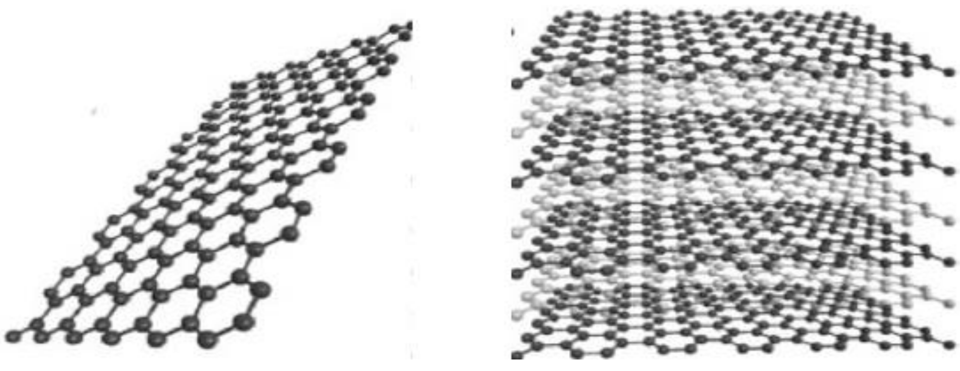

Figure 2.1: Simple scheme of (a) graphene and (b) graphite [6]

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