DEVELOPMENT OF PORTABLE ESCHERICHIA COLI BACTERIA DETECTION USING RESISTIVE GRAPHENE-BASED IMMUNOSENSOR

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

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ABSTRACT

Escherichia coli (E. coli) bacteria comes from the human and animal faeces. It is hazardous to human health and could cause death when not immediately treated. Therefore, it is crucial to detect *E. coli* bacteria in water before consuming it. Since many cases of water contaminated *E. coli* bacteria are in rural areas, a portable *E. coli* bacteria detector is needed. However, many existing biosensors to detect *E. coli* bacteria are not portable, while the conventional methods are time-consuming and need specialists to perform the task. In this work, an *E. coli* bacteria biosensor based on the resistivity method was developed using graphene nanostructure as the sensing layer, since carbon materials such as graphene have been known to have biocompatibility and excellent electrical properties. As a result, the concentration of *E. coli* bacteria can be measured through the change of conductivity caused by the negative charge produced by the *E. coli* bacteria's surface. Anti-*E. coli* antibodies and a blocking agent were employed on the graphene's surface for selectivity purposes. Characterisations using FESEM, AFM, Raman spectroscopy and contact angle were successfully conducted to study the properties and to verify the presence of the immunosensing properties on the sensing layer. The full system was developed with a standalone user interface and does not require any alternating current (AC) outlet or personal computer (PC) connection. An Arduino microcontroller was used to operate the new device. The circuit was functionalised to detect resistance change with the help of Wheatstone bridge for device calibration. Results show that resistivity change that can be detected by the device was as low as 0.0426 Ω. For *E. coli* bacteria detection, the system has a linear relationship between the device reading and the concentration of *E. coli* bacteria. The conductivity of Graphene increased with the increasing of *E. coli* bacteria concentration. Finally, the validation of the device was performed by comparing the data obtained from the device with plate culture method. The device was found to detect the concentration of *E. coli* bacteria selectively with LOD between 145 CFU/ml to 7966 CFU/ml with 104 seconds detection time.

ABSTRAK

Bakteria *Escherichia coli (E. coli)* berasal daripada najis manusia dan haiwan. Ia bahaya kepada kesihatan manusia dan boleh menyebabkan kematian apabila tidak dirawat segera. Oleh itu, adalah penting untuk mengesan bakteria *E. coli* dalam air sebelum mengunakannya. Oleh kerana banyak kes-kes air tercemar oleh bakteria *E. coli* berlaku di kawasan luar bandar, pengesan bakteria *E. coli* yang mudah alih diperlukan. Walaubagaimanapun, banyak biopenderia sedia ada untuk mengesan bakteria *E. coli* tidak mudah alih, sementara kaedah konvensional memakan masa dan memerlukan kepakaran untuk mengunakannya. Dalam penyelidikan ini, biopenderia bakteria *E. coli* berdasarkan kaedah pengesanan perubahan rintangan telah dibangunkan mengunakan struktur nano grafin sebagai lapisan penginderaan, kerana bahan karbon seperti grafin telah diketahui mempunyai sifat keserasian-bio dan sifat pengalir elektrik yang baik. Hasilnya, kepekatan bakteria *E. coli* dapat diukur melalui perubahan kekonduksian yang disebabkan oleh caj negatif yang dihasilkan oleh pemukaan bakteria *E. coli*. Antibodi anti-*E. coli* dan ejen penyekat digunakan pada permukaan grafin untuk tujuan selektiviti. Pencirian mengunakan FESEM, AFM, spektometer Raman dan sudut sentuh telah berjaya dijalankan untuk mengkaji sifat dan mengesahkan kehadiran sifat-sifat imuno pada lapisan penderiaan. Sistem lengkap dibangunkan dengan ruang hubung kait pengguna yang berdiri sendiri yang tidak memerlukan sambungan arus ulang alik (AC) atau sambungan pada komputer peribadi (PC). Micropengawal ARDUINO digunakan untuk mengendalikan peranti baru ini. Litar telah dfungsikan untuk mengesan perubahan rintangan dengan bantuan jambatan Wheatston sebagai penentuukuran peranti. Hasil ujikaji menunjukkan perubahan rintangan dapat dikesan oleh peranti serendah 0.0426 Ω. Untuk mengesan bakteria *E. coli*, sistem ini mempunyai hubungan garis datar antara bacaan peranti dengan kepekatan bakteria *E. coli*. Kekonduktivitian Grafin meningkat dengan peningkatan kepekatan bakteria *E. coli*. Akhirnya, pengesanan peranti dilakukan dengan membandingkan data yang diperolehi dengan kaedah plat kultur. Peranti telah didapati dapat mengesan perubahan kepekatan bacteria *E. coli* dengan selektif dengan had pengesanan antara 145 ke 7966 CFU/ml dengan masa pengesanan 104 saat.

CONTENTS

TITLE		**i**
DECLARATION		**ii**
DEDICATION		**iii**
ACKNOWLEDGMENT		**iv**
ABSTRACT		**v**
ABSTRAK		**vi**
CONTENTS		**vii**
LIST OF TABLES		**xi**
LIST OF FIGURES		**xii**
LIST OF SYMBOLS AND ABBREVIATIONS		**xvii**
LIST OF APPENDICES		**xxii**
CHAPTER 1 INTRODUCTION		**1**
1.1	Overview	1
1.2	Background of study	3
1.3	Problem statement	5
1.4	Objectives	6
1.5	Project scopes	6
1.6	Thesis organisation	7
CHAPTER 2 LITERATURE REVIEW		**8**
2.1	Introduction	8
2.2	*Escherichia coli* bacteria	8
2.3	Conventional methods of *E. coli* bacteria detection	10

2.3.1	Culture based technique	10
2.3.2	Enzyme-linked immunosorbent assay (ELISA)	12
2.3.3	Polymerase chain reaction (PCR)	14
2.4	Available *E. coli* bacteria detectors in the market	16
2.5	Biosensor	19
2.5.1	Direct labelling strategy on biosensor	20
2.5.2	Immunosensing strategy on biosensor	24
2.6	Nanomaterial in biosensor	31
2.7	Graphene	32
2.7.1	Electrical properties	33
2.7.2	Mechanical properties	34
2.7.3	Thermal properties	35
2.8	Graphene-based material for biosensor	36
2.9	Wheatstone bridge	39
2.10	Summary of the chapter	41
CHAPTER 3 RESEARCH METHODOLOGY		**43**
3.1	Introduction	43
3.2	Preparation of immunosensing on graphene sensing layer	45
3.2.1	Cross linker assembly	46
3.2.2	Bioreceptor immobilisation and blocking agent assembly	46
3.3	Characterisations of the sensing layer	47
3.3.1	Morphological characterisation	48
3.3.2	Topological characterisation	49
3.3.3	Wettability test	50

3.3.4 Raman spectroscopy characterisation 51
3.3.5 Two-point probe I-V measurement 51
3.4 Development of device and system design 53
3.4.1 Fabrication of electrode in sensing part 53
3.4.2 Optimisation of device sensitivity 56
3.4.3 Electrical circuit design 57
3.4.4 Software programming 60
3.4.5 Mechanical hardware and design 61
3.5 *E. coli* bacteria preparation 62
3.5.1 Sterilisation process 63
3.5.2 Culturing *E. coli* bacteria on solid medium 64
3.5.3 *E. coli* bacteria suspension 65
3.5.4 *E. coli* bacteria dilution and cell viability test 66
3.6 Device evaluation and testing 67
CHAPTER 4 RESULTS AND DISCUSSION 69
4.1 Characterisation of immunosensing on graphene layer 69
4.1.1 Morphological structure 69
4.1.2 Topological structure of sensing layer 73
4.1.3 Contact angle 76
4.1.4 Raman spectra of sensing layer 78
4.1.5 Electrical properties 80
4.2 Interaction properties of immunosensor on graphene 81
4.3 Device and sensing electrode assembling 83
4.4 *E. coli* bacteria sampling 86

4.4.2 Growing bacteria in solid medium 86

4.4.2 Growing bacteria in liquid medium 87

4.5 Electrical properties of immunosensing layer on different *E. coli* bacteria concentration 89

4.6 Device performance evaluation 92

4.6.1 Effect of fixed resistor value on device sensitivity and linear respond 92

4.6.2 Comparison between simulation and device measured reading 93

4.6.3 Stability and selectivity evaluation 95

4.6.4 Effect of *E. coli* bacteria concentration on device reading 96

4.6.5 Analysis of User/reader interface performance with Wheatstone bridge operation 99

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS 104

5.1 Conclusion 104

5.2 Recommendations 105

REFERENCES 106

VITA 134

LIST OF TABLES

2.1	Features of *E. coli* bacteria detectors available on market	19
2.2	Characteristics of the common enzyme-substrate technique for simultaneous detection of *E. coli* bacteria	22
2.3	Comparison between nanomaterial for *E. coli* bacteria detection using electrical approach	32
2.4	Existing graphene antibody-based biosensors for *E. coli* bacteria detection using electrical approach	38
3.1	The connection port between Arduino and LCD	58
4.1	The variables R_a, R_q, and R_z based on surface treated	75
4.2	Raman spectra of anti-*E. coli* antibodies and BSA as the protein	79
4.3	Driving current before and after immobilisation process	80
4.4	Sensor device packaging with its features	85
4.5	Growth of *E. coli* bacteria on original nutrient broth and after 1:1000 dilution factor	88
4.6	I-V curves of the graphene immunosensor with differences *E. coli* bacteria concentrations	90
4.7	Gradient results of simulation with difference value of fixed resistor	93
4.8	Steady state of the device changing reading	98

LIST OF FIGURES

1.1	TEM image of *E. coli* bacteria cell	2
1.2	Solid and liquid classical cultivation	3
2.1	*Escherichia coli* bacteria cells	9
2.2	Interpretation culture methods as the bacterial indicator (a) MTF method, (b) plate counting method, (c) MF method	11
2.3	ELISA microplate	12
2.4	Schematic representation of the ELISA assay procedure	13
2.5	A microplate reader from Thermo Fisher scientific	13
2.6	The PCR technique operation steps	14
2.7	The multiplex PCR amplified products from bacterial DNA	15
2.8	The Speedy Breedy device	16
2.9	PCR device with IQ-Check® *E. coli* O157H7 PCR detection kit	17
2.10	The PalmPCR™ from Ahram biosystems, Inc.	18
2.11	The E.coREADi™ field-ready assay kit	18
2.12	The biosensor operations	20
2.13	GUD activity of *E. coli* bacteria with specific fluorogenic substrates	21
2.14	Prototyping of *E. coli* bacteria fluorescent biosensor using cell-phone	22
2.15	Generalised of immunosensor method, (a) protein immobilised probe (b) target analyte (c) second probing label is added to increase the sensitivity	24
2.16	The classification of Immunosensor-based	24

2.17	Schematic illustration of *E. coli* bacteria detection using (a) fibre optics, (b) planar waveguides and (c) surface plasmon resonance	27
2.18	Schematic diagram of the piezoelectric biosensor I (a) detection well; (b) detection plate; II: data processing system; III: PC interface	29
2.19	Chemiresistive biosensor mechanism (a) integrated sensor array (b)(c)(d) readout interface	30
2.20	The Pristine graphene (pure carbon atoms) with carbon atoms sp^2-hybridised	33
2.21	Graphene-based materials which can be immobilised as the receptor with biomolecules	37
2.22	Scheme of graphene modified with antibodies for the recognition of pathogens	37
2.23	Schematic of standard Wheatstone bridge	40
2.24	Schematic of a full bridge GMR sensor	41
2.25	The oscillator and detector integrating with the Wheatstone bridge	41
3.1	Overall process flow for the development of portable *E. coli* bacteria detection using resistive graphene-based immunosensor	44
3.2	Graphene thin film (a) the packaging (b) used substrate	45
3.3	Composition step of the bioreceptor and the blocking agent on graphene/SiO_2/Si thin film	45
3.4	Anti-*E. coli* antibodies linker's agents (a) 1-pyrenebutiric acid (PBA) (b) N-hydroxysuccinimide	46
3.5	Linker assembly process	46
3.6	Immunoassay agents, (a) anti-*E. coli* O & K antibody (b) bovine serum albumin	47
3.7	Bioreceptor immobilisation and blocking agent assembly process	47
3.8	FESEM at MiNT-SRC, UTHM	48
3.9	AFM at MiNT-SRC, UTHM	49

3.10	Contact angle machine at MiNT-SRC, UTHM	50
3.11	Raman spectrometer at MiNT-SRC, UTHM	51
3.12	I-V measurement setup with Oriel instruments software	52
3.13	Two voltage divider that connected in parallel to form wheatstone bridge	53
3.14	Process flow for electrode sensing part asembling	54
3.15	Legs for making electrical connection	55
3.16	Substrate holder and casing for sensing chip	56
3.17	Custom design of PCB electrode	56
3.18	Arrangement of voltage divider circuit for simulation	57
3.19	Mini ATmega328 Arduino Nano board	57
3.20	I^2c Module	58
3.21	Circuit schematic for PCB design	59
3.22	PCB layout design for circuit development	59
3.23	Proposed messages displayed on the LCD-screen	60
3.24	Design the device using Sketch up software	61
3.25	Import to Cubicreator 3D software	61
3.26	Flow chart for preparing *E. coli* bacteria and testing.	62
3.27	Autoclave machine	63
3.28	Medium Agar preparation	64
3.29	Cell culture for *E. coli* bacteria stock preparation	64
3.30	Nutrient broth medium preparation	65
3.31	*E. coli* bacteria suspension preparation	65
3.32	Dilution step of bacteria suspension	66
3.33	The colony counter device for counting the bacteria colony	67
4.1	FESEM image of (a) graphene/SiO_2/Si (b) anti-*E. coli* antibodies with BSA/PBA-NHS/graphene/SiO_2/Si	71
4.2	Captured *E. coli* bacteria on sensing layer at (a) 1,000x magnification (b) 30,000x magnification	72
4.3	Gathered Streptococcus bacteria on sensing layer at (a) 3,000x magnification (b) 10,000x magnification	73
4.4	2D and 3D topology of (a) graphene/SiO_2/Si (b) anti-*E. coli* antibodies with BSA/PBA-NHS/graphene/SiO_2/Si	75

4.5	Height profiles of the line scans (arrows) on Figure 4.4	75
4.6	The behaviour of water droplet on the surface of (a) graphene film, (b) anti-*E. coli* antibodies with BSA/PBA-NHS/graphene/SiO_2/Si	77
4.7	Raman image of graphene before and after undergo immobilisation	79
4.8	Raman shift of graphene and after added anti-*E. coli* antibodies and BSA	79
4.9	Schematic interaction of 1-pyrenebutanoic acid succinimidyl ester (PBA-NHS) with graphene in cross linker assembly	82
4.10	Schematic interaction of anti-*E. coli* antibody with the linker	82
4.11	Schematic reaction of free amide linkage with the BSA	82
4.12	Soldered sensing connector to PCB (a) front (b) back	83
4.13	Fabricated PCB and electronic component (a) front (b) back	84
4.14	Fully assembling of casing on the sensing part	84
4.15	Genuine *E. coli* bacteria strain in medium Agar	86
4.16	(a) Nutrient broth solution + *E. coli* bacteria strain, (b) Nutrient broth solution + *E. coli* bacteria strain + incubated for 10 hours	87
4.17	*E. coli* bacteria growth curve up to ten hours after 11000 of dilution factor on Nutrient broth solution [sample for electrical measurement]	89
4.18	Optimisation results from simulation with difference value of fixed resistor	93
4.19	Evaluation between Proteus software simulation and device measurement	94
4.20	Device calibration and selectivity test	96
4.21	Respond time of the device reading on Arduino serial monitor software	97
4.22	Linear fit graph between CFU/ml and device reading	98
4.23	LOD of the device reading	99

4.24	Half bridge principle used in this research	100
4.25	Condition before inserting the sensor	101
4.26	Result on LCD when no sensor was inserted	101
4.27	Condition after inserting the sensor	102
4.28	Calibration display by adjusting to zero value	102
4.29	Device display when no *E. coli* bacteria presented (a) Output voltage ($V_{out} = V_C - V_D$) (b) number of colonies (c) result from CFU/ml reading	103
4.30	Device display when *E. coli* bacteria presented (a) Output voltage ($V_{out} = V_C - V_D$) (b) number of colonies (c) result from CFU/ml reading	103

LIST OF SYMBOLS AND ABBREVIATIONS

πα	-	Absorption coefficient
μm	-	Micrometer
α	-	constant
ΔR	-	Change of resistance
π	-	pi
2D	-	2 dimensional
3D	-	3 dimensional
4MU	-	4-methylumbelliferyl
A	-	Absorption
a.u.	-	Relative emission intensity
AC	-	Alternating current
ADC	-	Analogue-digital-converter
AFM	-	Atomic force microscopic
APHA	-	American Public Health Association
BSA	-	Bovine serum albumin
C	-	Carbon
CAD	-	Computer-aided design
CCD	-	Charge-coupled device
CFU	-	Colony forming unit
CL	-	Chemiluminescence

CMOS	-	Complementary metal-oxide-semiconductor
CNTs	-	Carbon nanotubes
CRF	-	Crystal resonance frequency
CVD	-	Chemical vapor deposition
D	-	Defect of graphene
DC	-	Direct current
DI	-	Deionised
DNA	-	Deoxyribonucleic acid
E. coli	-	*Escherichia coli*
e.g.	-	for example
EDC	-	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
ELISA	-	Enzyme-linked immunosorbent assay
EPA	-	Environmental Protection Agency
FESEM	-	Field emission scanning electron microscope
G	-	Graphitise of graphene
GMR	-	Giant magnetoresistive
GO	-	Graphene oxide
GUD	-	β-d-Glucuronidase
H_2O	-	Water
I^2C	-	Inter-Integrated circuit
IoT	-	Internet of things
ISFET	-	Ion-sensitive field-effect transistor
I-V	-	Current-Voltage
K	-	Kelvin

LCD	-	Liquid cristal display
LOD	-	Limit of detection
m-TAS	-	micro total analysis system
MF	-	Membrane filtration
MHDA	-	16-mercaptopropanoic acid
MiNT-SRC	-	Microelectronics and Nanotechnology- Shamsuddin Research Centre
mm	-	Millimeter
MPN	-	Most probable number
MTF	-	Multiple tube fermentation
MUG	-	4-methylumbelliferyl-b-D-glucuronide
N	-	Number of atoms
NHS	-	N-hydroxysuccinimide
PBA	-	1-pyrenebutanoic acid
PBA-NHS	-	1-pyrenebutanoic acid succinimidyl ester
PC	-	Personal computer
PCB	-	Printed circuit board
PCR	-	Polymerase chain reaction
PLA	-	polylactic acid
PMA	-	Propidium monoazide
PMMA	-	Polymethyl methacrylate
PLA	-	Polylactic acid
POC	-	Point - of - care
PPM	-	parts per million
PSPD	-	Position-sensitive photo diode

QCM	-	Quartz crystal microbalance
R_{adj}	-	Adjusted resistor
R_C	-	calibrating resistor
R_a	-	Average roughness
rGO	-	Reduced graphene oxide
RP	-	Rapid prototyping
R_q	-	Root mean square roughness
RT	-	Reverse transcriptase
R_s	-	Resistance of the sensor
R_z	-	Ten-point average roughness
SAM	-	Self-assembly monolayer
SAW	-	Surface acoustic wave
SCL	-	Serial clock line
SDA	-	Serial data line
SEM	-	Scanning electron microscopy
Si	-	Silicon
SiO_2	-	Silicon dioxide
SPR	-	Surface plasmon resonance
STL	-	Standard transform language
STW	-	Surface transverse wave
TEM	-	Transmission electron microscopy
TMAO	-	Trimethylamine N-oxide
USB	-	Universal serial bus
US	-	United Stated

UTHM	-	Universiti Tun Hussein Onn Malaysia
V_c	-	Voltage at point C
V_d	-	Voltage at point D
V_G	-	Gate voltage
V_{in}	-	Input voltage
V_{out}	-	Output voltage
W	-	Watt
WHO	-	World Health Organisation

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A		128
B	List of Awards	129
C	Arduino coding for the prototype system programme	130

CHAPTER 1

INTRODUCTION

1.1 Overview

Some of the major health risks, constituted by microorganisms as a source of bacteria or pathogen contamination. It may survive, reproduce and disperse in water systems [1]. Contaminated water can transmit diseases such diarrhoea, cholera, dysentery, typhoid, and polio [2]. Safety management and water treatment for drinking water is crucial for human consumption. Occasionally, water monitoring is necessary to provide a decision making on managing water quality and water treatment today and in the future. Water quality monitoring is used to alert the user to the current, ongoing and emerging problems so that the drinking water standard can be comply. On today's trajectory, 785 million people in this world use untreated water supplies and estimated of 144 million people collect untreated surface water from lakes, ponds, rivers and streams [2]. This mostly happens in the developing & third world countries, such as Myanmar, Cambodia, Bolivia, India, Bangladesh, Nepal, Somalia, Ethiopia, Kenya and most of countries in Africa [2].

Globally, at least two billion people use drinking water sources that are contaminated with faeces, which contribute to faecal contaminants. Faecal contaminants come from human and animal faeces and can spread around the water sources [3]. Unsafe water supplies, inadequate sanitation and poor hygiene attribute to 88% of deaths, which is more compared with deaths from AIDS, malaria and measles combined [4]. Most of the death cases involve children under five years old, which comprise 297,000 deaths [2]. It kills almost 800 children per day, or one child every two minutes. A person living in a third world country typically cannot afford to pay

money for usable water. This forces the people in poorer countries to use water from any available source, even if the water is contaminated and unsafe.

Human and animal waste in water resource can cause the growing of various types of harmful bacteria cells such as *E. coli, Campylobacter Jejuni, Salmonella, Vibriocholerae* and *Shigellae* in water resource [5]. A method or approach that can be used to identify all of the interest pathotypes selectively, are almost none and directly measuring a large variety of pathotypes will be costly, difficult and time consuming [6]. Therefore, a method to specifically detect one type of pathotype species as indicator to faecal contaminant need be to developed. *Escherichia coli* or *E. coli* bacteria, as shown in Figure 1.1 is the outstanding coliform bacteria, known as the important indicator for faecal contamination because it can be found almost exclusively in human and animal faeces and some of it became pathogenic outside of the intestinal tract [7].

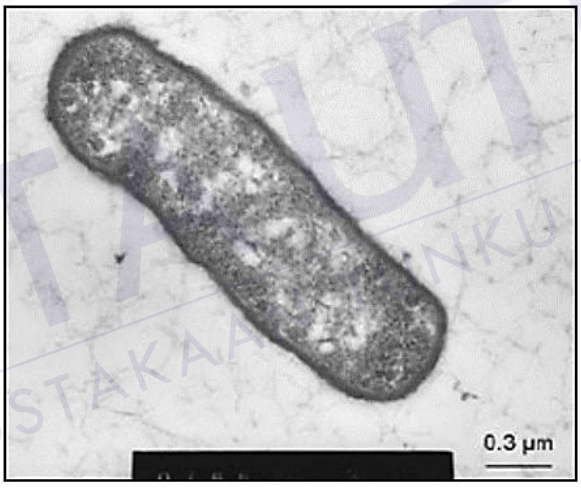

Figure 1.1: TEM image of *E. coli* bacteria cell [8]

Therefore, measuring *E. coli* bacteria become critical issue for indicating faecal contaminant from human and animal faeces that spread in the water sources. The monitoring device to detect amount of *E. coli* bacteria is crucial for indicating the quality of water and to protect public health. It remain significant risk to people for minimising the spreading of infectious disease from *E. coli* bacteria because it can lead to diseases such as bloody diarrhoea, kidney failure and can cause death if not treated immediately [9].

People living in third world countries cannot just turn on a faucet and be immediately rewarded with clean water. In order to have any water, the inhabitants

may have to go acquire it. In many third world countries, this task falls to the responsibility of the women of the household while the men work to provide financially. Every day women throughout the world will spend a collective 200 million hours collecting water. Some women walk up to three hours a day to get water to bring back to their families. They will have to travel, often on a dangerous path, to a natural water sources like rivers. The rivers can be filthy-full of waste which the whole families will use that day for drinking, cleaning, cooking and other activities [10]. There has a possibility that the water collection that they acquire has faecal contaminant. A standalone *E. coli* bacteria biosensor is needed for easy water quality monitoring. The user can test the water sample directly on sites before collecting it. Subsequently, this monitoring will lead to suitable water treatment to be built in the area. The role of label-free biosensor is also importance in order to make the monitoring process simpler.

1.2 Background of study

Conventional bacteria detection methods largely rely on microbiological and biochemical cultivation techniques [11]. Typically, the methods need to have bacteria cultured on selected chromogenic medium that are specifically framed to allow the growth of the concerned species and to obstruct the growth of other organisms. Figure 1.2 shows an example of solid and liquid microbial cultures for testing the presence of *E. coli* bacteria. The determination of enzymatic activity by the cultivation method is considered very accurate but lacking in terms of rapidity.

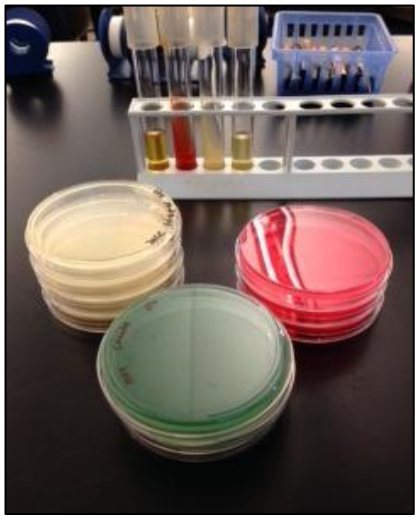

Figure 1.2: Solid and liquid classical cultivation [12]

REFERENCES

1. W. H. Organization and UNICEF, “Progress on drinking water, sanitation and hygiene: 2017 update and SDG baselines,” 2017. [Online]. Available: https://www.who.int/mediacentre/news/releases/2017/launch-version-report-jmp-water-sanitation-hygiene.pdf. [Accessed: 27-Nov-2019].
2. “Drinking water,” *World Health Organization*, 2019. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/drinking-water. [Accessed: 27-Nov-2019].
3. I. P. and H. V. Ana I. Gil, Claudio F. Lanata, Stella M. Hartinger, Daniel Mäusezahl, Beatriz Padilla, Theresa J. Ochoa, Michelle Lozada, “Fecal contamination of food, water, hands, and kitchen utensils at the household level in rural areas of Peru,” *Journal of environmental health*, vol. 76, no. 6, pp. 102–107, 2014.
4. UNICEF and WHO, *Progress on household drinking water, sanitation and hygiene 2000-2017: Special focus on inequalities*. 2019.
5. S. A. Abdallah, “Detection and differentiation of Escherichia coli populations from human, animal and avian feces, and different water sources,” *Polish Journal of Environmental Studies*, vol. 14, no. 5, pp. 639–646, 2005.
6. N. J. Ashbolt, “Microbial contamination of drinking water and disease outcomes in developing regions,” *Toxicology*, vol. 198, no. 1–3, pp. 229–238, 2004.
7. V. Tyagi and A. Chopra, “Alternative microbial indicators of faecal pollution: Current perspective,” *Iranian Journal of Environmental health Science and Engineering*, vol. 3, no. 3, pp. 205–216, 2006.
8. C. Saulou, “Synchrotron FTIR microspectroscopy of Escherichia coli at single-cell scale under silver-induced stress conditions,” *Analytical and bioanalytical chemistry*, vol. 405, no. 8, pp. 2685–2697, 2013.

9. L. Slutsker, A. A. Ries, K. D. Greene, J. G. Wells, L. Hutwagner, and P. M. Griffin, "Escherichia coli O157: H7 diarrhea in the United States: clinical and epidemiologic features," *Annals of internal medicine*, vol. 126, no. 7, pp. 505–513, 1997.

10. J. P. Graham, M. Hirai, and S.-S. Kim, "An analysis of water collection labor among women and children in 24 sub-Saharan African countries," *PloS one*, vol. 11, no. 6, pp. 1–14, 2016.

11. M. A. Shogren-Knaak, P. J. Alaimo, and K. M. Shokat, "Recent advances in chemical approaches to the study of biological systems," *Annual review of cell and developmental biology*, vol. 17, no. 1, pp. 405–433, 2001.

12. Holly Ahern, "Bacteriological Culture Methods," *Milne Publishing*, 2017. [Online]. Available: https://milnepublishing.geneseo.edu/suny-microbiology-lab/chapter/bacteriological-culture-methods/. [Accessed: 06-Jan-2017].

13. A. Rompré, P. Servais, J. Baudart, M. R. De-Roubin, and P. Laurent, "Detection and enumeration of coliforms in drinking water: Current methods and emerging approaches," *Journal of Microbiological Methods*, vol. 49, no. 1, pp. 31–54, 2002.

14. J. Y. Yoon and B. Kim, "Lab-on-a-chip pathogen sensors for food safety," *Sensors (Switzerland)*, vol. 12, no. 8, pp. 10713–10741, 2012.

15. P. Daly, T. Collier, and S. Doyle, "PCR-ELISA detection of Escherichia coli in milk," *Letters in Applied Microbiology*, vol. 34, no. 3, pp. 222–226, 2002.

16. T. Guo, "An Optical System towards In-line Monitoring of Bacteria in Drinking Water," Doctoral dissertation, Mcmaster University, 2016.

17. M. Xu, R. Wang, and Y. Li, "Electrochemical biosensors for rapid detection of Escherichia coli O157:H7," *Talanta*, vol. 162, no. 17, pp. 511–522, 2017.

18. Y. C. Lu, Y. S. Chuang, Y. Y. Chen, and A. C. Shu, "Bacteria detection utilizing electrical conductivity," *Biosensors and Bioelectronics*, vol. 23, no. 12, pp. 1856–1861, 2008.

19. S. Leahy and Y. Lai, "A cantilever biosensor exploiting electrokinetic capture to detect Escherichia coli in real time," *Sensors and Actuators, B: Chemical*, vol. 238, no 1, pp. 292–297, 2017.

20. P. D'Orazio, "Biosensors in clinical chemistry," *Clinica Chimica Acta*, vol.

334, no. 1–2, pp. 41–69, 2003.

21. D. Wildeboer, L. Amirat, R. G. Price, and R. A. Abuknesha, “Rapid detection of Escherichia coli in water using a hand-held fluorescence detector,” *Water research*, vol. 44, no. 8, pp. 2621–2628, 2010.

22. R. A. Deshmukh, K. Joshi, S. Bhand, and U. Roy, “Recent developments in detection and enumeration of waterborne bacteria: a retrospective minireview,” *MicrobiologyOpen*, vol. 5, no. 6, pp. 901–922, 2016.

23. A. Benvidi, N. Rajabzadeh, M. Mazloum-Ardakani, M. M. Heidari, and A. Mulchandani, “Simple and label-free electrochemical impedance Amelogenin gene hybridization biosensing based on reduced graphene oxide,” *Biosensors and Bioelectronics*, vol. 58, no. 1, pp. 145–152, 2014.

24. S. Sang, Y. Wang, Q. Feng, Y. Wei, J. Ji, and W. Zhang, “Progress of new label-free techniques for biosensors : a review,” *Critical Reviews in Biotechnology*, vol. 36, no. 3, pp. 465–481, 2015.

25. E. B. Bahadir and M. K. Sezgintürk, “Applications of graphene in electrochemical sensing and biosensing,” *TrAC - Trends in Analytical Chemistry*, vol. 76, no 1, pp. 1–14, 2016.

26. J. Wang, “Carbon-nanotube based electrochemical biosensors: A review,” *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, vol. 17, no. 1, pp. 7–14, 2005.

27. A. H. C. Neto, F. Guinea, N. M. R. Peres, K. S. Novoselov, and A. K. Geim, “The electronic properties of graphene,” *Reviews of modern physics*, vol. 81, no. 1, pp. 109–162, 2009.

28. E. Gerstner, “Nobel prize 2010: Andre geim & konstantin novoselov,” *Nature Physics*, vol. 6, no. 11, pp. 836–836, 2010.

29. K. S. Novoselov, A. K. Geim, and S. V Morozov, “Electric field effect in atomically thin carbon films,” *science*, vol. 306, no. 5696, pp. 666–669, 2004.

30. K. M. Wibowo, M. Z. Sahdan, and N. I. Ramli, “Detection of Escherichia Coli Bacteria in Wastewater by using Graphene as a Sensing Material,” *Journal of Physics: Conference Series*, vol. 995, no. 1, pp. 12063–12070, 2018.

31. K. M. Wibowo, “Fabrication of Graphene-Based Resistive Sensor For Eschericia Coli Bacteria Sensing,” Master Thesis, Universiti Tun Hussein Onn

Malaysia, 2018.

32. N. Cheeveewattanagul, "Straightforward immunosensing platform based on graphene oxide-decorated nanopaper: a highly sensitive and fast biosensing approach," *Advanced Functional Materials*, vol. 27, no. 38, pp. 1702741–1702750, 2017.

33. N. Saucedo, Y. Gao, T. Pham, and A. Mulchandani, "Lectin-and saccharide-functionalized nano-chemiresistor arrays for detection and identification of pathogenic bacteria infection," *Biosensors*, vol. 8, no. 3, pp. 63–74, 2018.

34. A. da S. Arcas, F. da S. Dutra, R. C. S. B. Allil, and M. M. Werneck, "Surface plasmon resonance and bending loss-based U-shaped plastic optical fiber biosensors," *Sensors (Switzerland)*, vol. 18, no. 2, pp. 1–16, 2018.

35. F. Tong, Y. Lian, and J. Han, "On-Line Monitoring the Growth of E . coli or HeLa Cells Using an Annular Microelectrode Piezoelectric Biosensor," *International journal of environmental research and public health*, vol. 13, no. 12, pp. 1254–1264, 2016.

36. R. Kochan, O. Kochan, and M. Chyrka, "Approaches of voltage divider development for metrology verification of ADC," *International Conference on Intelligent Data Acquisition and Advanced Computing Systems (IDAACS)*, vol. 1, no. 1, pp. 70–75, 2013.

37. Y. Yamamoto, Y. Ohno, K. Maehashi, and K. Matsumoto, "Noise reduction of carbon nanotube field-effect transistor biosensors by alternating current measurement," *Japanese Journal of Applied Physics*, vol. 48, no. 6S, pp. 1–6, 2009.

38. K. Rijal, A. Leung, P. M. Shankar, and R. Mutharasan, "Detection of pathogen Escherichia coli O157:H7 at 70 cells/mL using antibody-immobilized biconical tapered fiber sensors," *Biosensors and Bioelectronics*, vol. 21, no. 6, pp. 871–880, 2005.

39. S. T. Odonkor and J. K. Ampofo, "Escherichia coli as an indicator of bacteriological quality of water: an overview," *Microbiology Research*, vol. 4, no. 1, pp. 2–9, 2013.

40. "Escherichia coli bacteria," *Britannica*. Encyclopædia Britannica, inc., 2020.

41. C. Payus, N. Haziqah, N. Basri, and V. L. Wan, "Faecal Bacteria

Contaminations in Untreated Drinking Water (Groundwater Well and Hill Water) from Rural Community Areas," *International Journal of Advances in Science and Technology*, vol. 1, pp. 215–218, 2018.

42. S. C. Edberg, M. J. Allen, and D. B. Smith, *Comparison of the Colilert method and standard fecal coliform methods*. Amer Water Works Assn, 1998.

43. M. E. Bayer and J. L. Sloyer Jr, "The electrophoretic mobility of Gram-negative and Gram-positive bacteria: an electrokinetic analysis," *Microbiology*, vol. 136, no. 5, pp. 867–874, 1990.

44. D. A. Lytle, E. W. Rice, C. H. Johnson, and K. R. Fox, "Electrophoretic mobilities of Escherichia coli O157: H7 and wild-type Escherichia colistrains," *Appl. Environ. Microbiol.*, vol. 65, no. 7, pp. 3222–3225, 1999.

45. A. Terada, K. Okuyama, M. Nishikawa, S. Tsuneda, and M. Hosomi, "The effect of surface charge property on Escherichia coli initial adhesion and subsequent biofilm formation," *Biotechnology and bioengineering*, vol. 109, no. 7, pp. 1745–1754, 2012.

46. J. Li and L. A. McLandsborough, "The effects of the surface charge and hydrophobicity of Escherichia coli on its adhesion to beef muscle," *International journal of food microbiology*, vol. 53, no. 2–3, pp. 185–193, 1999.

47. M. T. Madigan and J. Martinko, *Brock Biology of Microorganisms*, 11th ed. SciELO Espana, 2005.

48. E. J. Baron, *Medical Microbiology*, 4th ed. University of Texas Medical Branch at Galveston, 1996.

49. J. W. F. Joan L. Slonczewski, *Microbiology an evolving science*, 5th ed. Norton Ebook, 2013.

50. R. W. Adler, J. C. Landman, and D. M. Cameron, *The clean water act 20 years later*. Island Press, 1993.

51. S. C. Edberg, M. J. Allen, and D. B. Smith, "National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and Escherichia coli from drinking water: comparison with the standard multiple tube fermentation method.," *Applied and Environmental Microbiology*, vol. 54, no. 6, pp. 1595–1601, 1988.

52. A. G. Bambang, “Analisis cemaran bakteri coliform dan identifikasi Escherichia coli pada air isi ulang dari depot di Kota Manado,” *Pharmacon*, vol. 3, no. 3, pp. 2721–4923, 2014.

53. V. P. Gannon, M. Rashed, R. K. King, and E. J. Thomas, “Detection and characterization of the eae gene of Shiga-like toxin-producing Escherichia coli using polymerase chain reaction.,” *Journal of Clinical Microbiology*, vol. 31, no. 5, pp. 1268–1274, 1993.

54. United States Environmental Protection Agency, “Method 1603: Escherichia coli (E. coli) in water by membrane filtration using modified membrane-thermotolerant Escherichia coli agar (modified mTEC),” EPA-821-R-02–023 US Environmental Protection Agency, 2002.

55. A. P. Dufour, E. R. Strickland, and V. J. Cabelli, “Membrane filter method for enumerating Escherichia coli,” *Applied and Environmental Microbiology*, vol. 41, no. 5, pp. 1152–1158, 1981.

56. G. Tchobanoglous and E. E. Schroeder, “Water quality: characteristics, modeling, modification,” 1985.

57. I. Hubner and D. Steinmetz, I., Obst, U., Giebel, D., Bitter-Suermann, “Rapid determination of members of the family Enterobac- teriaceae in drinking water by an immunological assay using a monoclonal antibody against enterobacterial common antigen.,” *Appl. Environ. Microbiol.*, vol. 58, no. 9, pp. 3187–3191, 1992.

58. R. M. Lequin, “Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA),” *Clinical Chemistry*, vol. 51, no. 12, pp. 2415–2418, 2005.

59. J. R. Crowther, *The ELISA Guidebook*, vol. 149. Springer Science & Business Media, 2001.

60. Thermo Fisher Scientific, “ELISA Instruments and Equipment.” [Online]. Available: https://www.thermofisher.com/my/en/home/life-science/protein-biology/protein-assays-analysis/elisa/elisa-instruments-equipment.html. [Accessed: 01-Apr-2019].

61. K. Mullis, F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich, “Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction,” *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 51, no. 1, pp. 263–

273, 1986.

62. F. Molina, E. López-Acedo, R. Tabla, I. Roa, A. Gómez, and J. E. Rebollo, "Improved detection of Escherichia coli and coliform bacteria by multiplex PCR," *BMC biotechnology*, vol. 15, no. 1, pp. 48–57, 2015.

63. A. Vierstraete, "Principle of the PCR," *University of Ghent, Belgium*, 1999.

64. C. Ferreira, V. Thiemy, M. Moreira, D. Schippers, and A. Greene, "Reverse Transcription and Polymerase Chain Reaction : Principles and," *Journal of applied oral science : revista FOB*, vol. 12, no. 1, pp. 1–11, 2004.

65. L. Soto-Muñoz, N. Teixidó, J. Usall, I. Viñas, A. Crespo-Sempere, and R. Torres, "Development of PMA real-time PCR method to quantify viable cells of Pantoea agglomerans CPA-2, an antagonist to control the major postharvest diseases on oranges," *International Journal of Food Microbiology*, vol. 180, no 1, pp. 49–55, Jun. 2014.

66. M. J. Taylor, R. H. Bentham, and K. E. Ross, "Limitations of Using Propidium Monoazide with qPCR to Discriminate between Live and Dead Legionella in Biofilm Samples," *Microbiology insights*, vol. 7, no. 7, pp. 15–24, 2014.

67. L. Roth, K. B. Biggs, and D. K. Bempong, "Substandard and falsified medicine screening technologies," *AAPS Open*, vol. 5, no. 1, pp. 2–14, 2019.

68. "Speedy breedy," *Orangutan Outreach*. [Online]. Available: https://redapes.org/projects-partners/ovaid/speedy-breedy/. [Accessed: 16-Dec-2019].

69. W. F. Lauer, S. Tymciu, C. D. Sidi, and P. Sonigo, "Validation of iQ-Check E. coli O157: H7 real-time PCR test kit for detection of Escherichia coli O157: H7 in selected foods," *Journal of AOAC International*, vol. 92, no. 4, pp. 1095–1104, 2009.

70. "iQ-Check E. coli O157:H7 PCR Detection Kit," *Bio-Rad*. [Online]. Available: https://www.bio-rad.com/en-ch/product/iq-check-e-coli-o157-h7-pcr-detection-kit?ID=b4010ea4-ddf4-46d1-968e-7c6d3bf42159. [Accessed: 16-Dec-2019].

71. H.-L. Cheah, N. Ravichantar, and T.-H. Tang, "Evaluation of the Palm PCR™ G1-12 System: a portable battery-operated PCR thermal cycler," *Songklanakarin Journal of Science and Technology*, vol. 190, no. 3, pp. 1–21,

2016.

72. "Palm PCR F1-12 Portable PCR System, Economy Version from Ahram Biosystems," *Biocompare*, 2019. [Online]. Available: https://www.biocompare.com/23398-Thermal-Cyclers-Thermocyclers-PCR-Machine/2759716-Palm-PCR-F1-12-Portable-PCR-System/. [Accessed: 16-Dec-2019].

73. C. Tison, "Luna's Assay Tests Water Quality Four Times Faster than Existing Methods," *Luna*, 2016. [Online]. Available: https://lunainc.com/lunas-assay-tests-water-quality-times-faster-existing-methods/. [Accessed: 16-Dec-2019].

74. J. Á. Álvarez, "Development of a polarimetric based optical biosensor using a free standing porous membrane." Universitat de València, 2013.

75. K. Kivirand, M. Kagan, and T. Rinken, "Calibrating Biosensors in Flow-Through Set-Ups: Studies with Glucose Optrodes," in *State of the Art in Biosensors-General Aspects*, InTech, 2013.

76. M. Manafi, "New developments in chromogenic and fluorogenic culture media," *International Journal of Food Microbiology*, vol. 60, no. 2–3, pp. 205–218, 2000.

77. G. Caruso, E. Crisafi, and M. Mancuso, "Development of an enzyme assay for rapid assessment of Escherichia coli in seawaters," *Journal of Applied Microbiology*, vol. 93, no. 4, pp. 548–556, 2002.

78. L. Fiksdal and I. Tryland, "Application of rapid enzyme assay techniques for monitoring of microbial water quality," *Current Opinion in Biotechnology*, vol. 19, no. 3, pp. 289–294, 2008.

79. B. Heery, C. Briciu-Burghina, D. Zhang, and G. Duffy, "ColiSense, today's sample today: A rapid on-site detection of Glucuronidase activity in surface water as a surrogate for E. coli," *Talanta*, vol. 148, no. 1, pp. 75–83, 2016.

80. L. Yang and Y. Li, "Simultaneous detection of Escherichia coli O157：H7 and Salmonella Typhimurium using quantum dots as fluorescence labels," *Analyst*, vol. 131, no. 3, pp. 394–401, 2006.

81. W.-I. Lee, S. Shrivastava, L.-T. Duy, B. Y. Kim, Y.-M. Son, and N.-E. Lee, "A smartphone imaging-based label-free and dual-wavelength fluorescent biosensor with high sensitivity and accuracy," *Biosensors and Bioelectronics*,

vol. 94, no 1, pp. 643–650, 2017.

82. L. Cinquanta, D. E. Fontana, and N. Bizzaro, "Chemiluminescent immunoassay technology: what does it change in autoantibody detection?," *Autoimmunity Highlights*, vol. 8, no. 1, pp. 9–17, 2017.

83. T. Geng, J. Uknalis, S.-I. Tu, and A. K. Bhunia, "Fiber-Optic Biosensor Employing Alexa-Fluor Conjugated Antibody for Detection of Escherichia coli O157:H7 from Ground Beef in Four Hours," *Sensors*, vol. 6, no. 8, pp. 796–807, 2006.

84. Y. Zhang, C. Tan, R. Fei, and X. Liu, "Sensitive chemiluminescence immunoassay for E. coli O157: H7 detection with signal dual-amplification using glucose oxidase and laccase," *Analytical chemistry*, vol. 86, no. 2, pp. 1115–1122, 2014.

85. A. Wolter, R. Niessner, and M. Seidel, "Detection of Escherichia coli O157: H7, Salmonella typhimurium, and Legionella pneumophila in water using a flow-through chemiluminescence microarray readout system," *Analytical chemistry*, vol. 80, no. 15, pp. 5854–5863, 2008.

86. X.-J. Huang, Q.-S. Pu, and Z.-L. Fang, "Capillary electrophoresis system with flow injection sample introduction and chemiluminescence detection on a chip platform," *Analyst*, vol. 126, no. 3, pp. 281–284, 2001.

87. Y. Lv, Z. Zhang, and F. Chen, "Chemiluminescence biosensor chip based on a microreactor using carrier air flow for determination of uric acid in human serum," *Analyst*, vol. 127, no. 9, pp. 1176–1179, 2002.

88. Y. Xu, F. G. Bessoth, J. C. T. Eijkel, and A. Manz, "On-line monitoring of chromium (III) using a fast micromachined mixer/reactor and chemiluminescence detection," *Analyst*, vol. 125, no. 4, pp. 677–683, 2000.

89. B. Li and Z. Zhang, "Chemiluminescence flow biosensor for determination of total D-amino acid in serum with immobilized reagents," *Sensors and Actuators B: Chemical*, vol. 69, no. 1–2, pp. 70–74, 2000.

90. B. Li, Z. Zhang, and Y. Jin, "Plant tissue-based chemiluminescence flow biosensor for glycolic acid," *Analytical chemistry*, vol. 73, no. 6, pp. 1203–1206, 2001.

91. M. C. Ramos, M. C. Torijas, and A. N. Dıaz, "Enhanced chemiluminescence

biosensor for the determination of phenolic compounds and hydrogen peroxide," *Sensors and Actuators B: Chemical*, vol. 73, no. 1, pp. 71–75, 2001.

92. B. Schweitzer, P. Predki, and M. Snyder, "Microarrays to characterize protein interactions on a whole-proteome scale," *Proteomics*, vol. 3, no. 11, pp. 2190–2199, 2003.

93. Y. Bai, Y. Sun, and C. Sun, "Pt–Pb nanowire array electrode for enzyme-free glucose detection," *Biosensors and Bioelectronics*, vol. 24, no. 4, pp. 579–585, 2008.

94. Y. Liu, Z. Matharu, M. C. Howland, A. Revzin, and A. L. Simonian, "Affinity and enzyme-based biosensors: recent advances and emerging applications in cell analysis and point-of-care testing," *Analytical and bioanalytical chemistry*, vol. 404, no. 4, pp. 1181–1196, 2012.

95. Martin M. F. Choi, "Fundamental Review Progress in Enzyme-Based Biosensors Using Optical Transducers," vol. 132, no. 1, pp. 107–132, 2004.

96. T. Xie, A. Wang, L. Huang, H. Li, Z. Chen, and Q. Wang, "Recent advance in the support and technology used in enzyme immobilization," vol. 8, no. 19, pp. 4724–4733, 2009.

97. M. A. Prieto and J. García, "Identification of the 4-hydroxyphenylacetate transport gene of Escherichia coli W: construction of a highly sensitive cellular biosensor," *FEBS letters*, vol. 414, no. 2, pp. 293–297, 1997.

98. S. Sang and H. Witte, "A novel PDMS micro membrane biosensor based on the analysis of surface stress," *Biosensors and bioelectronics*, vol. 25, no. 11, pp. 2420–2424, 2010.

99. R. Jain, S. Miri, V. L. Pachapur, and S. K. Brar, "Advances in antibody-based biosensors in environmental monitoring," in *Tools, Techniques and Protocols for Monitoring Environmental Contaminants*, Elsevier, 2019.

100. D. Wang, T. Hinkley, J. Chen, J. N. Talbert, and S. R. Nugen, "Phage based electrochemical detection of Escherichia coli in drinking water using affinity reporter probes," *Analyst*, vol. 144, no. 4, pp. 1345–1352, 2019.

101. M. Holzinger, A. Le Goff, and S. Cosnier, "Nanomaterials for biosensing applications: a review," *Frontiers in chemistry*, vol. 2, no. 1, pp. 63–73, 2014.

102. B. M. Beam, J. L. Burnett, N. A. Webster, and S. B. Mendes, "Applications of

the Planar Fiber Optic Chip," *Recent Progress in Optical Fiber Research*, vol. 1, no. 5, pp. 387–411, 2008.

103. H. Mukundan, A. S. Anderson, W. K. Grace, and K. M. Grace, "Waveguide-based biosensors for pathogen detection," *Sensors*, vol. 9, no. 7, pp. 5783–5809, 2009.

104. P. Kozma, F. Kehl, E. Ehrentreich-förster, C. Stamm, and F. F. Bier, "Biosensors and Bioelectronics Integrated planar optical waveguide interferometer biosensors : A comparative review," vol. 58, no. 1, pp. 287–307, 2014.

105. N. Tawil, E. Sacher, R. Mandeville, and M. Meunier, "Surface plasmon resonance detection of E. coli and methicillin-resistant S. aureus using bacteriophages," *Biosensors and Bioelectronics*, vol. 37, no. 1, pp. 24–29, 2012.

106. E. Stenberg, B. Persson, H. Roos, and C. Urbaniczky, "Quantitative determination of surface concentration of protein with surface plasmon resonance using radiolabeled proteins," *Journal of colloid and interface science*, vol. 143, no. 2, pp. 513–526, 1991.

107. S. K. Mishra, S. N. Tripathi, V. Choudhary, and B. D. Gupta, "SPR based fibre optic ammonia gas sensor utilizing nanocomposite film of PMMA/reduced graphene oxide prepared by in situ polymerization," *Sensors and Actuators, B: Chemical*, vol. 199, pp. 190–200, 2014.

108. P. Pattnaik, "Surface plasmon resonance," *Applied biochemistry and biotechnology*, vol. 126, no. 2, pp. 79–92, 2005.

109. P. M. Fratamico, T. P. Strobaugh, M. B. Medina, and A. G. Gehring, "Detection of Escherichia coli 0157: H7 using a surface plasmon resonance biosensor," *Biotechnology techniques*, vol. 12, no. 7, pp. 571–576, 1998.

110. S. Roh, T. Chung, and B. Lee, "Overview of the characteristics of micro-and nano-structured surface plasmon resonance sensors," *Sensors*, vol. 11, no. 2, pp. 1565–1588, 2011.

111. H. Xin, Q. Liu, and B. Li, "Non-contact fiber-optical trapping of motile bacteria: dynamics observation and energy estimation," *Scientific Reports*, vol. 4, no. 1, pp. 6576–6582, 2014.

112. R. Bharadwaj, V. V. R. Sai, K. Thakare, and A. Dhawangale, “Evanescent wave absorbance based fiber optic biosensor for label-free detection of E. coli at 280nm wavelength,” *Biosensors and Bioelectronics*, vol. 26, no. 7, pp. 3367–3370, 2011.

113. Z. Sun, J., Wang, X., Yin, C., Xiao, P., Li, H., & Cao, “Optical transduction of E. Coli O157:H7 concentration by using the enhanced Goos-Hänchen shift,” *Journal of Applied Physics*, vol. 112, no. 8, pp. 083104–083109, 2012.

114. P. Singh, “SPR biosensors: historical perspectives and current challenges,” *Sensors and actuators B: Chemical*, vol. 229, pp. 110–130, 2016.

115. N. Trivellin, M. Yushchenko, and M. Buffolo, “Laser-based lighting: experimental analysis and perspectives,” *Materials*, vol. 10, no. 10, pp. 1166–1184, 2017.

116. M. Pohanka, P. Skládal, and O. Pavliš, “Label-Free Piezoelectric Immunosensor for Rapid Assay of Escherichia coli,” *Journal of Immunoassay and Immunochemistry*, vol. 29, no. 1, pp. 70–79, 2007.

117. X.-L. Su and Y. Li, “A self-assembled monolayer-based piezoelectric immunosensor for rapid detection of Escherichia coli O157: H7,” *Biosensors and Bioelectronics*, vol. 19, no. 6, pp. 563–574, 2004.

118. V. K. Thanh Ngo, D. G. Nguyen, and H. P. Uyen Nguyen, “Quartz crystal microbalance (QCM) as biosensor for the detecting of Escherichia coli O157:H7,” *Advances in Natural Sciences: Nanoscience and Nanotechnology*, vol. 5, no. 4–13, pp. 045004–045013, 2014.

119. J. H. Lei, J. W. Ding, and W. Qin, “A chronopotentiometric flow injection system for aptasensing of E. coli O157,” *Analytical Methods*, vol. 7, no. 3, pp. 825–829, 2015.

120. K. O. Colquhoun, S. Timms, and C. R. Fricker, “Detection of Escherichia coli in potable water using direct impedance technology,” *Journal of applied bacteriology*, vol. 79, no. 6, pp. 635–639, 1995.

121. Z. Li, Y. Fu, W. Fang, and Y. Li, “Electrochemical Impedance Immunosensor Based on Self-Assembled Monolayers for Rapid Detection of Escherichia coli O157:H7 with Signal Amplification Using Lectin,” *Sensors*, vol. 15, no. 8, pp. 19212–19224, 2015.

122. Z. Muhammad-Tahir and E. C. Alocilja, “Fabrication of a disposable biosensor for Escherichia coli O157: H7 detection,” *IEEE sensors Journal*, vol. 3, no. 4, pp. 345–351, 2003.

123. J. C.Whitaker, *The Electronic Handbook*, 2nd ed. CRC Press, 2005.

124. M. Ko, A. Aykanat, M. Smith, and K. Mirica, “Drawing sensors with ball-milled blends of metal-organic frameworks and graphite,” *Sensors*, vol. 17, no. 10, pp. 2192–2209, 2017.

125. Y. Li, H. J. Schluesener, and S. Xu, “Gold nanoparticle-based biosensors,” *Gold Bulletin*, vol. 43, no. 1, pp. 29–41, 2010.

126. J. M. Pingarrón, P. Yañez-Sedeño, and A. González-Cortés, “Gold nanoparticle-based electrochemical biosensors,” *Electrochimica Acta*, vol. 53, no. 19, pp. 5848–5866, 2008.

127. G.-J. Zhang and Y. Ning, “Silicon nanowire biosensor and its applications in disease diagnostics: a review,” *Analytica Chimica Acta*, vol. 749, no. 1, pp. 1–15, 2012.

128. F. Patolsky, G. Zheng, and C. M. Lieber, “Nanowire-based biosensors.” *ACS Publications*, 2006.

129. C. R. Howlett, M. D. M. Evans, K. L. Wildish, and J. C. Kelly, “The effect of ion implantation on cellular adhesion,” *Clinical materials*, vol. 14, no. 1, pp. 57–64, 1993.

130. C. I. L. Justino, A. R. Gomes, A. C. Freitas, A. C. Duarte, and T. A. P. Rocha-Santos, “Graphene based sensors and biosensors,” *TrAC Trends in Analytical Chemistry*, vol. 91, no. 1, pp. 53–66, 2017.

131. E. W. Hill, A. Vijayaragahvan, and K. Novoselov, “Graphene sensors,” *IEEE Sensors Journal*, vol. 11, no. 12, pp. 3161–3170, 2011.

132. T. Baron, M. Mongillo, L. Poupinet, and B. Salem, “Beyond CMOS Nanodevices for Adding Functionalities to CMOS,” *Nanofunction*, vol. 1, no. 9, pp. 1–16, 2011.

133. Y. Wang, P. A. Fewins, and E. C. Alocilja, “Electrochemical immunosensor using nanoparticle-based signal enhancement for Escherichia coli O157: H7 detection,” *IEEE Sensors Journal*, vol. 15, no. 8, pp. 4692–4699, 2015.

134. M. S. Webster “Detection of bacterial metabolites for the discrimination of bacteria utilizing gold nanoparticle chemiresistor sensors,” *Sensors and Actuators B: Chemical*, vol. 220, pp. 895–902, 2015.

135. M. Basu “Nano-biosensor development for bacterial detection during human kidney infection: use of glycoconjugate-specific antibody-bound gold NanoWire arrays (GNWA),” *Glycoconjugate Journal*, vol. 21, no. 8–9, pp. 487–496, 2004.

136. C. García-Aljaro, M. A. Bangar, E. Baldrich, F. J. Muñoz, and A. Mulchandani, “Conducting polymer nanowire-based chemiresistive biosensor for the detection of bacterial spores,” *Biosensors and Bioelectronics*, vol. 25, no. 10, pp. 2309–2312, 2010.

137. H. So, “Detection and titer estimation of Escherichia coli using aptamer-functionalized single-walled carbon-nanotube field-effect transistors,” *Small*, vol. 4, no. 2, pp. 197–201, 2008.

138. C. García-Aljaro, “Carbon nanotubes-based chemiresistive biosensors for detection of microorganisms,” *Biosensors and Bioelectronics*, vol. 26, no. 4, pp. 1437–1441, 2010.

139. E. Akbari, Z. Buntat, A. Afroozeh, A. Zeinalinezhad, and A. Nikoukar, “Escherichia coli bacteria detection by using graphene-based biosensor,” *IET nanobiotechnology*, vol. 9, no. 5, pp. 273–279, 2015.

140. Y. Huang, X. Dong, Y. Liu, L.-J. Li, and P. Chen, “Graphene-based biosensors for detection of bacteria and their metabolic activities,” *Journal of Materials Chemistry*, vol. 21, no. 33, pp. 12358–12362, 2011.

141. A. Pandey, Y. Gurbuz, V. Ozguz, J. H. Niazi, and A. Qureshi, “Graphene-interfaced electrical biosensor for label-free and sensitive detection of foodborne pathogenic E. coli O157: H7,” *Biosensors and Bioelectronics*, vol. 91, no. 1, pp. 225–231, 2017.

142. M. Pisarek, M. Holdynski, and M. Krawczyk, “Surface characterization of graphene based materials,” *Applied Surface Science*, vol. 388, no. 1, pp. 696–703, 2016.

143. S. V Morozov, K. S. Novoselov, M. I. Katsnelson, and F. Schedin, “Giant intrinsic carrier mobilities in graphene and its bilayer,” *Physical review letters*,

vol. 100, no. 1, pp. 16602–16606, 2008.

144. The Editors of Encyclopaedia Britannica, “Carbon,” *Encyclopaedia Britannica*. Encyclopædia Britannica, inc, 2019.

145. P. Suvarnaphaet and S. Pechprasarn, “Graphene-based materials for biosensors: a review,” *Sensors*, vol. 17, no. 10, pp. 2161–2185, 2017.

146. J.-C. Charlier, P. C. Eklund, J. Zhu, and A. C. Ferrari, “Electron and phonon properties of graphene: their relationship with carbon nanotubes,” in *Carbon nanotubes*, Springer, 2007.

147. K. Xu, C. Zeng, Q. Zhang, and R. Yan, “Direct measurement of Dirac point energy at the graphene/oxide interface,” *Nano letters*, vol. 13, no. 1, pp. 131–136, 2012.

148. C. Lee, X. Wei, J. W. Kysar, and J. Hone, “Measurement of the elastic properties and intrinsic strength of monolayer graphene,” *science*, vol. 321, no. 5887, pp. 385–388, 2008.

149. R. J. T. Nicholl, H. J. Conley, N. V Lavrik, and I. Vlassiouk, “The effect of intrinsic crumpling on the mechanics of free-standing graphene,” *Nature communications*, vol. 6, no. 1, pp. 8789–8796, 2015.

150. A. A. Balandin, S. Ghosh, and W. Bao, “Superior Thermal Conductivity of Single-Layer Graphene,” *Nano Lett.*, vol. 8, no. 1, pp. 902–907, 2008.

151. C. Yu, L. Shi, Y. Zao, D. Li, and A. Majumdar, “Thermal Conductance of an Individual Single-Wall Carbon Nanotube above Room Temperature,” *Nano Letters*, vol. 6, no. 1, pp. 96–100, 2005.

152. S. Ghosh, I. Calizo, D. Teweldebrhan, and E. P. Pokatilov, “Extremely high thermal conductivity of graphene: Prospects for thermal management applications in nanoelectronic circuits,” *Applied Physics Letters*, vol. 92, no. 15, pp. 2–4, 2008.

153. M. D. Stoller, S. Park, Z. Yanwu, J. An, and R. S. Ruoff, “Graphene-Based ultracapacitors,” *Nano Letters*, vol. 8, no. 10, pp. 3498–3502, 2008.

154. M. Kalbacova, A. Broz, J. Kong, and M. Kalbac, “Graphene substrates promote adherence of human osteoblasts and mesenchymal stromal cells,” *Carbon*, vol. 48, pp. 4323–4329, 2010.

155. M. Pumera, “Graphene in biosensing,” *Materials today*, vol. 14, no. 7–8, pp.

308–315, 2011.

156. N. Chauhan, T. Maekawa, and D. N. S. Kumar, “Graphene based biosensors—accelerating medical diagnostics to new-dimensions,” *Journal of Materials Research*, vol. 32, no. 15, pp. 2860–2882, 2017.

157. C. N. R. Rao, A. K. Sood, K. S. Subrahmanyam, and A. Govindaraj, “Graphene: the new two-dimensional nanomaterial,” *Angewandte Chemie International Edition*, vol. 48, no. 42, pp. 7752–7777, 2009.

158. D. Li, W. Zhang, X. Yu, Z. Wang, Z. Su, and G. Wei, “When biomolecules meet graphene: From molecular level interactions to material design and applications,” *Nanoscale*, vol. 8, no. 47, pp. 19491–19509, 2016.

159. R. S. Figliola and D. E. Beasley, *Theory and Design for Mechanical Measurements*, 4th ed. John Wiley and Sons, 2006.

160. D. Bitounis, H. Ali-Boucetta, B. H. Hong, D. Min, and K. Kostarelos, “Prospects and challenges of graphene in biomedical applications,” *Advanced Materials*, vol. 25, no. 16, pp. 2258–2268, 2013.

161. M. Carbone, L. Gorton, and R. Antiochia, “An Overview of the Latest Graphene-Based Sensors for Glucose Detection: the Effects of Graphene Defects,” *Electroanalysis*, vol. 27, no. 1, pp. 16–31, 2015.

162. J. Peña-Bahamonde, H. N. Nguyen, S. K. Fanourakis, and D. F. Rodrigues, “Recent advances in graphene-based biosensor technology with applications in life sciences,” *Journal of Nanobiotechnology*, vol. 16, no. 1, pp. 1–17, 2018.

163. B. Thakur, G. Zhou, and J. Chang, “Rapid detection of single E. coli bacteria using a graphene-based field-effect transistor device,” *Biosensors and Bioelectronics*, vol. 110, no. 1, pp. 16–22, 2018.

164. G. Wu, M. Meyyappan, and K. W. C. Lai, “Graphene field-effect transistors-based biosensors for Escherichia coli detection,” in *International Conference on Nanotechnology (IEEE-NANO)*, pp. 22–25, 2016.

165. F. Wang, S. Horikawa, J. Hu, H. Wikle, and I. Chen, “Detection of Salmonella typhimurium on spinach using phage-based magnetoelastic biosensors,” *Sensors*, vol. 17, no. 2, pp. 386–395, 2017.

166. S. Cheng, S. Hideshima, S. Kuroiwa, T. Nakanishi, and T. Osaka, “Label-free detection of tumor markers using field effect transistor (FET)-based biosensors

for lung cancer diagnosis," *Sensors and Actuators B: Chemical*, vol. 212, no. 1, pp. 329–334, 2015.

167. A. Morgenshtein, L. Sudakov-Boreysha, U. Dinnar, C. G. Jakobson, and Y. Nemirovsky, "Wheatstone-Bridge readout interface for ISFET/REFET applications," *Sensors and Actuators B: Chemical*, vol. 98, no. 1, pp. 18–27, 2004.

168. B. Behkam and M. Sitti, "E. coli inspired propulsion for swimming microrobots," *ASME 2004 International Mechanical Engineering Congress and Exposition*, vol. 47063, no. 1, pp. 1037–1041, 2004.

169. S. Yan, Z. Cao, and Z. Guo, "Design and Fabrication of Full Wheatstone-Bridge-Based Angular GMR Sensors," *Sensors*, vol. 18, no. 6, pp. 1832–1840, 2018.

170. K. S. Cole and H. J. Curtis, "Wheatstone bridge and electrolytic resistor for impedance measurements over a wide frequency range," *Review of Scientific Instruments*, vol. 8, no. 9, pp. 333–339, 1937.

171. Graphenea, "Multilayer layer Graphene on SiO_2/Si 300 nm," *Graphenea*, 2016. [Online]. Available: https://graphenea.com/products/bilayer-graphene-on-sio2-si-10-mm-x-10-mm?variant=51790528083. [Accessed: 16-Jun-2019].

172. F. Banhart, J. Kotakoski, and A. V Krasheninnikov, "Structural defects in graphene," *ACS nano*, vol. 5, no. 1, pp. 26–41, 2010.

173. P. B. Price, "Reevaluation of ethyl alcohol as a germicide," *Archives of Surgery*, vol. 60, no. 3, pp. 492–502, 1950.

174. R. R. L. De Oliveira, D. A. C. Albuquerque, T. G. S. Cruz, F. M. Yamaji, and F. L. Leite, "Measurement of the nanoscale roughness by atomic force microscopy: basic principles and applications," *Atomic force microscopy-imaging, measuring and manipulating surfaces at the atomic scale*, pp. 147–175, 2012.

175. K. Anselme, "Osteoblast adhesion on biomaterials," *Biomaterials*, vol. 21, no. 7, pp. 667–681, 2000.

176. K. Hoffmann, *Applying the Wheatstone bridge circuit*.

177. N. E. Birns and O. K. Mizrahi-Shalom, "Microcontroller system for performing operations of multiple microcontrollers." Google Patents, 23-Mar-1999.

178. Arduino, “Arduino Nano,” *Arduino*, 2019. [Online]. Available: https://store.arduino.cc/usa/arduino-nano. [Accessed: 03-Apr-2019].

179. M. Shankar, B. Lalitha, S. Tamilselvan, S. Vignesh, S. Thangarasu, and V. Kathirvel, “A microchip wireless based wearable physiological parameters monitoring system,” *International Journal of Latest Research in Science and Technology: April*, vol. 2, pp. 71–74, 2013.

180. 14core, “Wiring I2C module on 16×2 LCD with SCL/SDA,” *14core*. [Online]. Available: https://www.14core.com/wiring-i2c-module-on-16x2-lcd-with-sclsda/. [Accessed: 16-Apr-2019].

181. J. Blum, *Exploring Arduino: tools and techniques for engineering wizardry*. John Wiley & Sons, 2013.

182. R. W. Fransiska, E. M. P. Septia, W. K. Vessabhu, W. Frans, and W. Abednego, “Electrical power measurement using arduino uno microcontroller and labview,” in *2013 3rd International Conference on Instrumentation, Communications, Information Technology and Biomedical Engineering (ICICI-BME)*, pp. 226–229, 2013.

183. I. Stroud and P. C. Xirouchakis, “STL and extensions,” *Advances in Engineering Software*, vol. 31, no. 2, pp. 83–95, 2000.

184. A. Lussi, “Autoclave,” U.S. Patent No. 5,535, pp. 141-142, 09-Jul-1996.

185. Y. Tanaka and Y. Benno, “Application of a single-colony coculture technique to the isolation of hitherto unculturable gut bacteria,” *Microbiology and immunology*, vol. 59, no. 2, pp. 63–70, 2015.

186. H. F. Church, “The long-term stability of fixed resistors,” *Proceedings of the IEE-Part B: Electronic and Communication Engineering*, vol. 109, no. 21, pp. 19–27, 1962.

187. J. Kang, D. Shin, S. Bae, and B. H. Hong, “Graphene transfer: key for applications,” *Nanoscale*, vol. 4, no. 18, pp. 5527–5537, 2012.

188. M. Ahmad, S. A. Han, D. H. Tien, J. Jung, and Y. Seo, “Local conductance measurement of graphene layer using conductive atomic force microscopy,” *Journal of Applied Physics*, vol. 110, no. 5, pp. 054307–054314, 2011.

189. Z. S. Lodish H, Berk A, *Molecular Cell Biology*, 4th editio. New York: W. H. Freeman, 2000.

190. Y. Li, D. M. Weinberger, C. M. Thompson, K. Trzciński, and M. Lipsitch, "Surface charge of Streptococcus pneumoniae predicts serotype distribution," *Infection and immunity*, vol. 81, no. 12, pp. 4519–4524, 2013.

191. G. Wu, X. Tang, Z. Lin, M. Meyyappan, and K. W. C. Lai, "The effect of ionic strength on the sensing performance of liquid-gated biosensors," in *International Conference on Nanotechnology (IEEE-NANO)*, pp. 242–245, 2017.

192. A. Huang, W. Li, S. Shi, and T. Yao, "Quantitative fluorescence quenching on antibody-conjugated graphene oxide as a platform for protein sensing," *Scientific reports*, vol. 7, no. 1, pp. 40772–40779, 2017.

193. C.-H. Kim, J.-H. Ahn, J.-Y. Kim, J.-M. Choi, T. J. Park, and Y.-K. Choi, "Improvement of sensitivity and limit of detection in a nanogap biosensor by controlling surface wettability," *BioNanoScience*, vol. 3, no. 2, pp. 192–197, 2013.

194. J.-Y. Kim, K. Choi, D.-I. Moon, and J.-H. Ahn, "Surface engineering for enhancement of sensitivity in an underlap-FET biosensor by control of wettability," *Biosensors and Bioelectronics*, vol. 41, pp. 867–870, 2013.

195. P. Hu, G. Du, W. Zhou, and J. Cui, "Enhancement of ethanol vapor sensing of TiO2 nanobelts by surface engineering," *ACS applied materials & interfaces*, vol. 2, no. 11, pp. 3263–3269, 2010.

196. N. E. Allen, D. L. LeTourneau, and J. O. E. N. HOBBS, "The role of hydrophobic side chains as determinants of antibacterial activity of semisynthetic glycopeptide antibiotics," *The Journal of antibiotics*, vol. 50, no. 8, pp. 677–684, 1997.

197. M. Dathe, T. Wieprecht, H. Nikolenko, and L. Handel, "Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides," *FEBS letters*, vol. 403, no. 2, pp. 208–212, 1997.

198. S. Wang, Y. Zhang, N. Abidi, and L. Cabrales, "Wettability and surface free energy of graphene films," *Langmuir*, vol. 25, no. 18, pp. 11078–11081, 2009.

199. C. J. Shih, M. S. Strano, and D. Blankschtein, "Wetting translucency of graphene," *Nature Materials*, vol. 12, no. 10, pp. 866–869, 2013.

200. X. Li, J. Feng, E. Wang, S. Meng, J. Klimeš, and A. Michaelides, “Influence of water on the electronic structure of metal-supported graphene: Insights from van der Waals density functional theory,” *Physical Review B*, vol. 85, no. 8, pp. 85425–85435, 2012.

201. X. Liu, L. Peng, J. Meng, Z. Zhu, B. Han, and S. Wang, “Protein-mediated anti-adhesion surface against oral bacteria,” *Nanoscale*, vol. 10, no. 6, pp. 2711–2714, 2018.

202. V. Skákalová and A. B. Kaiser, *Graphene: properties, preparation, characterisation and devices*. Elsevier, 2014.

203. R. Ganapathy, M. Sarmadi, and F. Denes, “Immobilization of α-chymotrypsin on oxygen-RF-plasma functionalized PET and PP surfaces,” *Journal of Biomaterials Science, Polymer Edition*, vol. 9, no. 4, pp. 389–404, 1998.

204. F. Grinnell and M. K. Feld, “Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody binding and analyzed during cell adhesion in serum-containing medium.,” *Journal of Biological Chemistry*, vol. 257, no. 9, pp. 4888–4893, 1982.

205. J. Wei, T. Igarashi, N. Okumori, and T. Igarashi, “Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts,” *Biomedical Materials*, vol. 4, no. 4, pp. 45002–45010, 2009.

206. S. Afsahi, M. B. Lerner, J. M. Goldstein, and J. Lee, “Novel graphene-based biosensor for early detection of Zika virus infection,” *Biosensors and Bioelectronics*, vol. 100, pp. 85–88, 2018.

207. O. Akhavan and E. Ghaderi, “Escherichia coli bacteria reduce graphene oxide to bactericidal graphene in a self-limiting manner,” *Carbon*, vol. 50, no. 5, pp. 1853–1860, 2012.

208. E. Smith and G. Dent, *Modern Raman spectroscopy: a practical approach*. Wiley, 2019.

209. T. Miyazawa, T. Shimanouchi, and S. Mizushima, “Normal vibrations of N-methylacetamide,” *The Journal of Chemical Physics*, vol. 29, no. 3, pp. 611–616, 1958.

210. R. C. Lord and N.-T. Yu, “Laser-excited Raman spectroscopy of biomolecules: I. Native lysozyme and its constituent amino acids,” *Journal of molecular*

biology, vol. 50, no. 2, pp. 509–524, 1970.

211. T. G. Spiro and B. P. Gaber, "Laser Raman scattering as a probe of protein structure," *Annual review of biochemistry*, vol. 46, no. 1, pp. 553–570, 1977.

212. P. Carey, *Biochemical applications of Raman and resonance Raman spectroscopes*. Elsevier, 2012.

213. A. T. Tu and A. T. Tu, "Raman spectroscopy in biology: principles and applications," *Wiley New York*, 1982.

214. Z. Wen, "Raman spectroscopy of protein pharmaceuticals," *Journal of pharmaceutical sciences*, vol. 96, no. 11, pp. 2861–2878, 2007.

215. M. Hinnemo, J. Zhao, and P. Ahlberg, "On monolayer formation of pyrenebutyric acid on graphene," *Langmuir*, vol. 33, no. 15, pp. 3588–3593, 2017.

216. W. Shen, S. Li, M.-K. Park, and Z. Zhang, "Blocking agent optimization for nonspecific binding on phage based magnetoelastic biosensors," *Journal of The Electrochemical Society*, vol. 159, no. 10, pp. 818–823, 2012.

217. E. Fernandes, P. D. Cabral, R. Campos, and G. Machado Jr, "Functionalization of single-layer graphene for immunoassays," *Applied Surface Science*, vol. 480, no 1, pp. 709–716, 2019.

218. A. Shabani, C. A. Marquette, R. Mandeville, and M. F. Lawrence, "Modern Probe-Assisted Methods for the Specific Detection of Bacteria," no. 2, pp. 104–121, 2015.

219. V. Chavasit, J. Photi, S. Purttiponthanee, and P. Saekoo, "Use of Bacterial Growth Curve for Assessing Risk of Microbiological Pathogens in Food Products," in *Microbial Contamination and Food Degradation*, Elsevier, pp. 341–365, 2018.

220. G. M. Alsop, G. T. Waggy, and R. A. Conway, "Bacterial growth inhibition test," *Water Pollution Control Federation*, vol. 2. no 1, pp. 2452–2456, 1980.

221. N. M. Salih, "Design And Development of Coliform Bacteria Detection System Integrated With Microfluidic and Optical Absorbance Measurement Device," Master Thesis, Universiti Tun Hussein Onn Malaysia, 2016.

222. E. O. Powell, "Growth rate and generation time of bacteria, with special reference to continuous culture," *Microbiology*, vol. 15, no. 3, pp. 492–511,

1956.

223. M. D. Rolfe, C. J. Rice, and Lucchini, “Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation,” *Journal of bacteriology*, vol. 194, no. 3, pp. 686–701, 2012.

224. H. M. Adelman and R. T. Haftka, “Sensitivity analysis of discrete structural systems,” *AIAA journal*, vol. 24, no. 5, pp. 823–832, 1986.

225. H. O. Jacobs and G. M. Whitesides, “Submicrometer patterning of charge in thin-film electrets,” *Science*, vol. 291, no. 5509, pp. 1763–1766, 2001.

226. N. Toyoda, “Semiconductor integrated circuit device having an integrally formed bypass capacitor.” Google Patents, 15-Nov-1988.