NOVEL DNA-BASED ELECTRONIC PROFILING METHOD FOR SELECTED ALGAE

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NOVEL DNA-BASED ELECTRONIC PROFILING METHOD FOR SELECTED ALGAE

ABSTRACT

The utilization of deoxyribonucleic acid (DNA) in electronics has become significant and gradually accepted by researchers due to its remarkable characteristics. There are several devices and sensors that employ DNA in their fabrication process. The standard methods to detect and recognize any species of living organism are polymerase chain reaction (PCR), sequencing and microarray techniques. However, there are several drawbacks pertaining to these methods such as sample contamination, misleading results as well as being costly and involving complicated procedures. As an alternative method, this study presents a simple, fast, high sensitivity and economical novel identification method for algae-derived DNA using the electronic properties of DNA. Novel current-voltage (I-V) characteristics of chosen algal species using DNA-specific diodes were obtained and its corresponding diode parameters (turn-on voltage, shunt and series resistance, knee voltage, breakdown voltage as well as breakdown current) were then calculated in this study. Each algal species exhibits specific turn-on voltage values for example *Chlorella* sp. had a value of 1.40 V, *Synechococcus* sp. with 1.15 V and *Amphora* sp. with 1.36 V. This novel technique demonstrates an exciting potential that may have huge impact in various fields, especially in pathology and taxonomy.

Keywords: Indium tin oxide, DNA, Schottky diode, biosensor, diode parameters.

NOVEL DNA-BASED ELECTRONIC PROFILING METHOD FOR SELECTED ALGAE

ABSTRAK

Penggunaan asid deoksiribonukleik (DNA) dalam elektronik menjadi lebih penting dan semakin diterima pada masa kini oleh penyelidik kerana ciri-cirinya yang luar biasa. Terdapat beberapa peranti dan pengesan yang menggunakan DNA dalam proses fabrikasinya. Kebanyakan kaedah piawai untuk mengesan dan mengenali mana-mana spesies organisma hidup adalah reaksi berantai polymerase (PCR), teknik penjujukan dan teknik microarray. Sebaliknya terdapat beberapa kekurangan berkaitan kaedah tersebut seperti pencemaran sampel, keputusan mengelirukan serta mahal dan prosedur yang rumit. Sebagai kaedah alternatif, tesis ini membentangkan kaedah pengenalpastian baru yang mudah, cepat, sensitiviti tinggi dan praktikal digunakan untuk DNA yang berasal dari alga yang menggunakan sifat-sifat elektronik DNA. Ciri-ciri arus voltan (I-V) spesies alga yang terpilih menggunakan diod khusus DNA ditunjukkan dan parameter diod yang berkenaan (voltan putar, peredaran dan rintangan siri, voltan lutut, voltan kerosakan serta arus pecahan) kemudian dikira dalam kajian ini. Setiap spesis alga menunjukkan nilai voltan pemula yang khusus seperti *Chlorella* sp. mempunyai nilai 1.40 V, *Synechococcus* sp. dengan 1.15 V dan *Amphora* sp. dengan 1.36 V. Teknik novel ini mungkin mempunyai impak yang besar dalam pelbagai bidang, terutamanya dalam patologi dan taksonomi.

Kata kunci: Indium tin oksida, DNA, diod Schottky, pengesan bio, parameter diod.

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LIST OF SYMBOLS AND ABBREVIATIONS

k	:	Boltzmann constant
A	:	Effective diode area
A^*	:	Effective Richardson constant
q	:	Electronic charge
n	:	Ideality factor
I_o	:	Reverse saturation current
$Ø_b$	:	Zero-bias barrier height
A	:	Adenine
AFM	:	Atomic Force Microscopy
A1	:	Aluminium
BaP	:	Benzo(a)pyrene
BBM	:	Bold's Basal Medium
BPV	:	Biological Photovoltaic
C	:	Cytosine
DNA	:	Deoxyribonucleic Acid
ELISA	:	Enzyme-linked immunosorbent Assay
FESEM	:	Field Emission Scanning Electron Microscope
FIS	:	Fluoroimmuno-sensor
G	:	Guanine
GMOs	:	Genetically Modified Organisms
ITO	:	Indium Tin Oxide
PCR	:	Polymerase Chain Reaction
PDMS	:	Polydimethylsiloxane
RH	:	Relative Humidity

RFLP	:	Restriction Fragment Length Polymorphism
T	:	Thymine
TCO_s	:	Transparent Conducting Oxides
UMACC	:	University of Malaya Algae Culture Collection

CHAPTER 1: INTRODUCTION

1.1 Overview

In recent years, a great amount of literature regarding molecular nanotechnology and nanoelectronics has emerged since biological macromolecules such as proteins and nucleic acid have shown good potential to be utilized in construction of nanostructured systems (Niemeyer, 1999). For example, biosensor related research have increased tremendously towards production of inexpensive, easy to handle, rapid and highly sensitive sensors (Downs et al., 1987). Enzyme sensors (Alvarez-Icaza et al., 1995), immunosensors (DeSilva et al., 1995), microbial sensors (Karube, 1990) as well as deoxyribonucleic acid (DNA) biosensors are some of the various types of biosensors currently in the mainstream research.

These sensors have been shown to overcome the limitations presented by conventional methods due to its increased stability and sensitivity. Techniques such as real time polymerase chain reaction (PCR), DNA sequencing and also DNA microarray available nowadays are often unsuccessful due to the high similarities in conserved regions of closely related species (Filipe et al., 2008). Besides that, there are a lot of possible applications of biosensors in numerous important fields such as in food production industry, environmental monitoring and also medical diagnostics (Mehrotra, 2016). Therefore, continuous enhancement of the biosensor works to improve its efficiency, especially related to type of transducer used, sensing layer, design of sensor and most importantly its operating simplicity, is essential (Dong & Chen, 2002). The technology presented in this study however is a DNA biosensor based on a Schottky diode design. This type of diode is based on a combination of metal and semiconductor materials forming a metal-semiconductor junction (Tung, 2001). As a result, the sensor will exhibit specific current-voltage (I-V) characteristics that could be used to detect any

type of DNA species. Furthermore, various diode parameters can be extracted and calculated to verify the identification process.

Currently, algae are organisms that are known to have great industrial applications especially in energy production. Currently utilized fossil-based fuels are non-renewable energy that needs replacement as this resource is in severe decline (Demirbas, 2010). Biofuel has therefore emerged as a substitute for petroleum fuels due to its sustainable energy supply while being environmentally friendly by minimizing greenhouse effect and is more economical in terms of foreign exchange (Balat, 2009). Many algal species have high lipid contents and can serve as potential feedstocks for biofuel production. In addition, algae produce diverse biochemicals that can serve as nutraceuticals, biopharmaceuticals, etc. Thus, it is necessary to have an accurate electronic detection technique for algae; especially the ability to recognize and distinguish any closely related species. In this work, three species of algae-derived DNAs will be utilized to obtain fingerprinting I-V profiles and its corresponding solid-state parameters calculated for utilization in a proposed fully electronic detection method.

1.2 Problem Statement

There are many ways to identify and detect DNA; i.e. real time PCR, DNA sequencing as well as DNA microarray. However, there is a demand for a new approach of DNA detection because the existing method is unable to differentiate the DNA of closely related species. Furthermore, the conventional methods usually have some drawbacks such as time consuming, expensive and also difficult to handle.

1.3 Research Objectives

The main purpose of this research is to develop a novel DNA based electronic profiling method for selected algae (*Chlorella* sp., *Synechococcus* sp. and *Amphora* sp.). In order to achieve the target, the objectives of this research are divided into four important parts;

1. To fabricate Al/indium tin oxide (ITO)/DNA/Al junction diode using the Schottky junction structure.
2. To study the surface morphology of DNA film on ITO surface.
3. To obtain current-voltage characteristics for the *Chlorella* sp., *Synechococcus* sp. and *Amphora* sp.
4. To acquire and calculate various diode parameters such as turn-on voltage, shunt and series resistance, ideality factor, barrier height, knee voltage, breakdown voltage as well as breakdown current of the three algae DNAs.

1.4 Motivation of Study

Nowadays, it is undeniable that algae are essential to humans as well as to the environment in various aspects. Thus, it is necessary to have a good and accurate identification tool for algal species. In this research, a new electronic technique to help identify the DNA of algal species is proposed and also developed by utilizing the electronic properties of DNA using I-V profiles and diode parameters. This method may provide a sensor that is cost-effective, rapid and require only a trace amount of DNA strand or highly sensitive.

1.5 Organisation of Thesis

The thesis is written in five chapters; Literature Review (Chapter 2), Experimental Methodology (Chapter 3), Results and Discussion (Chapter 4) and Conclusions and Future Works (Chapter 5).

In Chapter 2, a brief introduction about the DNA molecule, historical background on discovery of DNA, its conductivity, applications of DNA and traditional method of DNA detection are discussed. Also, an elaborate discussion related to the biosensor and its components are presented in this chapter. In addition, concepts of Schottky diode and an introduction to algae are provided in detail.

Chapter 3 describes the information related to the materials and techniques used in this work. These includes the source of DNA used, the extraction method, the steps involved in cleaning the ITO substrate, the preparation of Al/DNA/ITO/Al Schottky barrier diode, the procedure of acquisition of I-V profiles and calculation of electronic diode parameters together with the characterization analyses are thoroughly explained.

Chapter 4 discusses the I-V profiles generated under the two different bias regions and various important electronic diode parameters. The investigation on morphology as well as structural properties of DNA film on ITO surface are further elaborated.

Finally, Chapter 5 lists all conclusions of the current work and future research mainly involving optimization of the biosensor developed.

CHAPTER 2: LITERATURE REVIEW

This chapter presents and discusses related literature and works from previous studies besides identifying the current state of research pertaining to biosensors, especially in relation to DNA detection.

2.1 Deoxyribonucleic Acid

2.1.1 Structure of DNA

DNA found in the cell nucleus or sometimes in mitochondria is an important biomolecule for all living organisms and also various types of viruses. It contains genetic data (Taniguchi & Kawai, 2006) that are useful in important processes such as growth, development, functioning and reproduction (Rau et al., 2012). This is due to the complementarity nature of the pairing between the bases; adenine (A) with thymine (T) and guanine (G) with cytosine (C). In addition, this enables DNA to make an exact copy of the information that is stored in each strand through a semi-conservative mechanism (Seeman, 2005). Semi-conservative mechanism or replication process is where two exact copies of the original double stranded molecule is produced (Meselson & Stahl, 1958).

The structure of DNA molecule is described as a double helix structure as shown in Figure 2.1 by Watson & Crick (1953). There are four main types of macromolecules; nucleic acids, proteins, carbohydrates and also polyphenols. Since DNA is a nucleic acid, it is also defined as macromolecules which are important in life et al., 2002). DNA is made up from four different monomers termed as nucleotides. The nucleotides meanwhile consist of phosphate group, single ring sugar which is a deoxyribose together with one of the four nitrogen-containing nucleobases (A, T, G or C). The nucleotides are connected with the sugar of another nucleotide and phosphate of the next by covalent bond, which forms the sugar-phosphate bond. Hydrogen bond ties the

two nitrogenous bases of the two separate polynucleotide strands together to create the double-stranded DNA (Katan & Mashaghi, 2013).

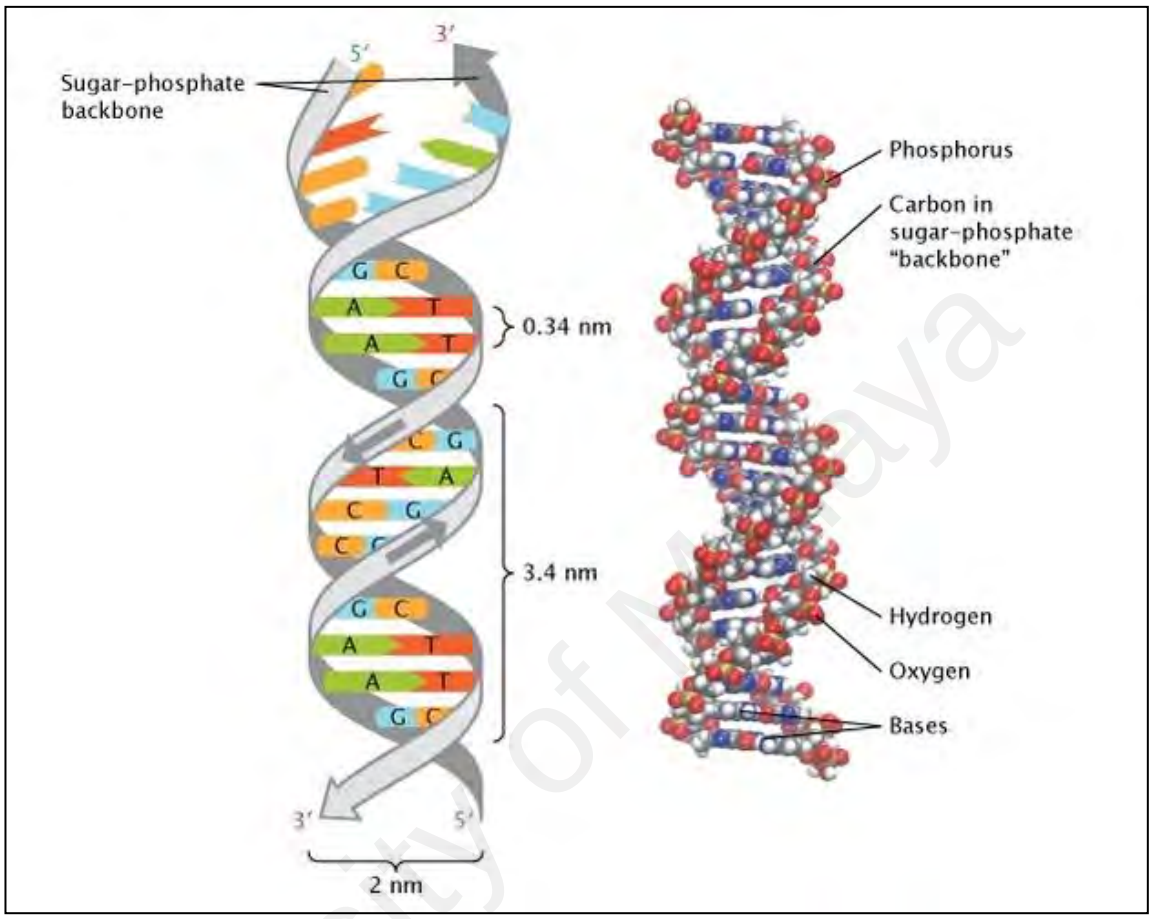


Figure 2.1: The structure of DNA (Pray, 2008).

A DNA molecule has a diameter of 2 nm and the length between two bases is 0.34 nm. The orientation of the nucleotide strands is opposite and antiparallel to each other where one strand is from 5' to 3' while the other is vice versa (Seeman, 2003). There are two categories of DNA nucleobases, purines and pyrimidines. Adenine and guanine are classified as purines since they have two rings while pyrimidines such as cytosine and thymine has only one ring as shown in Figure 2.2 (Tseng & Yang, 2013).

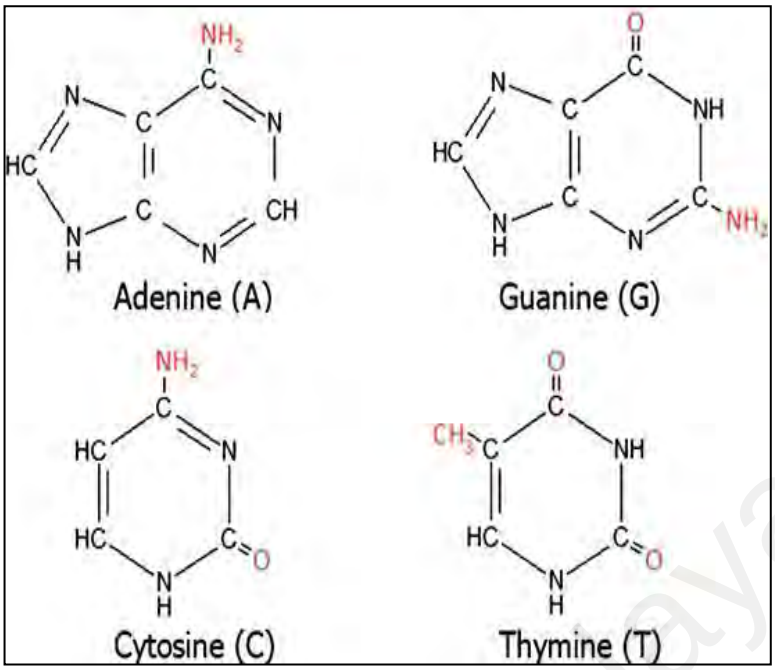


Figure 2.2: The four types of DNA bases (Tseng & Yang, 2013).

2.1.2 Discovery of DNA

In 1944, Avery et al. carried-out a pioneering work as an attempt to understand the fundamentals of DNA. They concluded that DNA transmit hereditary information without the existence of protein as was previously believed (Avery et al., 1944). Later, Hershey & Chase through their work in 1952 also agreed that DNA is a genetic material. Study by Chargaff (1951) found that each species would have a fixed ratio of base configuration in DNA. That is similar amount of adenine to thymine bases and also similar amount of cytosine to guanine bases (Chargaff et al., 1949), despite having its own specific base composition of DNA (Chargaff, 1951). Watson & Crick (1953) later, successfully described the structure of DNA based on X-ray diffraction patterns obtained by Rosalind Franklin and Maurice Wilkins, which earned them the Nobel Prize in Chemistry.

In 1977, Sanger method or chain termination method of DNA sequencing that can determine exactly the sequence of nucleobases (A, T, C and G) in DNA molecules was

developed by Frederick Sanger (Sanger et al., 1977). This method offers advantages such as simplicity and reduction of errors as compared with previous configuration about the sequence of nucleobases.

2.1.3 Conductivity of DNA

Extensive studies associated with charge transfer mechanism in DNA molecules are being carried out continuously to allow a better understanding of various biological processes and cell repair mechanism. One of the studies involves the determination of DNA conductivity where research groups have reported insulating, conducting and semiconducting behaviors. However, recent reports indicated the possibility of DNA conductivity to be highly influenced by the environmental conditions (Bag et al., 2016).

Okahata et al. reported that there is electron conduction occurring in DNA strand constituting a current flow when aligned-DNA film was connected on a comb-type electrode (Okahata et al., 1998). In another work, DNA molecules was reported to demonstrate ohmic electric conduction under condition of minimum temperature level at distance around few hundred nanometers (Kasumov et al., 2001) as shown in Figure 2.3. In this work, it was proposed that the type of substrate may affect the conductivity of DNA.

Fink & Schonenberger meanwhile reported that DNA behaving as a semiconductor suggest application as a potential material in the fabrication of mesoscopic electronic devices (Fink & Schonenberger, 1999). In addition, DNA also behaves as a large band gap semiconductor (Figure 2.4) when connected to two metal nanoelectrodes as demonstrated in an investigation done by Porath et al. (Porath et al., 2000). Experimental results from Rakitin et al. indicated that at room temperature, B-DNA (a typical form of DNA) exhibits semiconducting behavior with a few hundred meV band

gap. M-DNA created by addition of metal ion to the imino proton group of each base pair of DNA molecules meanwhile displayed metallic-like conduction (Rakitin et al., 2001).

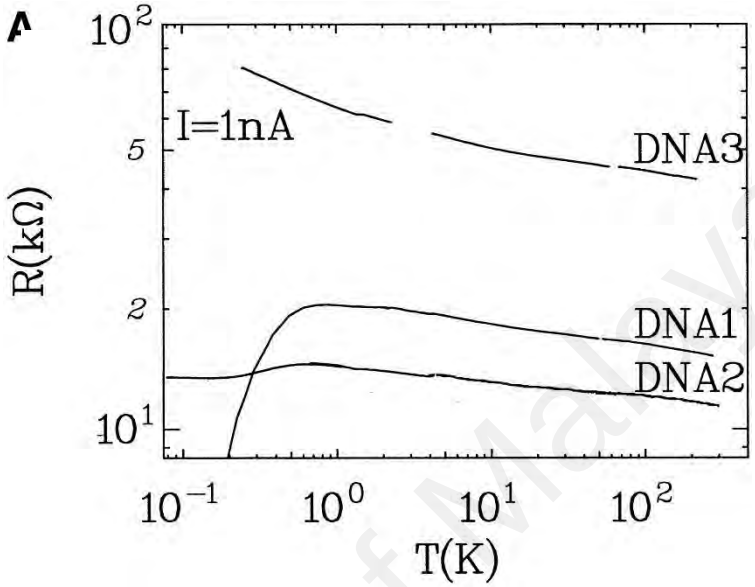


Figure 2.3: Linear transport measurements on the three samples. DC resistance as a function V_0H (T) of temperature on a large temperature scale, showing the power law behavior down to 1 K (Kasumov et al., 2001).

Storm et al. (2001) on the other hand reported DNA behaving as an insulator at different length and base pair sequences and also assumed that many factors determined the conductivity of DNA molecules. In 1997, Braun and group claimed that DNA molecule displayed good mechanical properties and has suitable molecular-recognition in contrast to poor electric conduction (Braun et al., 1998). Besides that, a study done by de Pablo and group revealed that DNA act as an excellent insulator and many factors may affect electron transport in DNA molecules for example different base sequence composition, effect of counter ions as well as thermal vibration (de Pablo et al., 2000).

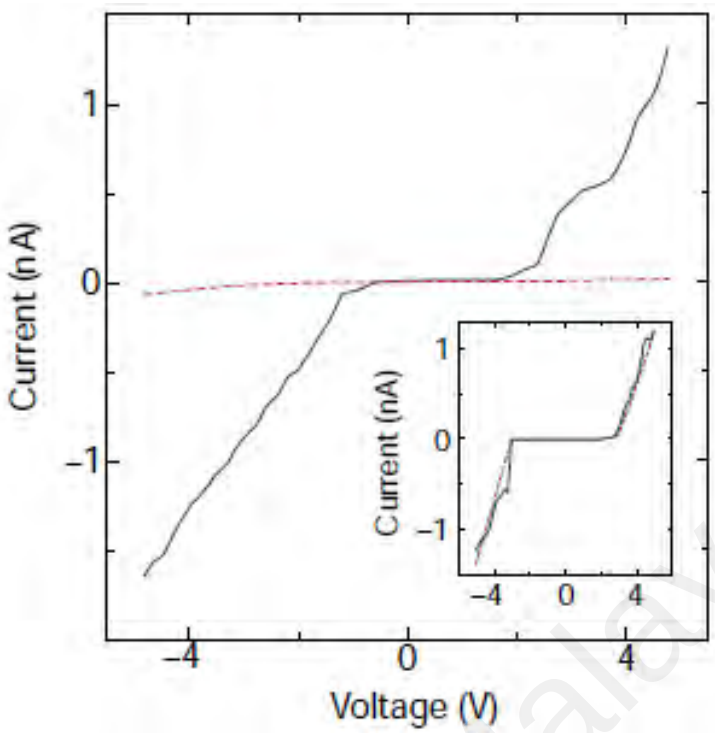


Figure 2.4: A large band gap semiconductor reported (Porath et al., 2000).

Despite the contrasting reports, DNA has been generally accepted to be semiconductor-like in recent works. These works conclude that DNA may behave as a semiconducting material at right conditions as also shown by our research group (Al-Ta'ii et al, 2015a; Periasamy et al., 2016). Using this semiconducting property as the basic principle, we fabricated and demonstrated a novel electronic technique for detecting the selected algae-derived DNA molecules as detailed and explained in Chapter 3.

2.1.4 Applications of DNA

The unique properties of DNA molecules allow its wide usage in numerous applications, especially in nanotechnology, genetic engineering, DNA profiling, food industry, medical and agricultural sectors. Study related to manipulation of matter on an atomic and molecular scale to fabricate materials, structures and also devices not more than 100 nm is defined as nanotechnology (Anisa et al., 2003). As the size of DNA is in

the nanometer range, fabrication of DNA assisted nano-devices became feasible as was initiated by Seeman (2007). Also, the advancement of biosensors are highly influenced by the existence and availability of nanotechnology (Vo-Dinh et al., 2001).

Rothemund (2006) successfully established the techniques of scaffolded DNA origami formation with a diameter of 100 nm (Figure 2.5) followed by spatial resolution of about 6 nm. The scaffolded DNA origami may be utilized to produce other structures that are larger and more complex. Ko et al. (2008) created DNA nanotube systems using synthetic DNA molecules as shown in Figure 2.5(b) as a transport to deliver certain chemicals into cancer cells. This method has several benefits such as being natural and higher biocompatibility. Conventional synthetic polymer based delivery systems are foreign to cell and the effect of their toxicity on human in prolonged periods remain uncertain.

Genetic technology is a technique for producing new variations of organisms via modification and transformation of DNA molecules of particular species into different species and the new DNA strand created is known as recombinant DNA. The new organism developed by this technology is called genetically modified organisms (GMOs) (Uzogara, 2000). In 1990, genetic modification was performed on tomato plant by incorporating a coat protein gene from a cucumber mosaic virus which then produced tomatoes that are resistant to bacterial infections and prolonged shelf life (Domingo, 2007).

(a)

(b)

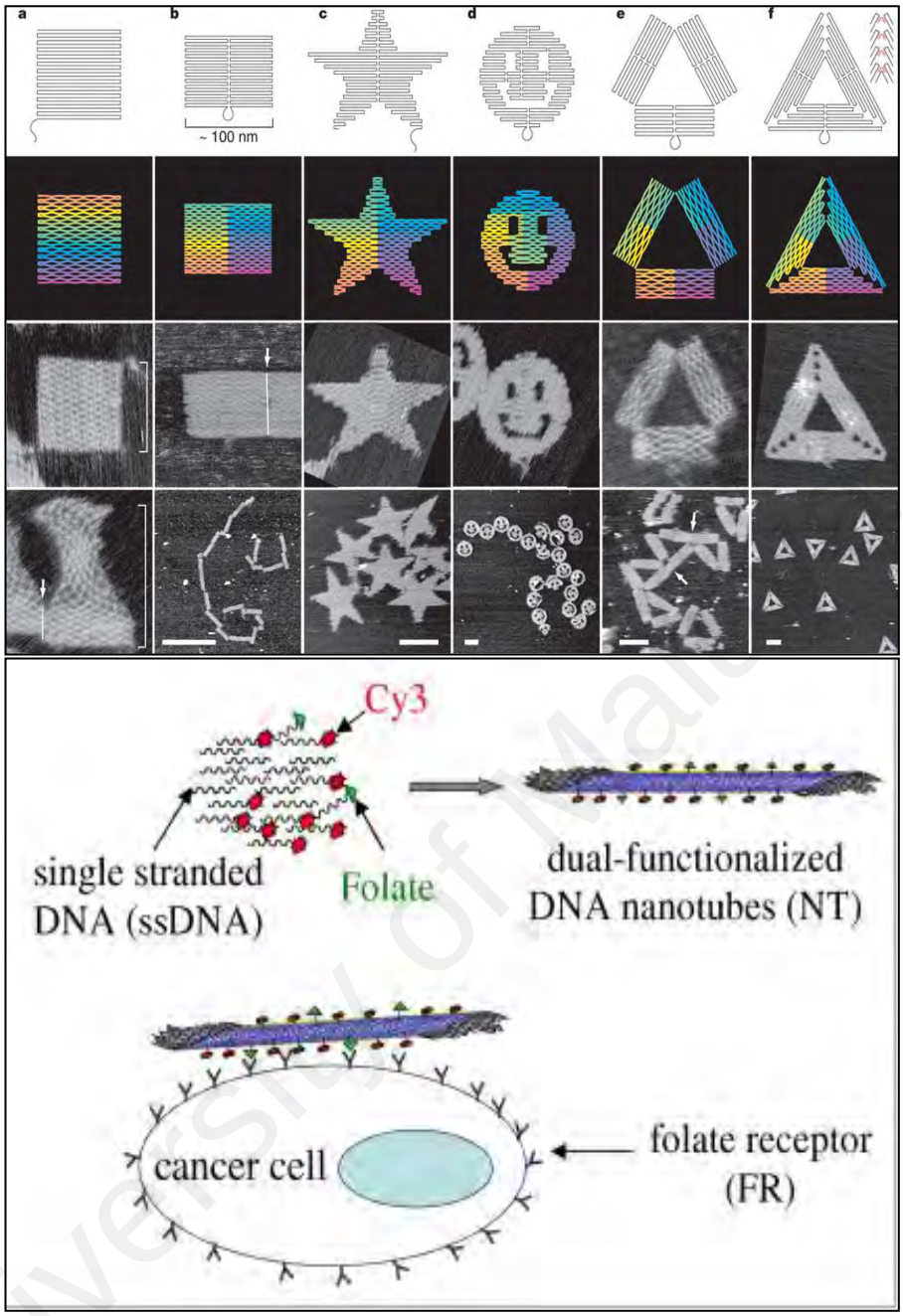


Figure 2.5: (a) DNA origami structures (square, rectangle, star, disk with three holes and triangle with rectangular domains fabricated by DNA folding). Top row of diagram is for folding paths while second row from top, diagrams showing the bend of helices at crossovers (where helices touch) and away from crossovers (where helices bend apart) with corresponding AFM images at the bottom (Rothemund, 2006) and (b) Schematic diagram on how DNA nanotube works (Ko et al., 2008).

DNA can also be utilized in other applications such as DNA profiling. DNA profiling (also called as DNA fingerprinting) is a method to recognize and distinguish between individuals based on their DNA profile. The DNA can be obtained from hair as

well as bodily fluids (for example blood, semen or saliva) and tests carried out by scientist usually in medical and forensic fields (Roeder, 1994). This procedure introduced by Jeffreys et al. (1985) at University of Leicester, United Kingdom using restriction fragment length polymorphism (RFLP) technique found that each individual have its own highly specific DNA profile.

2.2 DNA Detection

2.2.1 Introduction

Before the emergence of biosensors, several well-established methods were employed to identify and detect DNAs. These include techniques such as PCR, microarray, fluorescent measurement, electrophoresis, ELISA and also colony count, which were widely used despite numerous limitations (Lazcka et al., 2007).

2.2.2 Polymerase Chain Reaction

The polymerase chain reaction (PCR) technique was originally developed by Kary Mullis in 1983, which earned him a Nobel Prize in Chemistry ten years later. This method is able to produce 100 billion exact DNA molecules from only a piece of DNA molecule (Mullis, 1990). The detection or characterization of a disease can be achieved by using this method and is frequently used in modern diagnostic microbiology laboratories since DNA can be easily manipulated through PCR technique (Carter et al., 2010). Pötsch et al. (1992) successfully reported the application of PCR in forensic dentistry particularly in identification of DNA situated in human tooth pulp tissue obtained mostly from forensic casework. In forensics, DNA can also be obtained from other sources besides the tooth pulp tissue as shown in Figure 2.6 below.

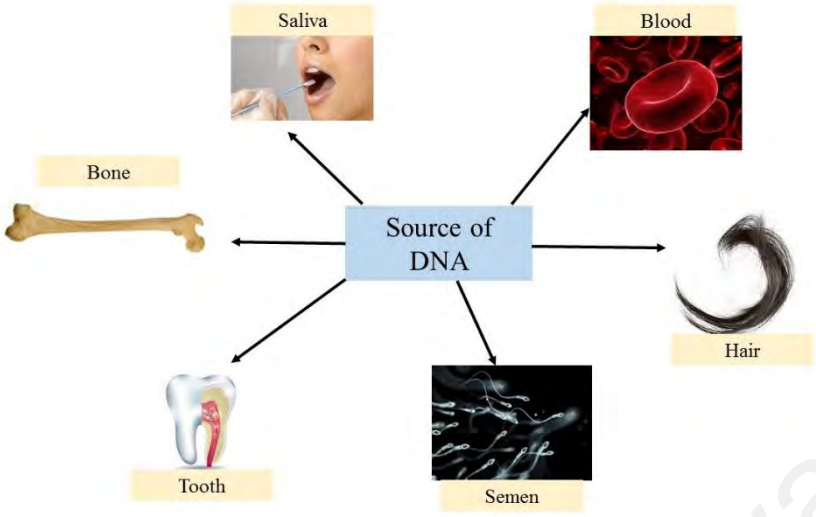


Figure 2.6: Various sources of DNA.

There are several advantages of PCR method which includes high sensitivity, relatively quick analysis (take only a day) and only need a little amount of starting DNA strand (Ashimoto et al., 1996). However, this technique still requires the information of target DNA besides being costly and at the same time requiring expertise to handle the equipment (Maheaswari et al., 2016).

2.2.3 DNA Microarray

The detection of a great number of gene expression for example DNA or protein samples can be achieved by a technology known as DNA microarray (Afshari, 2002) as illustrated in Figure 2.7. The principle of this technology is based on hybridization process between probe DNA strand and target DNA strand collected from clinical specimen (Yoo et al., 2009). This technique relies on two main principles, which are immobilization of the probe molecules onto solid surface (such as micro-printing standard glass or silicon slide) and secondly recognition of their complementary DNA target sequence via (Hughes et al., 2001; Lipshutz et al., 1999). Transmittable and hereditary diseases, forensic and genetic identification purposes followed by cancer

diagnostics are examples of molecular biologically related analyses and genomic study applications that utilize the microarray system (Heller, 2002).

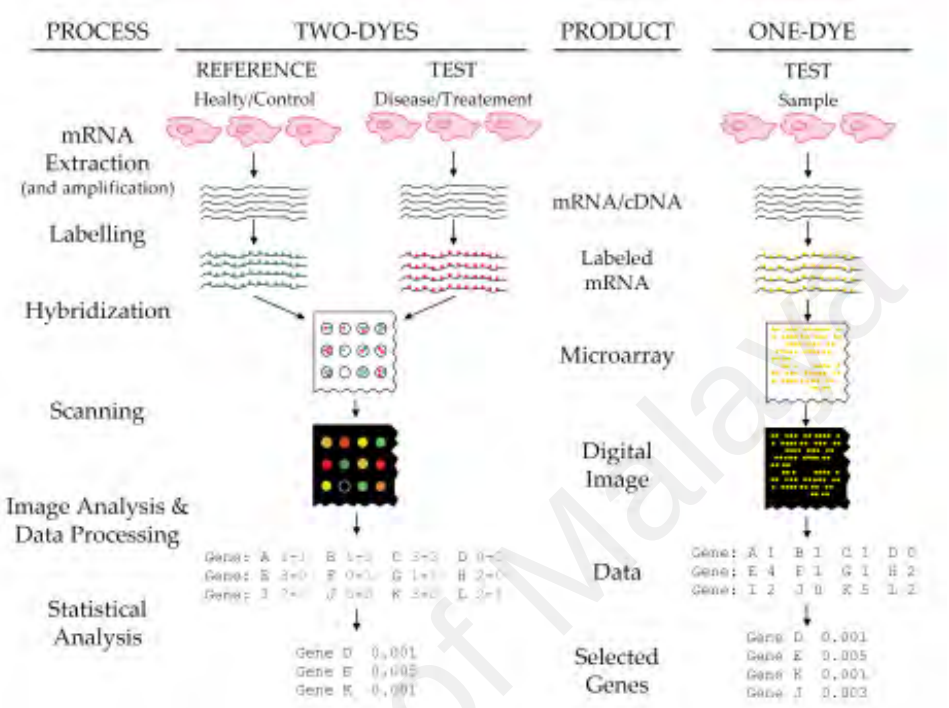


Figure 2.7: Schematic diagram represents microarray system (Trevino et al., 2007).

2.2.4 Biosensors

Research related to biosensors generally began in 1962 (Zhang et al., 2000). It became a popular topic among researchers working towards developing high quality biosensors. Biosensors are described as devices that consist of a transducer element attached to a sensing layer comprised of a biological element as shown in Figure 2.8 (Dong & Chen, 2002). Zhai et al. (1997) meanwhile stated that biosensors are another example of analytical devices that generate signal corresponding to the concentration of the target chemical substance being measured or analyte.

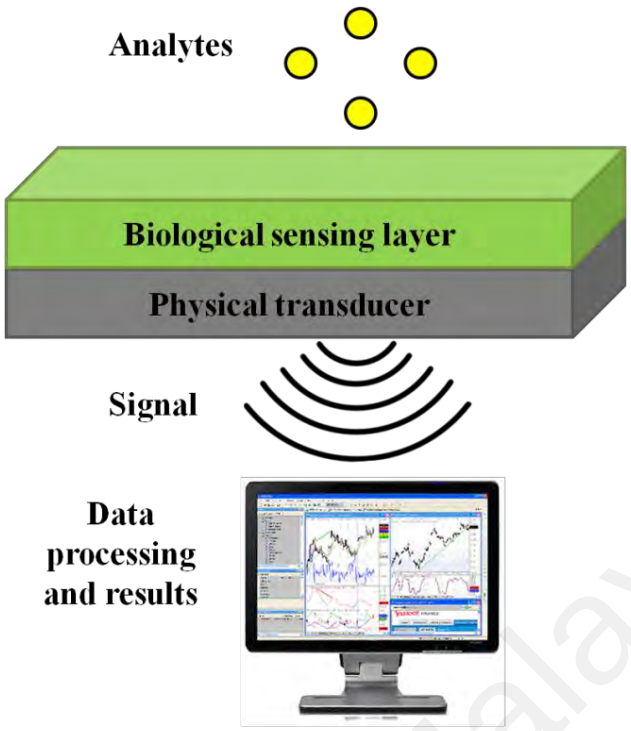


Figure 2.8: Schematic diagram of a biosensor (Dong & Chen, 2002).

There are two main components of the biosensor which is the biological element followed by the transducer (Kumar et al., 2015). Examples of biological elements being used as sensing material for example are nucleic acid, enzymes, whole cell or receptor proteins (Solanki et al., 2011). The function of transducer is to convert biological response into measurable signal where the type of transducer normally used and reported in many literature is the electrochemical, optical, electrical and also thermometric type (Teles & Fonseca, 2008). Biosensors are more preferred nowadays compared to traditional methods of identifying and detecting DNA such as PCR and microarray technique because it provides several advantages including shortened window period, relatively lower cost, highly sensitive, non-complicated and have tendency for miniaturization (Lazcka et al., 2007).

Blood glucose biosensor developed in 1962 by Clark (known as the father of biosensor) was the first biosensor invented. The working principle is entrapping of

enzyme glucose oxidase located at oxygen electrode done by using dialysis tubing. The role of this invention is to monitor blood oxygen level of a person in real time (Clark & Lyons, 1962). Seven years later, George Guilbault from Louisiana State University introduced the first potentiometric biosensor to detect the level of urea in body fluids by immobilization of urease enzyme on ammonia electrode (Guilbault & Montalvo, 1969).

Lactate normally is produced when there is insufficient oxygen in blood. The conventional process of detection of lactate level usually is costly to carry out frequently for patients. It is also a time consuming procedure that will result in inaccurate results since the state of the patient may be different because of the delay. This situation motivated Racine to develop a simple device based on electrochemical enzymatic sensor for lactate or lactate electrode that could detect lactate level rapidly and overcome the problem faced previously (Racine et al., 1975).

Another type of biosensor is the fiber optic pH sensor to monitor pH in studies of respiration and tissue oxygenation. This type of biosensor was first reported by Peterson et al. (1980) and promoted a non-electrical probe harmless to the human body since it does not involve any electrical connection to the body and also as an alternative to conventional micro-pH electrodes. The probe is designed based on fiber optics together with a dye sensor and suitable for tissue and blood pH measurements. The immobilization of the indicator that is surrounded by a hydrogen-ion-permeable membrane is made on the microsphere at the end of the optical fibre (Peterson et al., 1980).

The essential building block of the biosensor is the biological element. The biological materials are extracted from living organism synthesized in laboratory due to their difficulty to be obtained naturally. Molecular recognition elements usually involve enzymes, antibodies, nucleic acids, whole cell, tissue as well as receptors (Chambers et

al., 2008). The selectivity of the sensors depends on the type of biological elements used that binds and interact selectively and specifically with the target molecules. A good isolation procedure of biomaterials is crucial to maintain their role in creating an efficient biosensor (Castillo et al., 2004).

In order for the biosensor to perform well, Turner illustrated that there should not be any contaminant within the biological elements to prevent any disturbance occurring to the reaction that will take place when the biomaterial react with the electrode of the biosensor (Turner, 2015). Furthermore, the biological elements need to be in stable condition in terms of temperature, ionic strength, pH, redox potential, and chemical composition. Another important aspect to be considered in fabricating an efficient biosensor is how the biological element is immobilized. A method related to the formation of attachment of whole cells, enzymes, nucleic acid or antibodies onto solid support system or structure or stored by a membrane to sustain and enable them to be used again to react with target molecules is called immobilization. The immobilization procedure varies according to the type of the biological element used (Saxena, 2015). Various available techniques of immobilization are as shown in Figure 2.9; generally adopted in designing biosensors for example adsorption, covalence, entrapment and cross-linking or affinity (Andreescu et al., 2002). However, each technique has its own advantages and limitations. Hence, the selection of a wise and relevant method relies on the nature of the biological element, the characteristics of the transducer followed by the detection approach (Sassolas et al., 2012).

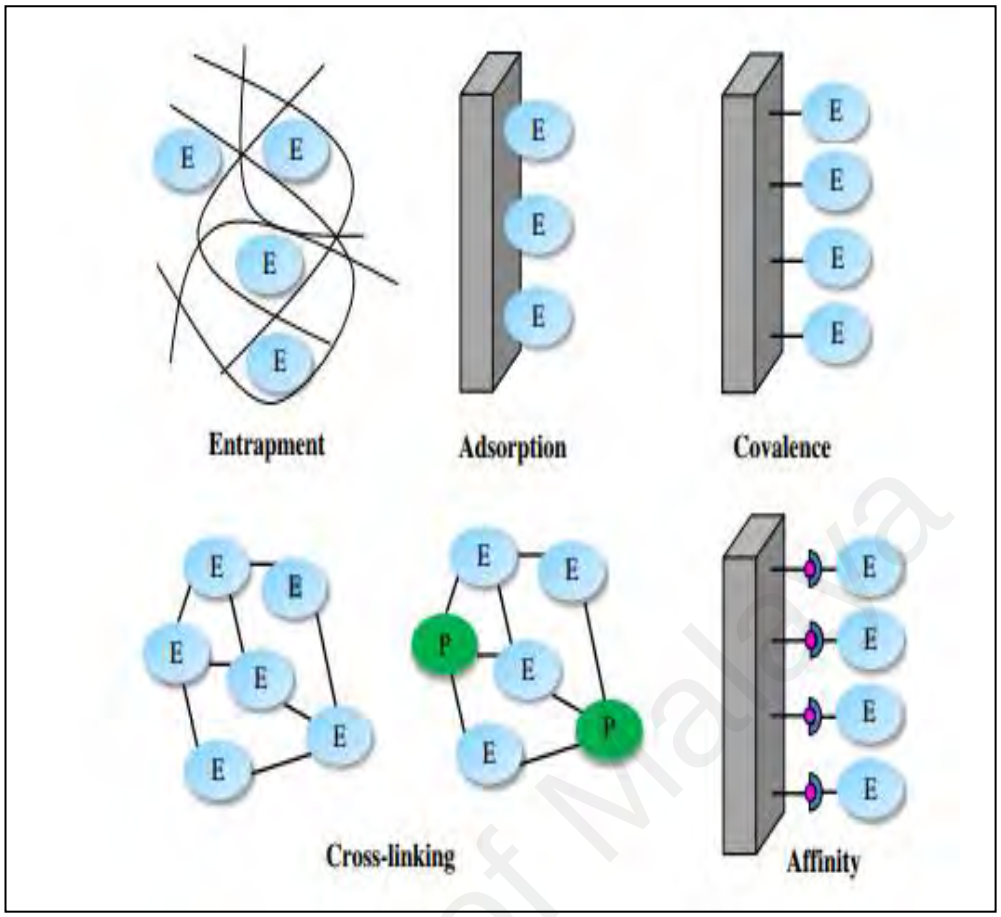


Figure 2.9: Illustration of different methods of enzyme immobilization (E: enzyme, P: inert protein) (Sassolas et al., 2012).

One of the non-destructive methods for maintaining biomolecule activity during immobilization is adsorption process. To carry out this method, the biological elements need to be dissolved in a solution before being made to contact with a solid support with the biomaterial solution at a specific period. Removal of unabsorbed biomolecules can be achieved by using washing solutions such as buffer. Weak bonds, such as Van der Waal's forces, electrostatic and hydrophobic interactions are involved in this adsorption process. However, the drawbacks for this method are that the biomolecule's lifetime stability will decrease, biosensor performance results in poor operation and also desorption of biomaterial from the solid support may occur since there is a change in temperature, pH and ionic strength (Ahuja et al., 2007).

Covalent linking between biological elements and solid supports promote strong and efficient bonding that result in increase of lifetime stability of the biomaterial. Besides that, the biosensor starts to operate efficiently and become more stable even in unexpected conditions because only a low diffusional resistance will occur. Although this is an effective immobilization technique, however it still required high amounts of bioreagent to purify the biological element and the solid support sometimes faces some problem in replicating the experiment because of its low reproducibility (Cao, 2006).

Another way to immobilize biological components is by using entrapment method. This procedure enable biomaterials to be immobilized in 3D surrounding medium for example in electropolymerized film, amphiphilic network composed of polydimethylsiloxane (PDMS), photopolymer, silica gel, polysaccharide or carbon paste. Since no additional chemicals are involved in this method, the behavior of the biological elements is maintained and hence contributes to the efficiency of the biosensor and increase storage stability. Nevertheless, this technique still requires improvement because the biomaterials can be easily drained away (Sassolas et al., 2012).

Fabrication of biosensors can also be achieved with cross-linking immobilization approach that requires the use of bi-functional reagents such as glutaraldehyde. Biological elements and the supporting material will have a strong chemical binding. The limitations for this method are low quality in terms of mechanical strength and usually it will influence the activity of the biomaterials (Eggins, 1996).

Biosensors are categorized according to their type of transducer or detection mode. There are several detection approaches available such as electrochemical, optical and piezoelectric biosensors. Besides the biological component and the way to immobilize them, another important factor to take into account in the development of the biosensor

is the type of the transducer and specifically its surface. This is due to the fact that area of the attachment of the biomaterial also plays an important role in biosensor performance (Goode et al., 2015). The most commonly used biosensor is the electrochemical biosensors. The concept of this type of biosensor is based on the changes occurring in the electrical properties of the solution. These changes are considered as a measuring parameter due to the chemical reactions that take place and generate or consume ions or electrons. Amperometric, conductimetric as well as potentiometric are examples of different electrochemical sensing. These biosensors are usually applied in detection of hybridized DNA, DNA-binding drugs, and glucose concentration (Mohanty & Kougianos, 2006). Optical techniques such as measurement of absorbance, fluorescence, chemiluminescence and surface plasmon resonance (SPR) are employed as the detection method for optical biosensors. The existence of this kind of biosensors will overcome some problems faced by other types of biosensors because of its benefits for example rapid, real time measurement, detection of multi parameters and also high selectivity and specificity (Martins et al., 2013).

Economical and non-complex assay devices can be developed using piezoelectric biosensors. This type of biosensor can detect biomaterials through affine interactions carried out in the absence of additional chemical reagents. The mechanism of this sensor is that crystals will experience elastic deformation or vibration in the presence of electric field. The detection method is based on the changes occurring in the resonance frequency (Pohanka, 2016). Since biosensors offered many benefits in terms of sensitivity and stability, various applications extensively applied and utilized biosensor as an alternative to conventional techniques mainly in medical field, food industry as well as environmental monitoring (Mehrotra, 2016).

One example of biosensor that is popular and often used by people suffering from diabetes or hypoglycemia is the home blood glucose biosensor. The function of this sensor is to determine the level of blood glucose accurately of a person. Another advantage of this device is that the patient can just simply monitor the amount of glucose in blood at home without going to hospital and seek expert advice (Mehrotra, 2016).

The world's demand for high quality food has led to increase of research efforts related to developing an efficient system to ensure food quality and safety. With the emergence of biosensors, examination on food composition particularly about the content of pathogens and toxins can be carried out in an economical way within a short period of time (Scognamiglio et al., 2014). For example, bacteria usually found in vegetables such as *Escherichia coli* can be detected by using antibody-based biosensors. The measurement of change in pH caused by ammonia produced by urease or *Escherichia coli* antibody conjugate is the working principle for this potentiometric alternating biosensing system (Ercole et al., 2003).

An antibody-based fiber optics sensor for determination of benzo(a)pyrene (BaP) based on laser-induced fluorescence is introduced by one research group in 1987 since there is a growing amount of hazardous environmental pollutants. BaP is a harmful pollutant that can be found mostly in industrial and residential environments. The excitation source used in this technique is helium-cadmium laser and polyclonal techniques were used to create an antibody for BaP and then bind with fiber optics sensing probe of a fluoroimmuno-sensor (FIS) by a covalent bond. The advantages of this sensor includes ability to trace the desired pollutant within complex matrices and can be applied to detect other compounds if suitable antibodies are available (Vo-Dinh et al., 1987).

2.3 Schottky Diode

2.3.1 Introduction

Schottky barrier occurs when a junction is formed from a combination of any metal with semiconductor material. It was originally proposed in 1939 by a physicist from German named Walter Hermann Schottky. He discovered that there is a rectifying effect similar with conventional semiconductor resulting from this type of contact (Tung, 2001). Usually, metal is evaporated or sputtered on a cleaned semiconductor surface to create a metal/semiconductor junction (Di Bartolomeo, 2016). This semiconductor diode has been widely used in various applications for example transistors, microwave diodes, solar cells and photodetectors as it possesses two important characteristics such as low forward voltage drop and very fast switching action (Van Meirhaeghe et al., 1994).

In 1874, F. Braun successfully demonstrated the rectifying effect of metallic contacts on several materials; iron, copper and also lead sulfide crystals (Rhoderick, 1982). Another research group also reported that a combinational contact of graphene and silicon constructs a Schottky diode that successfully exhibits I-V characteristic. They reported barrier gap values of 0.41 eV and 0.45 eV on n-type and p-type silicon at room temperature, respectively. In this work, graphene obtained from mechanical exfoliation was deposited on top of silicon substrates and it was observed that several factors affected the ideality factor of graphene–silicon Schottky diodes such as temperature as well as graphene layers (Chen et al., 2011) (Figure 2.10).

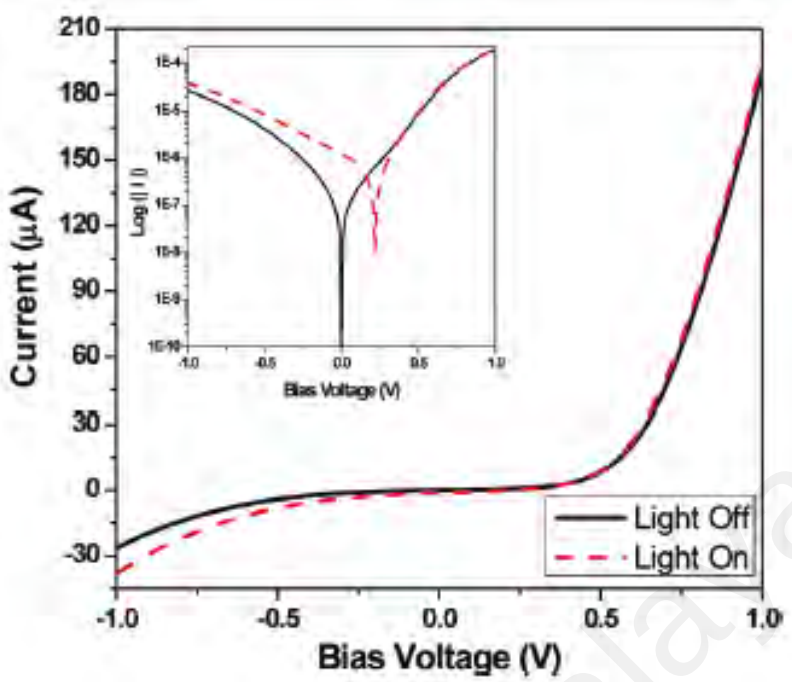


Figure 2.10: Current-voltage characteristics of a graphene on n-Si device with and without illumination. The inset figure shows current on a log scale (Chen et al., 2011).

2.3.2 DNA-specific Schottky Diodes

2.3.2.1 Introduction

Recently, there are several published literatures regarding fabrication of Schottky diode that are composed of DNA-metal (semiconductor-metal) junction and revealed I-V profile similar to a normal semiconductor (Al-Ta'ii et al., 2015a; Al-Ta'ii et al., 2015b; Periasamy et al., 2016). DNA being an organic semiconductor material was used in this work together with an aluminium metal contact due to the metal being more readily available besides being a good electric conductor compared to most other metals. In this work, this DNA/Al Schottky junction was utilized as a radiation sensor for the detection of alpha particles for environmental monitoring. Selected electronic diode parameters calculated using the I-V profiles obtained (Figure 2.11) such as ideality factor, series resistance and also barrier height were determined and utilized as the basis for the alpha particle detection.

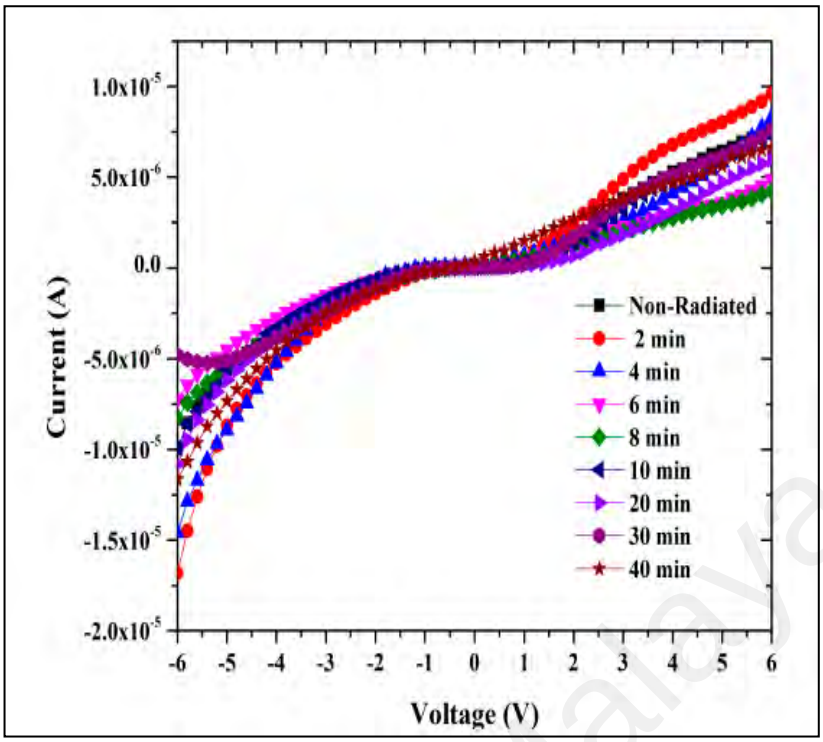


Figure 2.11: I-V graph for both forward and reverse biases of DNA sample irradiated by alpha particles for different periods of time (Al-Ta'ii et al., 2015b).

In most of the DNA-specific Schottky diodes reported and discussed in previous paragraphs are based on ITO substrates or electrodes. The obvious choice of ITO as the semi-transparent conducting material for the construction of DNA based sensors are mainly due to its advantages which will be discussed in-depth in the proceeding section.

2.3.2.2 Indium Tin Oxide

Optoelectronic industry especially involving the development of various efficient devices such as solar cells, displays, opto-electrical interfaces and circuitries requires a material with two important properties that are electrical conductivity and low absorption of light. Transparent conducting oxides (TCOs) material is the ideal choice since their characteristics fulfill the demand in that field (Stadler, 2012). ITO thin film is one of the examples of TCOs materials and being frequently used. Cadmium oxide (CdO) was the first generation of TCOs but this material presents harmful effect

towards the organism and hence ITO becomes an alternative material (Mattox & Mattox, 2007).

In order to have better conductivity, ITO thin film should have a low resistivity value. This is achievable when oxygen vacancies and substitutional tin dopants caused a degeneration to occur and indirectly high carrier concentration takes place when the Fermi level (E_F) is situated above the conduction level (E_C) (Kim et al., 1999). Generally, the resistivity value of this thin film is in the range of 10^{-4} Ω cm and its electron mobility value is 10 to 30 cm^2/Vs (Ishibashi et al., 1990). Different deposition methods of ITO thin film are for example direct current sputtering, radio frequency sputtering, electron beam evaporation, chemical vapor deposition as well as spray pyrolysis (Lien, 2010). The most preferred technique of producing ITO thin film is direct current magnetron sputtering since this method offer advantages which includes a well uniform film, important for applications in display devices (Ishibashi et al., 1990).

Recently, ITO thin film as the anode material was employed in biological photovoltaic (BPV) devices and extensively used in studies of biofilm growth. Ng et al. (2014) reported power output obtained from biofilms of selected algal species in BPV devices using ITO thin film as the substrate for algae growth. This demonstrates that ITO is an excellent and stable substrate for immobilization of algae and may be possible for other biomaterials.

2.3.2.3 Algae

Algae belong to a large group of aquatic and marine organisms categorized into two types; microalgae and macroalgae. Microalgae thrive generally in ponds and other aquatic habitats and may be mass cultivated in photobioreactors which are examples of enclosed production systems. Microalgae are single celled algae while seaweeds which

are large-sized species of algae or macroalgae can be found in the sea. *Chlorella* is one of the examples of microalgae that belong to the Phylum *Chlorophyta*. Algae also behave similar to plants by capturing sunlight to convert carbon dioxide, water and nutrients to produce oxygen together with biomass by a process called photosynthesis (Dyer-Smith, 2011).

In the early 1940s, Harder and Von Witsch developed an idea to utilize microalgae for example, diatoms, as a source of lipids to be applied mostly in the production of food as well as fuels (Harder & Witsch, 1942). Generally, most of research works regarding microalgae involved the *Chlorella* strain (Vejeysri et al., 2014). Furthermore, waste water could be treated by algae in terms of reduction of both chemical and biochemical oxygen demand and elimination process of heavy metals as well as by coliform bacteria as first suggested by Oswald and Gotaas (Abdel-Raouf et al., 2012).

The improvement of fruit quality for example grapes can also be achieved by using green alga *Chlorella vulgaris* cells extract as a bio-fertilizer. This type of fertilizer is environmental friendly, cost-effective and also increases the yield production and at the same time enhances the nutritional content of the fruit (El-Moniem & Abd-Allah, 2008).

Algae are one of the living microorganisms that could produce electricity from its photosynthetic activity and create a form of biological solar cells known as Biophotovoltaic (BPV) devices. Electricity is generated when the charge separation take place in the BPV device. The process starts when a series of reactions occur after the algae is exposed to light; process of breaking down water into three elements which are photons (H^+), electrons and oxygen. These are the important and compulsory substances for algae to utilize for converting carbon dioxide and inorganic matter into carbohydrate and protein (Driver & Bombelli, 2011). Figure 2.12 explains the mechanism that occurs.

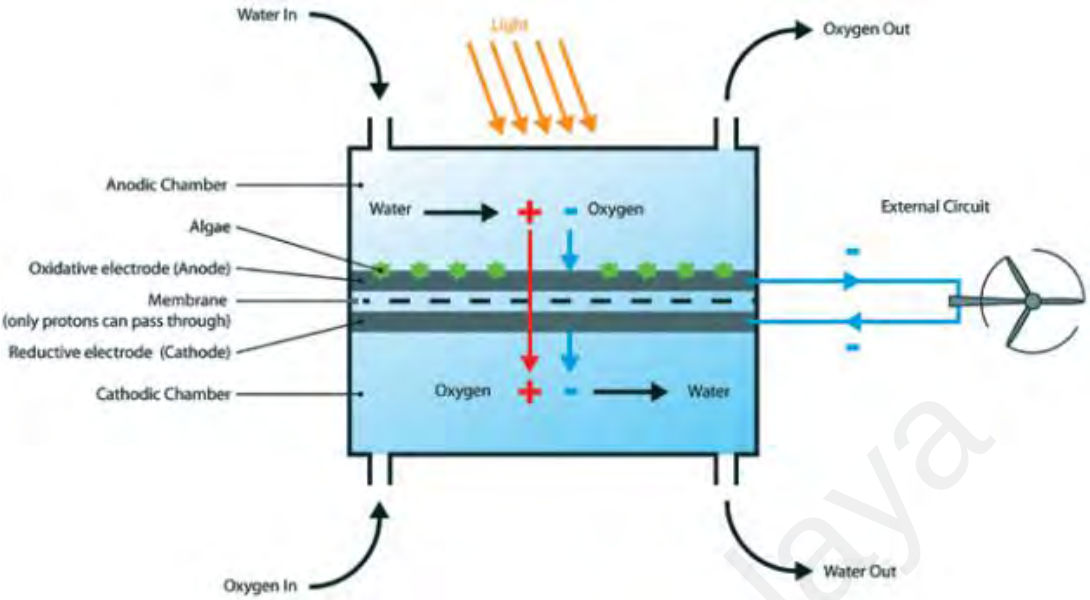


Figure 2.12: A schematic diagram of BPV device (Driver & Bombelli, 2011).

2.3.3 DNA-specific Schottky Diode for Detecting Algae

It is no doubt that algae in recent times have proved to be an important and exciting source of biomass, bioenergy and bioelectricity. Due to the large number of algae, both freshwater and marine, the need for an in-situ and rapid detection method for identification would prove beneficial for accelerating advances in algal biotechnology. In this work, we propose and employ the DNA-specific Schottky diode for the detection and identification of DNA from three different algae (*Chlorella* sp., *Synechococcus* sp. and *Amphora* sp.).

CHAPTER 3: EXPERIMENTAL METHODOLOGY

This chapter provides a description of research methodology used for this study to achieve all related research objectives. This also comprises how sample is obtained, experimental setup that have been carried out as well as data collection and analysis method.

3.1 Source of DNA

In this work, three different species of algae; *Chlorella* sp. (Chlorophyta), *Synechococcus* sp. (Cyanophyta) and *Amphora* sp. (Bacillariophyta) were obtained from the University of Malaya Algae Culture Collection (UMACC) (Phang & Chu, 1999). The total DNA was extracted and analyzed. Figure 3.1 below shows the fluorescence microscopy images for the selected algae used in this study.

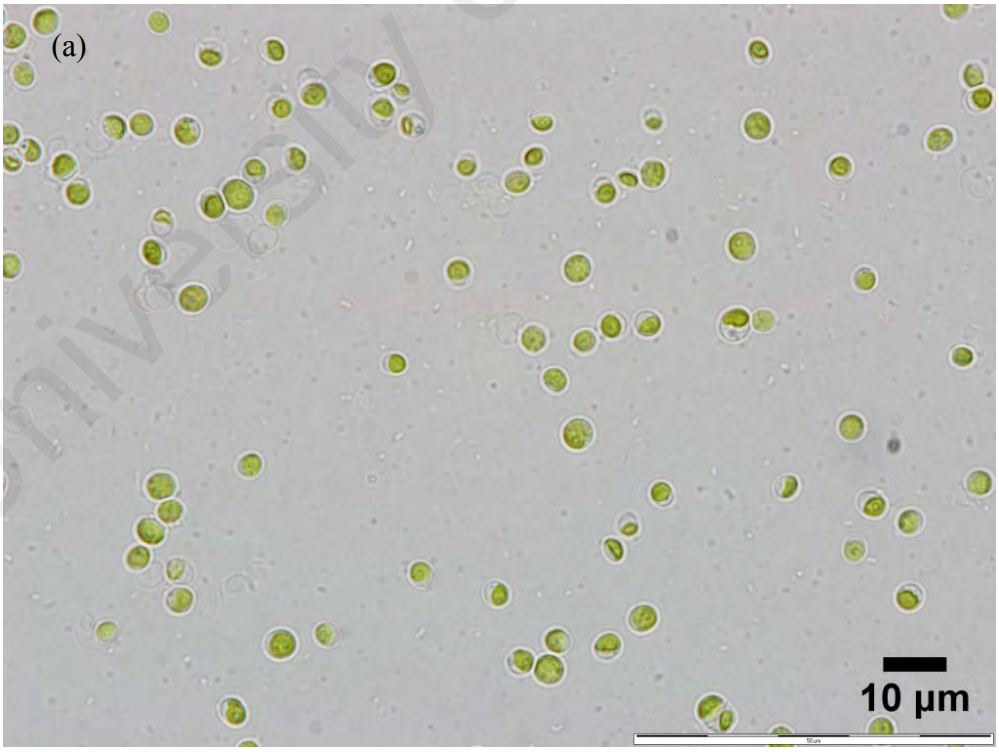
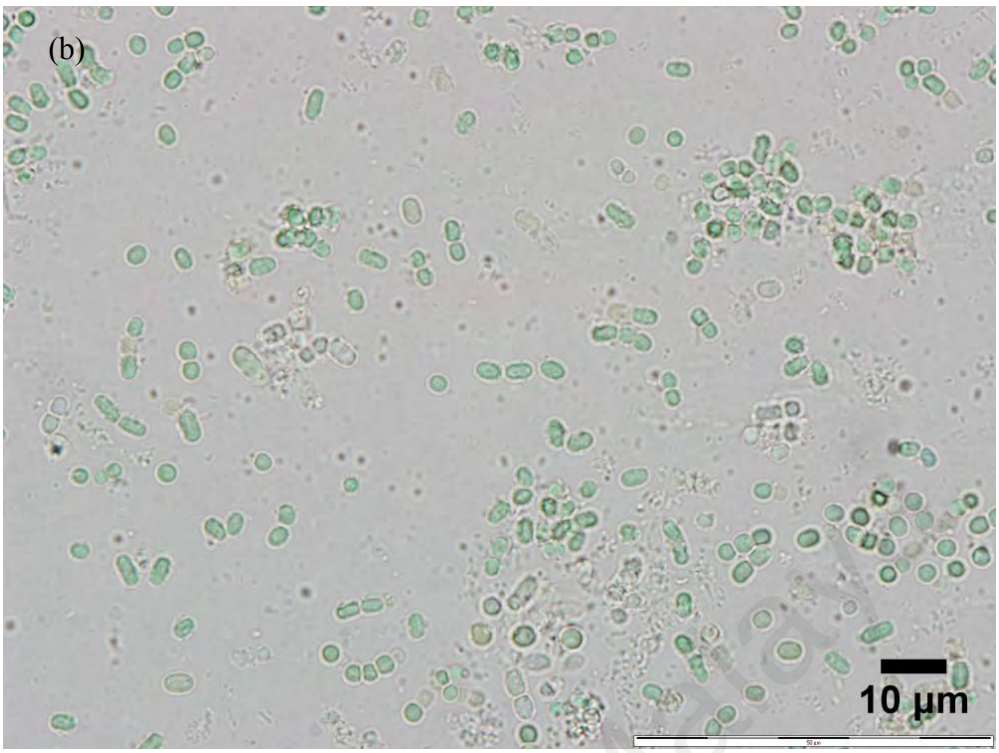


Figure 3.1: Fluorescence microscopy image for (a) *Chlorella* sp. (b) *Synechococcus* sp. and (c) *Amphora* sp.



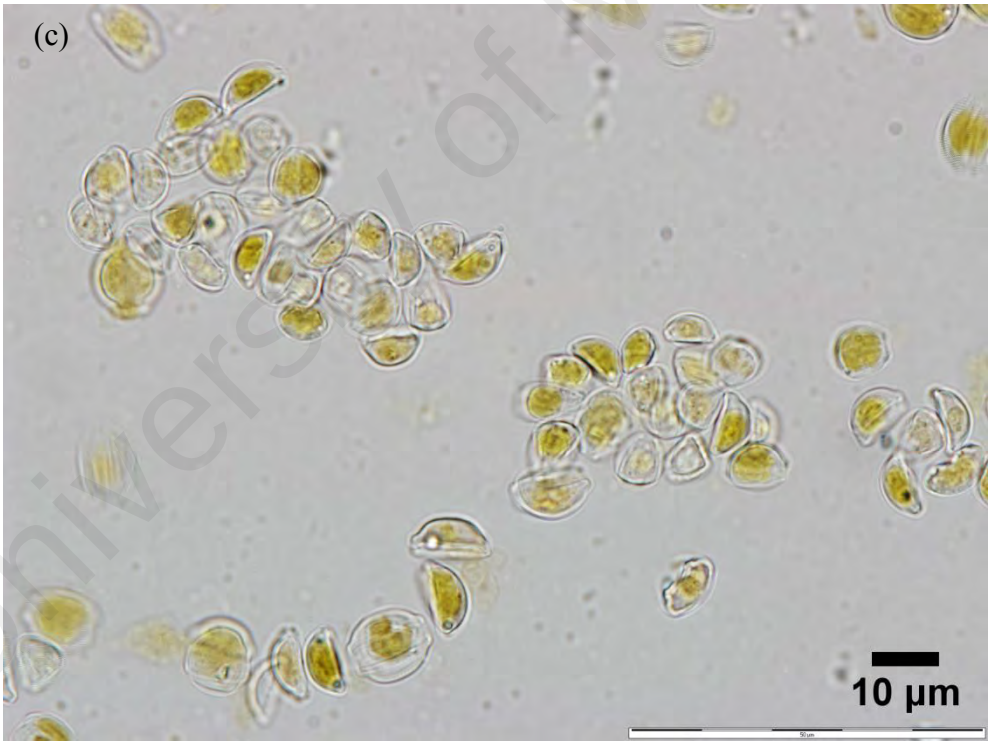


Figure 3.1, continued.

3.2 Culture Condition and Extraction of DNA

Three selected microalgal strains *Chlorella vulgaris* (UMACC 050), *Synechococcus* (UMACC 371) and *Amphora* sp. (UMACC 370) were obtained from the University of Malaya Algae Culture Collection (UMACC) (Phang & Chu, 1999). The cultures were subjected to purification by serial dilution followed by streaking on agar plates (20 g L^{-1} of Bacto™) to obtain individual colonies. This was to ensure that the algal cultures were not contaminated by other micro-organisms. The cultures were then set apart onto the agar plates or slants and kept in a culture chamber (25 ± 1 °C and irradiance of 30 µmol m^{-2} s^{-1}).

The individual colonies were inoculated into Bold's Basal Medium (BBM) for the freshwater *Synechococcus* and Provasoli medium buffered with 3 mM HEPES (pH 6.8-8.2), for the marine *Chlorella* and *Amphora* spp. An inoculum size of 10 % of total culture volume, standardized at an optical density at 620 nm (OD620 nm) of 0.4 (equivalent to 0.15 mg/L of chlorophyll-a, $r^2 = 0.9$) from exponential phase cultures was used. For growth studies, the microalgae were cultured in triplicate flasks with a working volume of 600 mL. The flasks were incubated in a controlled-environment at 25 ± 1 °C, illuminated with cool white fluorescent lamps (40 µmol m^{-2} s^{-1}) on a 12:12 h light-dark cycle and supplied with 100 ml min^{-1} of filtered ambient air. An aliquot of cultured cells (10 mL) was harvested in the mid to late exponential growth phase (4-6 days) by centrifugation (6,500x g for 3 min at 4 °C) in a sterile 1.5 mL microcentrifuge tube.

Genomic DNA was extracted using i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions and protocols. Initially, 390 µL lysis buffer, 7 µL Enhancer Solution, 20 µL Proteinase K, and 5 µL RNase. A solution was added and samples were vortexed vigorously for 5

min. The lysate was then incubated for 30 min at 65 °C. After brief centrifugation, 100 µL of precipitation buffer was added to the samples and incubated for 5 min on ice with occasional mixing. The samples were centrifuged for 5 min at 13,000x g to remove any droplets from the lids. Then, 200 µL of supernatant was transferred to new tube followed by 650 µL of binding buffer and mixed well by gently inverting 5 to 6 times or by pipetting. Next, 650 µL of the mixture plus any precipitate that may have formed were moved into the DNA binding spin column by inserted in a 2.0 mL collection tube. Mixtures were centrifuged for 1 min at 13,000x g and the flow-through liquid was discarded. The spin column was placed into a new 2.0 mL collection tube and 700 µL of washing buffer was added and centrifuged for 1 min at 13,000x g and the flow-through liquid was discarded. DNA was washed for second time by pipetting 700 µL of wash buffer into column and centrifuged at 13,000x g for 1 min. The flow-through liquid was discarded while collection tubes were stored. The columns were centrifuged at 13,000x g for 2 min to dry the columns.

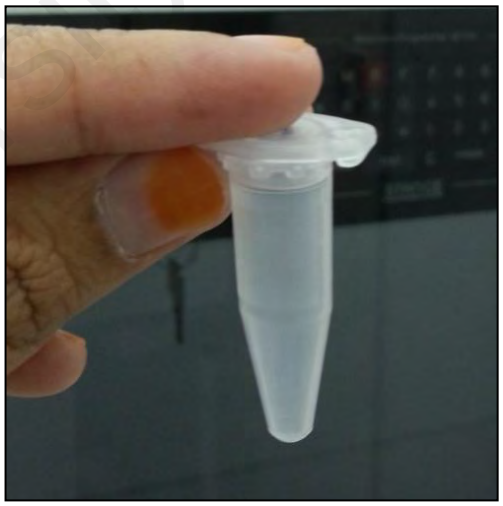


Figure 3.2: Photograph of extracted DNA solution of *Chlorella* sp., one of three types of algae used in this work.

All columns were transferred to new 1.5 mL Eppendorf tubes. The elution buffer (100 µL) was added to columns and left for 3 min at room temperature. Columns were then centrifuged at 13,000x g for 1 min for elution. DNA samples contained in Eppendorf tubes as shown in Figure 3.2 were then stored at – 20 °C freezer for future use. The DNA quality was finally measured using Nano Drop spectrophotometer.

3.3 ITO Substrate Cleaning Method

ITO slides (KINTEC, Hong Kong) as the substrates with specification of width 1.1 mm, layer thickness of 100 nm and dimension of (2.5 × 2.5) cm followed by a sheet resistance of 377.0 Ω/sq and conductivity of 10^4 S/cm. The substrates were thoroughly cleaned by using decon, acetone and iso-polypropanol and finally dried using nitrogen gas to remove any impurities.

3.4 Preparation of the Al/DNA Schottky Barrier Diode

The experiment is carried out in an industrial grade 1 K clean room to control and maintain the conditions in terms of temperature and humidity (about 20 °C and between range of 70 to 80 relative humidity RH%). 10 µl of DNA solution was then applied using a syringe (Hamilton, Nevada) onto the cleaned ITO surface. Self-assembly method is used for immobilization of DNA solution onto the ITO surface. This method is selected compared to other methods since the sensor fabricated will be cheapest, simple and mobile (Gong et al., 2014). Next, aluminium (Al) wire contacts are made as shown in Figure 3.3 to achieve good electrical contact. Al is chosen for the contact since aluminium is cheaper than other metals and is easily available. The thickness of the Al wire electrodes used was (0.50 ± 0.05) mm and purity 99.999 % (Sigma Aldrich, USA). Figure 3.3 below demonstrates the photo and schematic diagram of the fabricated sensor for the measurement of the I-V characteristics (Keithley Electrometer, SMU 236).

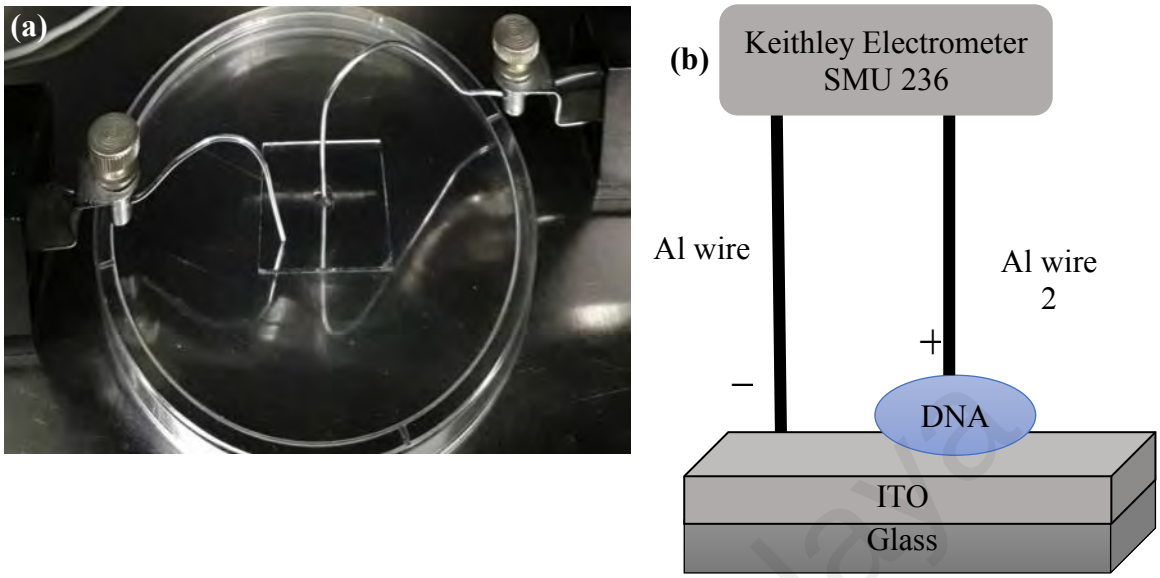


Figure 3.3: (a) Photograph of Al/DNA Schottky diode used in this work and (b) its schematic diagram.

3.5 Acquisition of I-V Characteristic Profiles and Calculation of Electronic Diode Parameters

A waiting period of 10 minutes was selected as the most suitable period for DNA to self-assemble on ITO substrate before being applied by any bias voltage (Periasamy et al., 2016). I-V characteristics were carried out in both the positive and negative biased regions. In order to obtain the I-V profile in the positive region, bias voltage was set from 0 to 3 V while in negative region; the measurements were carried out up to -20 V. The combination of I-V profile in both the positive and negative regions will form another profile, which is called the sweep profile. In this work, a bias voltage ranges between 2 and -4 V, respectively.

After all the necessary I-V profiles were obtained, various electronic diode parameters such as turn-on voltage, shunt and series resistance, knee voltage, breakdown voltage and breakdown current was calculated. These values could then be

analyzed and used to differentiate and identify specific DNA species since each DNA will have its own individual values.

The electrical parameters such as ideality factor, barrier height and series resistance of Schottky diode were obtained from the I-V characteristics using two different methods and the values were compared. The first method to calculate the electrical parameter was with the assumptions that the current in the Schottky diode are based on thermionic emission and the effects of series resistance can be ignored for low forward bias region (Tahir et al., 2012). The equation for voltage and current can be expressed as;

$$I = I_0 e^{\frac{qV}{nkT}} \left[1 - e^{\frac{-qV}{kT}}\right] \tag{3.1}$$

For $V > \frac{3kT}{q}$, equation (3.1) becomes as;

$$I = I_0 e^{\frac{qV}{nkT}} \tag{3.2}$$

where n is the ideality factor, q is the electronic charge, k is the Boltzmann constant, T is the temperature in Kelvin and I_o the reverse saturation current which can be expressed as;

$$I_0 = AA^* T^2 e^{\frac{-q\emptyset_b}{kT}} \tag{3.3}$$

where A is the effective diode area, A^* is the effective Richardson constant which is equal to 1.3 x 10^5 A/cm^{2}K^2 for ITO[23] and $Ø_b$ is the zero-bias barrier height. The I_o can be obtained by extrapolating the straight line of *ln I versus V* to intercept the axis at zero voltage.

$$\ln I = \ln I_o + \frac{q}{nkT} V \tag{3.4}$$

$$\emptyset_b = \frac{kT}{q} \ln\left(\frac{AA^*T^2}{I_0}\right) \tag{3.5}$$

Further, the deviation of Schottky diode from ideal thermionic emission model can be evaluated by calculating the ideality factor, *n* which can be extracted from the gradient of the straight-line *ln I* versus *V* plot and can be written from equation 3.2 as;

$$n = \frac{q}{kT}\left(\frac{\partial V}{\partial(\ln I)}\right) \tag{3.6}$$

To verify the values obtained for ideality factor and barrier height, Cheung's method was adopted which includes the series resistance effect to the calculation (Tahir et al., 2012). The equations involved in this method can be expressed as;

$$\frac{dV}{d\ln I} = n\frac{kT}{q} + IR_s \tag{3.7}$$

Using *dV/dln(I)* versus *I* graph, the axis intercept at zero current was obtained to calculate the ideality factor of the Schottky diode. On the other hand, to calculate the value of barrier height, *H(I)* function was used as defined by Cheung, which can be expressed as;

$$H(I) = V + n\frac{kT}{q}\ln\left(\frac{I}{AA^*T^2}\right) \tag{3.8}$$

$$H(I) = n\emptyset_b + IR_s \tag{3.9}$$

From the plot of *H(I)* versus *I*, the y axis intercept was used to estimate the barrier height of the diode. The values were compared with the first method to verify the effects of series resistance. Figure 3.4 summarizes and lists the steps involved in the experimental procedure.

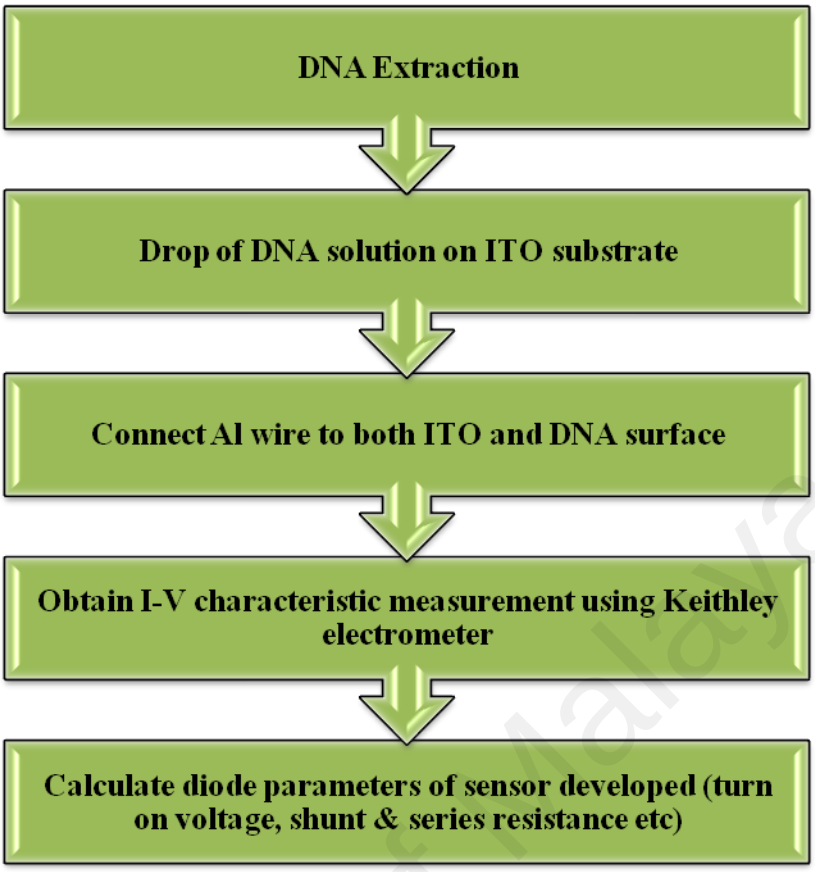


Figure 3.4: Flow chart of the experimental procedure of fabrication of Al/DNA Schottky diode sensor.

3.6 Characterization Analysis

Formations of DNA film on ITO surface by using self-assembly technique was further analyzed and investigated by carrying out selected characterization studies. The analyses involved are morphology, spectroscopy and structural studies. Spectroscopic study was performed using a UV NanoDrop Spectrophotometer to ensure there is a peak of maximum absorbance attributed to DNA and its purity. Field Emission Scanning Electron Microscope (FESEM) was selected to study the morphology of formation of DNA film.

3.6.1 NanoDrop Spectrophotometer Analysis

Generally, spectrophotometric quantification and UV fluorescence tagging are the standard approaches to quantify DNA, RNA or protein content as well as its purity. These methods only need trace amounts of sample between 0.5-2 μL for direct measurement and also avoid wasteful dilutions and expensive consumables. The procedure of nanoDrop spectrophotometer technique involves three simple steps; pipette, measure and wipe clean. It is very straightforward, non-complicated and give accurate results within seconds. Besides that, contaminating results of different samples analysis does not occur since the sample carryover is prevented by highly polished stainless steel base design (Desjardins & Conklin, 2010).

3.6.2 Atomic Force Microscopy

The characterization of microstructure at nanometer range was carried out using an AFM (Hitachi, Japan). This technique provides a simple, accurate and quantitative analysis of surface structure by the employment of a profilometer-based operating principle in this equipment (Göken & Kempf, 1999). This enables two main parameters to be acquired quantitatively from the image obtained which are width and height (Westra & Thomson, 1995). Several fields for example electronics, polymers, biomaterials, materials and manufacturing utilized this technique in order to perform analysis involving direct measurements of intermolecular forces with atomic-resolution characterization (Jalili & Laxminarayana, 2004). Fundamental studies on the atomic scale for both type of materials (insulators and conductors), could be done by using this method (Binnig et al., 1986).

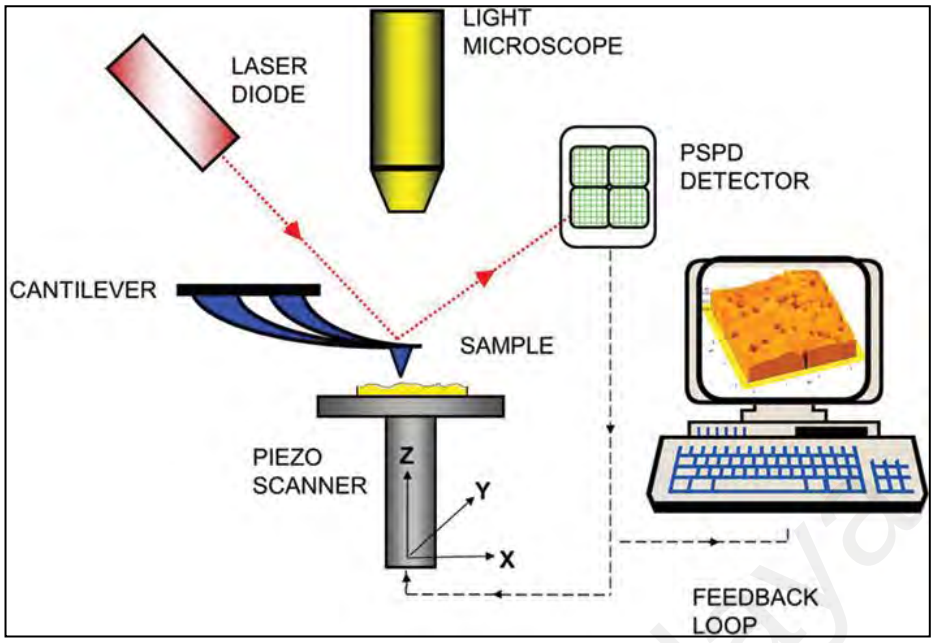


Figure 3.5: AFM schematic diagram (James et al., 2016).

The measurement of force present between a probe and the sample forms the concept of the AFM operation. Various measurements of forces can be done using AFM technique for example mechanical contact force, van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces, Casimir forces, and solvation forces (Binnig et al., 1986). There are three main components of standard AFM system, which are the micro-machined cantilever probe, a sharp tip mounted to a piezoelectric (PZT) actuator and a position sensitive photo detector as shown in Figure 3.5. In this instrument, the sharp tip attached at the end of a cantilever has an apex with a radius of curvature in nanometer range and placed very closely with the sample's surface. Its role in this system is to produce a topographical image by consistently scanning the surface of the sample. Then, the cantilever will be deflected closer to the surface of the sample due to the existence of an attractive force created between the tip and surface when the tip moves nearer to the surface (James et al., 2016).

CHAPTER 4: RESULTS AND DISCUSSION

This chapter demonstrates all the data gathered, the results of analysis done and the interpretations of findings. These are presented in tables and graph accordingly to the research objectives.

4.1 NanoDrop Spectrophotometer Analysis

Consideration on purity of the DNA content in the samples plays a vital role in the experiments carried out in this work. Inaccurate outcomes will be generated if there are any impurities in the samples and may affect the whole results. Therefore, NanoDrop spectrophotometer analysis was performed to examine the purity of the DNA samples. The concentration and ratio of absorbance at 230 to 280 nm is presented in Table 4.1.

Table 4.1: The purity and concentration of three selected algal DNA samples used in the experiment. The results were generated by using NanoDrop Spectrophotometer.

Samples	***Chlorella* sp.**	***Synechococcus* sp.**	***Amphora* sp.**
Nucleic Acid Concentration (ng/µl)	56.80	57.10	59.60
A260/A280	1.85	1.85	1.81
A260/A230	0.95	1.52	0.27

Normally, A260/A280 ratio indicates any protein or phenol contamination within the DNA samples. High purity corresponds to a range of 1.8 to 2.0 (Wilfinger et al., 1997). Another ratio to determine the purity of nucleic acid is A260/A230, which shows the existence of chaotropic salts, carbohydrates, phenolate ions, and guanidine compounds. This ratio should ideally lies between values more than 1.5 and close to 1.8 (Schrader et al., 2012). The main purity indicator for DNA is A260/A280 ratio which shows protein or phenol contamination, with pure DNA preparations having an A260/A280 ratio ≥ 1.8

to 2.0 (Wilfinger et al., 1997). In our samples, the values were from 1.81 to 1.85, which is in the acceptable range. The A260/A230 ratio meanwhile is used as a secondary measure of DNA purity, indicating the presence of chaotropic salts, carbohydrates, phenolate ions, and guanidine compounds. As a guideline, the A260/A230 ratio should be greater than 1.5, ideally close to 1.8 (Nichols & Harold, 1965). The lower A260/230 values originate from salt, ions and other contaminations from the buffer used to wash and elute the DNA during the extraction process. However, since such contaminants do not exhibit any semiconductive behavior (as shown by the insulating elution buffer profile in Figure 4.1) that may contribute to the rectification achieved by the DNA-based diodes, further purification is not required.

4.2 Positive Biased Region

It is observable in positive biased region (Figure 4.1) that every species of algae demonstrates a specific rectifying profile equivalent to a standard diode. One of the diode parameters involved in this region and may be applied to compare one species to another is the turn-on voltage (Table 4.1). This voltage is described as a certain amount of positive voltage needed by the diode to start functioning as a rectifier. It is indicated by voltage value of the x-axis when the conducting region of the profile is extrapolated towards the abscissa as shown in Figure 4.1. As a control, elution buffer used to dissolve the DNA was also tested for its conductivity behavior. We observed no rectification as shown in Figure 4.1. The profile shown indicated an insulating behavior and therefore has no influence on the semiconductive profiles generated in this work.

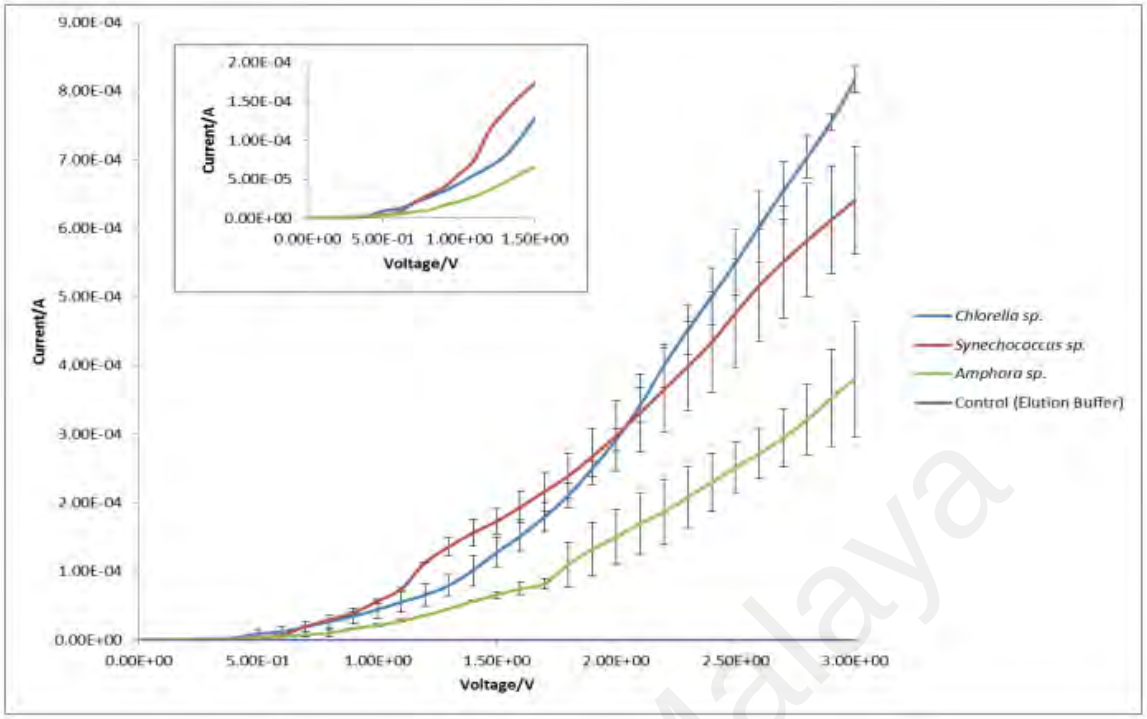


Figure 4.1: Positive biased I-V profiles derived from triplicate measurements for the three types of algal species. Insert shows the clear discrimination between the profiles within the region 1 to 2 V.

It was observed that each different algae species have their own unique turn-on voltage values. Furthermore, another two parameters were extracted from the I-V profiles, which are the shunt and series resistance. Table 4.2 listed all the values of shunt and series resistance belonging to all the three species of algae studied in this study. Shunt resistance usually takes place when high conductivity paths are present in the diode (Rockett, 2007) whereas the performance of Schottky-based devices is indicated by the series resistance (Zhijian et al., 2015).

Hence, identification of any type of algal species could potentially be achieved based on this information and might suggest an alternative electronic method to identify different algal species effectively and accurately. These characteristic values might be compiled to provide an electronic database or profile for each type of algal species. This

may also enable a novel method of understanding the fundamentals linked to in-depth studies of any DNA species.

Table 4.2: Electronic diode parameters calculated for all selected algal species.

Samples	***Chlorella* sp.**	***Synechococcus* sp.**	***Amphora* sp.**
Turn-on Voltage (± 0.05V)	1.40	1.15	1.36
Shunt Resistance (± 0.05kΩ)	164	1840	174
Series Resistance (± 0.05kΩ)	3.67	4.68	7.88

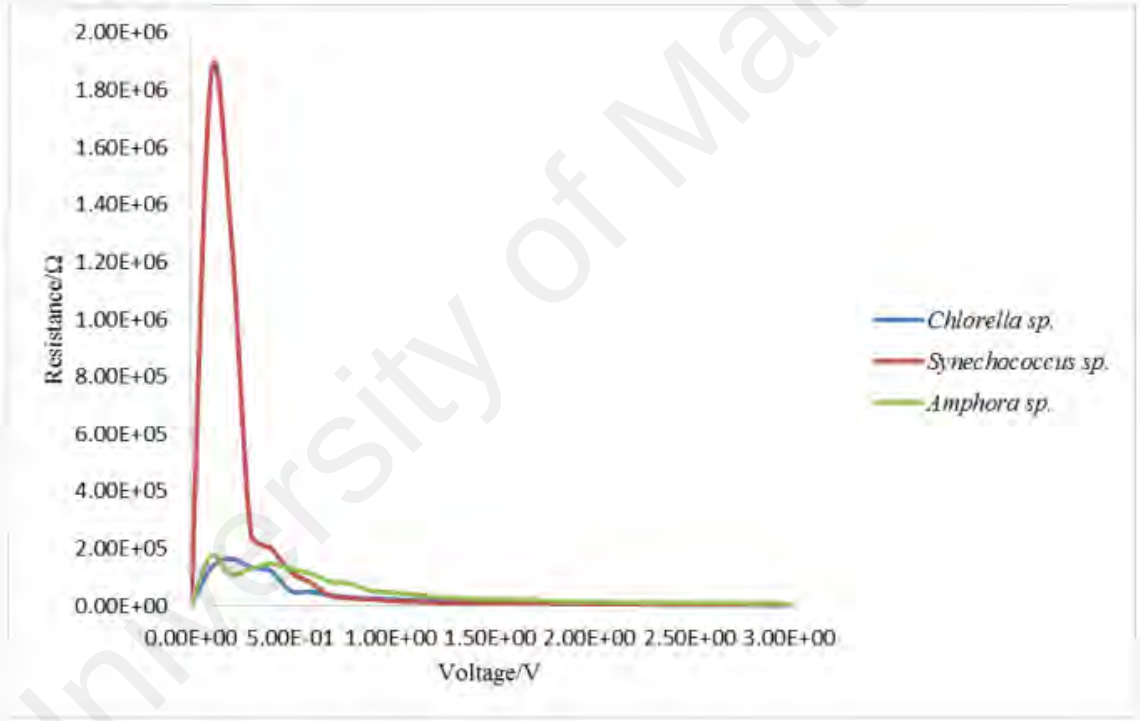


Figure 4.2: Resistance profile against bias voltage for the three selected algal species.

Figure 4.2 meanwhile demonstrate the resistance profile against bias voltage for the three selected algal species. The values of both shunt and series resistance based on the I-V characteristics in the positive or forward region could be visibly distinguished corresponding to each type of the algal species. By applying Ohm's Law ($\partial V/\partial I$), the

related calculations of resistance profiles of shunt and series resistances were measured by using the data obtained from the I-V profiles.

The maximum peak noticed in the resistance profile refers to the value of shunt resistance. It can be clearly seen that *Synechococcus* sp. demonstrated the highest value of shunt resistance, which is 1840 kΩ followed by *Amphora* sp. and *Chlorella* sp. which are 174 kΩ and 164 kΩ respectively. Series resistance, meanwhile, can be determined from the lowest saturating trend in the resistance against bias voltage graph. *Chlorella* sp. compared to another two algal species seems to demonstrate a lower value of series resistance with 3.67 kΩ of resistance followed by *Synechococcus* sp. and *Amphora* sp. with 4.68 kΩ and 7.88 kΩ respectively.

Different dynamics of the charge transfer mechanism within particular base pair sequences of the algal species investigated in this work might be the reason for the dissimilarity of the values of the resistance and other parameters as well. The accurate fundamental reasoning behind these observations however still remains elusive. In-depth research related to the DNA base-pairing mechanism itself is needed before further fundamental mechanisms can be unraveled and brought to light.

It is understood that there exist a variation of ideal to non-ideal diode behaviours responding to a host of different materials characterized by their corresponding electronic properties. The electronic processes could differ from pure thermionic in nature for ideal (ideality factor, $n = 1$) condition to ones that possibly engage with other unknown processes. This is indicative of the material, which was employed within the metal-semiconductor structure functioning as a tool for identifying the semiconductor material developed. In this work, these materials originated from the DNAs derived from different species of algae, which exhibit characteristic I-V profiles that may be utilized as a fingerprinting method. A few factors may take place when great amount of

voltage are supplied and possibly influence the deviation of the forward bias I–V characteristics of the Schottky diodes from linearity. For example, the existence of the interface layer between the metal and semiconductor will have an impact on the series resistance and therefore will lead to non-ideal forward biased I-V behaviour. This effect is significantly visible when the applied voltage is adequately large.

Cheung and Norde's functions were observed to be in conflict with each other in terms of the acquisition of a few parameters for example series resistance and barrier height. Cheung's functions are only appropriate and valid to the non-linear region (high voltage region) of the forward bias I–V curve but Norde's functions are applicable to the full forward bias I–V curve of the diodes. This situation leads to inconsistency for non-ideal diodes and display deviation from the thermionic emission theory as well. However, standard thermionic emission theory remains the most popular model preferred for characterizing diode parameters (Al-Ta'ii et al., 2015a; Al-Ta'ii et al., 2015b; Zhijian et al., 2015).

Other significant diode parameters derived from this region is the ideality factor and barrier height. Both of these parameters together with the series resistance for the three selected algae in this work were calculated using the two different methods and the values shown in Table 4.3. In method 1, the effects of series resistance was disregarded at lower region of the forward bias voltage. The value of ideality factor was determined from the slope of the linear region of the forward bias $ln\ I$ versus V graph shown in Figure 4.3 by using Equation 3.4 and Equation 3.6. It is obvious that the ideality factor measured for algal species display deviation from ideal Schottky diode in which the values are larger than 1. These values drop drastically when the effects of series resistance were considered using method 2 as illustrated in Equation 3.7. However, interfacial thin film, barrier inhomogeneity and other phenomena are expected to exist

since ideality factor demonstrate higher values for both methods (Gupta & Yakuphanoglu, 2012; Reddy et al., 2011).

Table 4.3: Electronic diode parameters calculated for the algal species.

Samples		***Chlorella* sp.**	***Synechococcus* sp.**	***Amphora* sp.**
Method 1	***n***	20.74	25.84	20.94
	Ø	0.8469	0.8257	0.8629
	R_s	-	-	-
Method 2	***n***	11.54	11.25	14.06
	Ø	0.8894	0.8634	0.8827
	R_s	1.4 kΩ	2.4 kΩ	2.9 kΩ

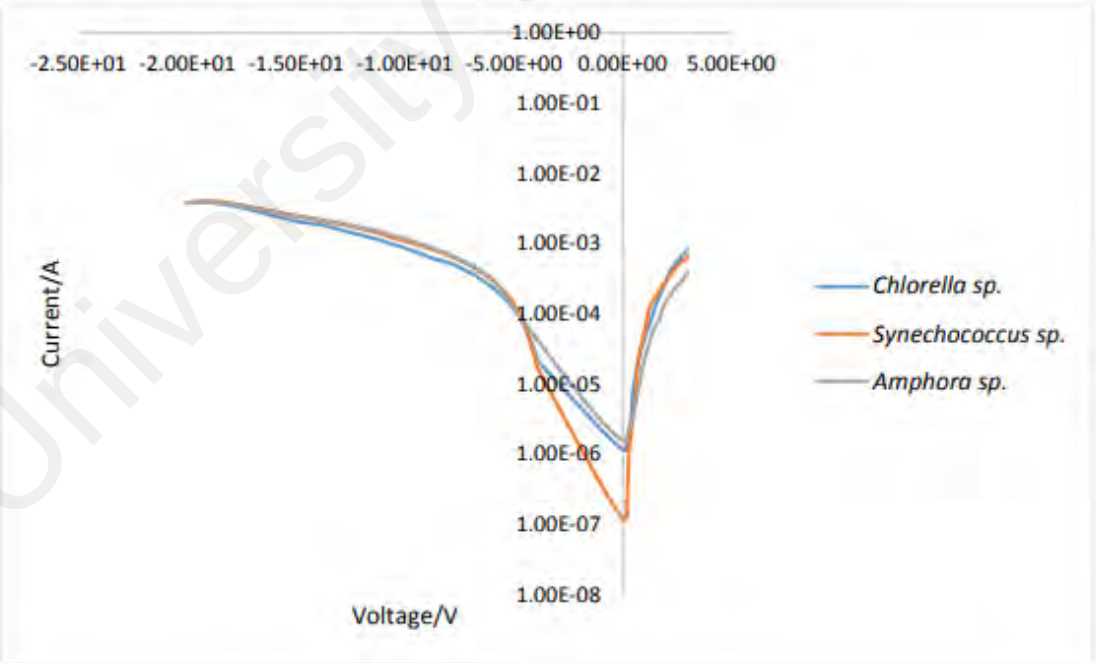


Figure 4.3: Semi-log current versus voltage.

On the other hand, barrier height calculated with method 1 using Equation 3.4 and Equation 3.5 shows similar results with method 2 calculated using Equation 3.8 and Equation 3.9, which illustrate that it was not influenced much by the series resistance. Moreover, the values for series resistance obtained from Cheung's method using *dV/dln I* versus *I* and *H(I)* versus *I* plots were in good agreement with the values obtained from resistance profile against bias voltage plot. It can be observed that by excluding the series resistance in the calculations of the Schottky diode parameters mainly for ideality factor might cause errors to take place. In conclusion, Cheung's method was observed to be the most reliable way to extract the diode parameters.

4.3 Negative Biased Region

Figure 4.4 shows the negative or reverse biased I-V characteristic for the selected algae involved in this study. Al/DNA Schottky sensors demonstrate significantly clear I-V profiles with breakdown current occurring at a voltage region between -18 to about -20 V in this work. Characteristics related to the mechanisms of current flow in negative region enable the extraction of certain electronic parameters for example knee voltage, breakdown voltage and breakdown current as listed in Table 4.4. These values are determined by the type of the species of algae DNA used and at the same time it may propose a possible DNA identification and detection tool following an in-depth research on the mechanism behind such observations.

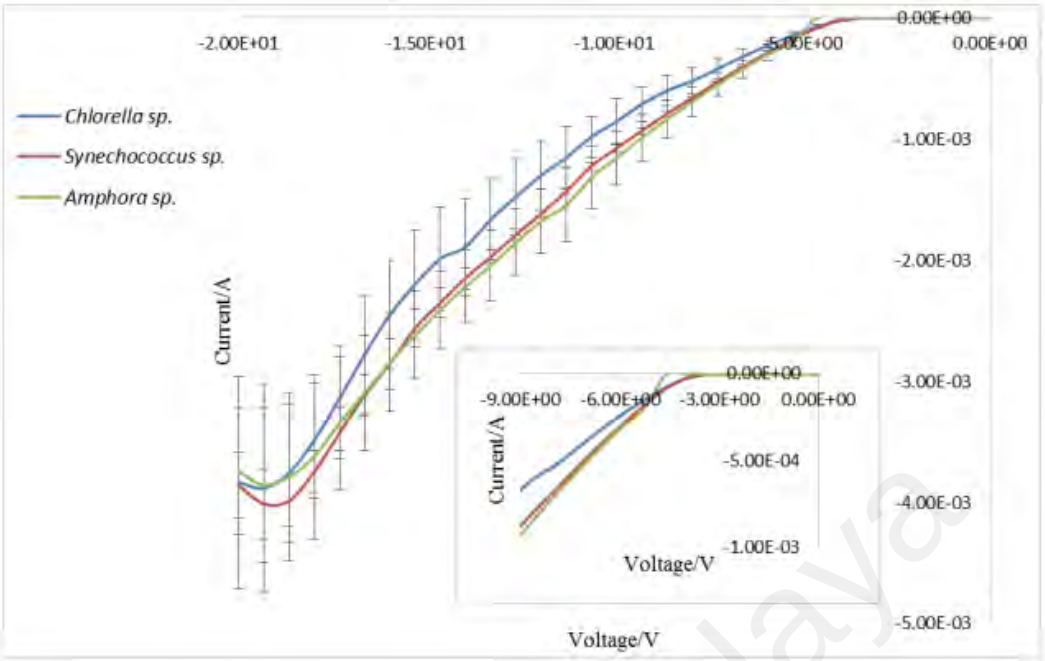


Figure 4.4: Negative biased I-V profiles for the three selected algal species carried-out in triplicates. The insert shows the magnified region between 0 and -9 V for clearer visualization.

The values recorded in Tables 4.3, 4.4 and 4.5 demonstrate significantly distinguishable electronic differences that could be observed between two different types of organism; algae (current work) and mushroom (Periasamy et al., 2016). For example, there is a significant difference from the values of turn-on voltage for both *Chlorella* sp. and Enoki mushroom, which are 1.40 V and 0.30 V, respectively. In addition, another obvious contrast in values is the series resistance values for *Synechococcus* sp. (6.10 kΩ) and White Oyster mushroom or *Pleurotus floridanus* (11.50 kΩ). These may conclude that they do not belong to the same species group.

4.4 Surface Morphology Analysis

Figure 4.5 displays the FESEM image of the distribution of DNA strands on the self-assembled DNA film. It shows the uniformly distributed formation of fractal-like DNA strands on the ITO surface achieved when using the simple but effective self-assembly

technique. The size of the strand was observed to be between tens of nanometers towards the center and in micrometer scale at the edges as a result of the compressive force of the DNA layer. No other foreign substance or impurities was observed on the film which could have disrupted the analysis carried out.

Table 4.4: Electronic diode parameters (knee voltage, breakdown voltage and breakdown current) calculated for the algal species.

Samples	***Chlorella* sp.**	***Synechococcus* sp.**	***Amphora* sp.**
Knee Voltage (± 0.05) V	-4.25	-4.25	-4.50
Breakdown Voltage (± 0.05) V	-19.33	-19.33	-19.33
Breakdown Current (± 0.05 x10^{-3}) A	-3.88	-4.01	-3.86

Table 4.5: Electronic diode parameters calculated for selected mushroom species from previous literature (Periasamy et al., 2016).

Diode Parameters	**White Oyster (KLU-M1382)**	**Enoki (KLU-M1387)**
Turn-on Voltage (± 0.05) V	1.00	0.30
Series Resistance (± 0.05) kΩ	11.50	1.90
Shunt Resistance (± 0.05) kΩ	189.00	4.60
Knee Voltage (± 0.05) V	-4.00	-10.90

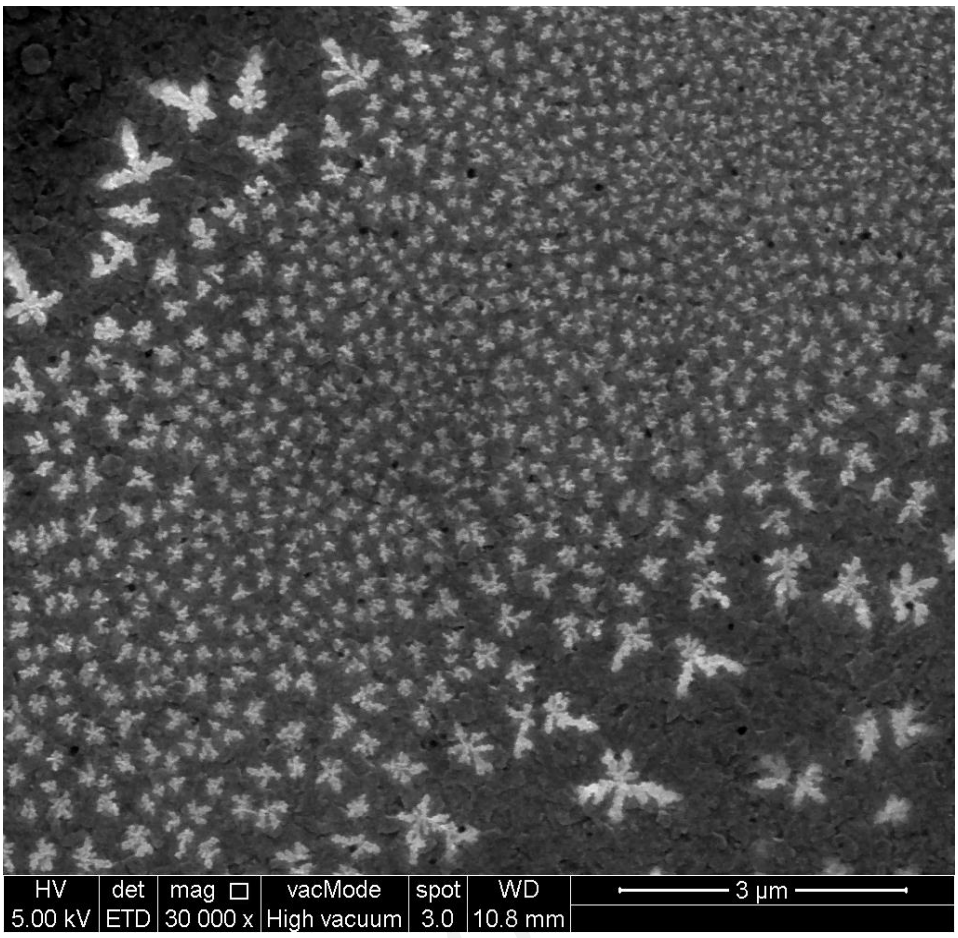


Figure 4.5: FESEM images of the distribution of DNA strands on the self-assembled DNA film.

4.5 Summary of Chapter

This chapter reveals that identification of selected algal species has been successfully achieved by using the electronic method of sensing using the DNA-specific Schottky junction or diode. Each algal species has been found to demonstrate a set of various diode parameters calculated using the characteristic I-V profiles. These data can in turn be utilized to identify and classify different species using the electronic approach. In addition, the results obtained have significant differences when compared between the algae utilized in this study and mushroom species from our previous study (Periasamy et al., 2016).

CHAPTER 5: CONCLUSIONS AND FUTURE WORKS

5.1 Conclusions

In this work, we have successfully developed a novel DNA-specific Schottky diode based electronic profiling technique. Selected DNA from three different algal species (*Chlorella* sp., *Synechococcus* sp. *Amphora* sp.) were used and its electronics profiles obtained and analyzed. The electronic technique developed offered several advantages, which includes simplicity, rapidness (10 minutes), sensitivity, practicality and economical viability. The results from this work highlight these advantages, which were also the objectives, listed in this study.

Primarily, Al/ITO/DNA/Al based sensors using the Schottky diode sensor structure was fabricated. We discovered that this sensor exhibited a specific I-V characteristic profile for every algal species used in this work. Besides that, various diode parameters were also extracted and calculated. Each species has its own values of turn-on voltage, shunt and series resistance, ideality factor, barrier height, knee voltage, breakdown voltage as well as breakdown current that can be utilized as a possible tool for detection and identification. For example, the turn-on voltage values for *Chlorella* sp., *Synechococcus* sp., and *Amphora* sp. were 1.44 V, 1.24 V and 1.30 V, respectively. The series resistance values meanwhile were calculated as 4.60 kΩ, 6.10 kΩ and 7.00 kΩ accordingly. This and other values pertaining to the solid-state parameters calculated in this work, significant differences could be observed suggesting potential electronic identification of the related species.

5.2 Future Works

The outcome of this pioneering work involving DNA electronics may provide a better and improved detection technique that is primarily based on electronics. An

electronic database of every species of algae and not only limited to the three species studied in this work could be generated by using this method leading to an accurate and efficient method of identifying algal species in future.

This rapid electronic profiling method may also be applied in other fields such as taxonomy, medical, forensic etc. due to its various advantages compared to other conventional techniques. Furthermore, this novel technology could be utilized as a tool in future for studying damage and repair mechanism within DNA molecules crucial towards understanding disease progress and mutation.

In summary, DNA electronics may allow fundamental studies related to in-depth understanding about DNA mechanism in all life processes and may well usher in a new approach in molecular biology. This current preliminary work aims to unconventionally demonstrate this statement; using DNA electronics as a mean to study molecular biology.

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LIST OF PUBLICATION AND PAPER PRESENTED

List of Publication

1. **Azmi, S. Z.**, Vejeysri, V., Rizan, N., Krishnasamy, J., Talebi, S., Gunaselvam, P., Mohamed Iqbal, S. N., Chan, Y. Y., Phang, S. -M., Iwamoto, M. & Periasamy, V. (2018). Electronic profiling of algae-derived DNA using DNA-specific Schottky diode. *Applied Physics A*, *124*(8), 559-569.

Paper Presented

1. **Azmi, S. Z.**, Rizan, N., Phang, S. -M., & Periasamy, V. (2016). *A novel electronic based DNA detection method.* Paper presented at the National Physics Conference (PERFIK), 21-22 December 2016, Kuala Lumpur, Malaysia.