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DETECTION OF AEROMONAS HYDROPHILA USING FIBER OPTIC SENSOR

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DETECTION OF *AEROMONAS HYDROPHILA* USING FIBER OPTIC SENSOR

By

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DETECTION OF AEROMONAS HYDROPHILIA MICROORGANISM USING FIBER OPTIC SENSOR

SAMLA GAURI A/P BALAKRISHNAN

JULY 2015

Chair: Assoc.Prof. Dr.Zurina Zainal Abidin, PhD
Faculty: Engineering

Pathogenesis including chronic infections, inflammation, malignancy, tissue break down, and immune system disorder are linked with pathogens that affect human health with massive diseases. *Aeromonas hydrophila* infections increasingly recognized as a serious worldwide public health concern. Although numerous antibiotics and vaccination have been introduced to protect against diseases, some pathogen continues to threaten living life. However, the current pathogen detection method which based on molecular culture and PCR techniques are fundamentally slow and time consuming. Consequently, these discoveries have created awareness in development of effective pathogen detecting tool to control the related quality and prevent further infections. In recent time, several researchers have attempted to develop rapid detecting tool. Despite advanced engineering, there is still need for an accurate and rapid pathogen detection tool. Thus, this research has been carried out to highlight mostly on detection of *Aeromonas hydrophila* by using optical biosensor as contribution of this study while *Escherichia coli* and *Saccharomyces cerevisiae* were used for justification of the findings as part of the experimental work. In this study, we proposed an optical based biosensor as relatively an accurate method of early effective detection of pathogen or infectious agent. Fiber optic flow cell and fiber optic microchannel were used in this study for detection of pathogens. Fiber optic flow cell was used as preliminary study for optical characteristic of the pathogens used. Then optical microchannel was fabricated with fiber optics by using photolithography method. Fiber optic was chosen as signal receiving and transmitting medium due to its excellent plus rapid signal delivering feature. Fiber optic biosensor is based on light scattering, absorption and optical properties of the microorganisms. The chemical composition, energy, the total nucleotides and photopigments will define the absorption properties of each microorganism. The difference in peak absorbance spectra of microorganism at particular region of wavelength will be manipulated for detection of the sample. Based on the experimental findings, the detection of *A.hydrophila* and *E.coli* of any phases (lag, exponential and stationary) were fall in UV spectral region while every phases of *S.cerevisiae* detection falls in visible spectral region. *A.hydrophila* was identified at between 350nm to 354nm and *E.coli* detected at region of 280nm to 285nm by using the microchannel. While, *S.cerevisiae* recognized in visible region of 570nm to 580nm. The entire detection can be done in less than 10 minutes. These detection regions for each sample have been compared with fiber optic flow cell measurements, spectrophotometer measurement plus theoretical calculations by using Beer Lambert Absorption Law and proved the detection regions were in approximately accurate spectral.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

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Pengurusi: Prof.Madya Dr.Zurina Zainal Abidin, PhD
Faculti: Kejuruteraan

Patogenesis merupakan sejenis jangkitan berasaskan mikroorganisma atau lebih dikenali sebagai patogen yang berpotensi untuk menjejaskan kesihatan manusia. Implikasinya mampu menyebabkan keradangan, kerosakan tisu, gangguan sistem imun dan jangkitan kronik. *Aeromonas hydrophila* merupakan salah satu patogen yang menyebabkan jangkitan bukan sahaja kepada manusia dan haiwan malah turut mempengaruhi sumber ekonomi Jabatan Perikanan di Malaysia. Terdapat banyak hasil penyelidikan yang telah diterbitkan mengenai implikasi patogen dan jangkitannya yang serius. Walaupun terdapat antibiotik dan vaksin untuk melindungi daripada jangkitan penyakit yang berasaskan patogen, namun kesihatan manusia dan haiwan tetap tidak terjamin dengan penyelesaian tersebut. Kaedah pengesanan patogen secara konvensional melalui pengenalpastian molekul dan teknik PCR adalah perlahan dan mangambil masa yang lama untuk menyelesaikan satu kitaran lengkap. Penyelidikan terkini lebih tertumpu ke arah perkembangan alat pengesan patogen untuk mengawal kualiti kehidupan dan mencegah jangkitan penyakit yang disebabkan oleh mikroorganisma. Justeru, kajian ini telah dijalankan untuk menitik beratkan aspek teknik pengesanan mikroorganisma. "*Fiber optic flow cell*" dan "*fiber optic* microchannel" merupakan dua jenis kaedah pengesanan optik yang digunakan. "Fiber optic flow cell" digunakan sebagai kajian awal untuk menganalisis ciri-ciri optik patogen yang digunakan dan "Fiber optic microchannel" dihasilkan melalui kaedah "photolithography". Fiber optic telah dipilih sebagai sumber penerima dan penghantar isyarat optik untuk mengesan patogen. Biosensor fiber optik ini berasaskan penyerapan dan ciri-ciri optik mikroorganisma sendiri. Komposisi kimia, tenaga, jumlah "nucleotides" dan "photopigments" akan menentukan sifat-sifat penyerapan optik setiap mikroorganisma. Perbezaan keserapan (absobance) optik yang berlainan pada kawasan (wavelength region) tertentu antara 200 nm hingga 900 nm akan dimanipulasikan untuk mengesan sampel sasaran tersebut. Hasil kajian dapat dikaitkan bahawa, *A.hydrophila* dan *E.coli* dari kesemua fasa pertumbuhan dapat dikesan pada sinaran gelombang ultraungu (UV) manakala pengesanan setiap fasa *S.cerevisiae* yang boleh dikesan di bawah sinaran UV. *A.hydrophila* telah dikenalpasti di antara 350nm kepada 354nm dan *E.coli* yang dikesan diantara 280nm ke 285nm dengan menggunakan "fiber optic microchannel". Sementara itu, *S.cerevisiae* dapat dikesan daripada 570nm hingga 580nm. Keputusan yang diperolehi untuk setiap sampel daripada kajian "fiber optic microchannel" telah dibandingkan dengan kajian keputusan daripada "fiber optic flow cell". Seterusnya keseluruhan keputusan dibandingkan dengan "*spectrophotometer*" dan teori keserapan "*Beer Lambert Law of Absorption*" untuk membuktikan pengesanan mikrorganisma tersebut.

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Declaration by graduate student

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LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
DNA	Deoxyribonucleic acid
MAb	Monoclonal antibody
AHL	H-acylhomoserine lactone
FF-2A	Fragrance and flavor analyzer
LOC	Lab-on-chip
EW	Evanescent wave
ATR	Attenuated total reflection
UV-Vis	Ultra violet to visible
LED	Light-emitting diodes
LD	Laser diode
RNA	Ribonucleic acid
PZ	piezoelectric
ECA	*Enterobacteriaceae* common antigen
EIS	Electrochemical impedance spectroscopy
Z	Impedance
C	Capacitance
BQ	Benzonquinone
TIR	Total internal reflection
NA	Numeric aperture
ILD	Injection laser diode
APD	Avalanche diode
SNR	Signal to noise ratio
GI	Gastrointestinal
STRIDE	Science & Technology Research Institute for Defense
MINDEF	Ministry Of Defense
TSA	Tryptic soy agar
TBS	Tryptic soy broth
LB	Luria broth
YPD	Yeast extract, Peptone, Dextrose
PBS	Phosphate buffered saline
AU	Absorbance unit
V	Voltage
OD	Optical density
dB	Decibels

LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
DNA	Deoxyribonucleic acid
MAb	Monoclonal antibody
AHL	H-acylhomoserine lactone
FF-2A	Fragrance and flavor analyzer
LOC	Lab-on-chip
EW	Evanescent wave
ATR	Attenuated total reflection
UV-Vis	Ultra violet to visible
LED	Light-emitting diodes
LD	Laser diode
RNA	Ribonucleic acid
PZ	piezoelectric
ECA	*Enterobacteriaceae* common antigen
EIS	Electrochemical impedance spectroscopy
Z	Impedance
C	Capacitance
BQ	Benzonquinone
TIR	Total internal reflection
NA	Numeric aperture
ILD	Injection laser diode
APD	Avalanche diode
SNR	Signal to noise ratio
GI	Gastrointestinal
STRIDE	Science & Technology Research Institute for Defense
MINDEF	Ministry Of Defense
TSA	Tryptic soy agar
TBS	Tryptic soy broth
LB	Luria broth
YPD	Yeast extract, Peptone, Dextrose
PBS	Phosphate buffered saline
AU	Absorbance unit
V	Voltage
OD	Optical density
dB	Decibels

CHAPTER 1

INTRODUCTION

1.1 Background

Pathogen which can be found in environment, food, waste water and others could cause massive health diseases especially to young children when it compromised immune system's ability to fight off such serious infections. Pathogenesis usually linked with pathogens that affect human health. Nowadays, there are several medical advances such as antibiotics and vaccinations to protect against diseases, food safety and hygiene as well as water treatment are available to reduce the rate of infections. However, being their nature some pathogens continue to threaten human life.

Basically, pathogens are opportunistic free living microorganisms and always present in water, soil as well as food. *A.hydrophila* is a Gram-negative aerobic and facultative anaerobic, oxidase-positive motile bacterium that mostly found in aquatic environments especially fresh stream and ponds. This bacterium contributes to internal infectious such as dermal ulceration, rotting of tails, inflammation, exophthalmia, scale protrusion and other in fish species (Austin B and Austin DA, 2007). Particularly, it has been associated with diseases complication and most pathogenic infections for Cyprinuscarpio (Koi) and Carassiusauratus (fancy gold fish), eels, tilapia, catfish, frogs, other vertebrates and invertebrates. Consequently, it caused high mortality and economic losses of fish farming (Siegel et al., 2004). For instance, the developing oyster farming in Malaysia faced economical critical due to *A.hydrophila* infection and contamination of fecal pathogens through raw oyster. According to a recent study conducted at an aquarium shop in Terengganu-Malaysia, it stated that an average ornamental fish caused internal complication by 60% of *A.hydrophila* (Musa, 2008). Moreover, these bacteria caused pathogenesis to human through freshwater fish hermorrhagic and zoonotic diseases, plus food borne infections. *Aeromonas* infections mainly related to gastrointestinal disease, diarrheal and cellulitis or wound infections. According to medical journal of Malaysia, several numbers of cases about *A.hydrophila* complications have been reported in Malaysia since 1985 (Laith and Najiah, 2013). Besides, the microorganism is resistant towards the usual antimicrobials (*ampicillin*, *cephalosporin* and *cloxacillin*) that mostly available for treatment. Thus it requires highly appropriate antimicrobial treatment to avoid and control potential serious consequences (Chugh, 2008).

Hence, pathogen detecting tool would be helpful to recognize pathogen at an early stage, consequently can avoid diseases and infections to animals and human plus economic losses can be solved indirectly. Detection of pathogen requires a high efficient, sensitive and rapid tool due to its pathogenicity and fast infection rate. In past years several methods have been developed for detection of opportunistic microorganism. Recently, an advance in molecular biology, micro-electronics, optical and computer technologies has led to the development miniaturized fiber optic biosensor for fast and effective detection of microorganisms. Research in the development of miniaturized biosensor system is mainly to replace a common microbial test that involves laborious steps and difficult instrumentations. Moreover, a rapid system is required to detect "on the spot" and "straight-forward detection" to replace the common method which was time consuming. An optical fiber in sensor technology especially in detection of micro particles, delivers a very high sensitivity either with applications of light-emitting diodes (LED) or laser diode (LD) as an excitation sources. The theory of fiber optic sensor is based on light scattering, absorption and waveguide or optical properties of the microorganisms. The absorption properties of each microorganism differ by their chemical composition which includes energy, the total nucleotides (DNA + RNA), non-chromophoric protein, and photopigments (Alupoaei and Garcia-Rubio, 2005). Thus, the targeted microorganism emits light at different wavelength from those emitted by other microorganisms.

In this research, fiber optic microchannel has used as analytical device for detection and identification of microorganism like *Aeromonas hydrophila*. A high-handed light source with wider region of spectrum with a built-in photodetector was used for this research to setup the optical detection system. The handheld as well as its unparalleled level of flexibility of light source integrated photodetector gives high efficient results with higher resolution and sensitivity during the experimental measurements. The portable and light weight detection system makes it very convenient. The transmission of light with higher intensity was directed to the sample through fiber optic and the signals collected by photodetector. The detection of microorganism is determined based on their absorbance spectrum and region.

1.2 Problem Statements

Aeromonas hydrophila is a free living pathogen that causes not only infections to human and animal but also influence the economics of fisheries department especially in Malaysia. A considerable amount of literature has been published on these pathogens and their infections with complications in these past years. Although numerous antibiotics and vaccination have been introduced to protect against diseases, some pathogen continues to threaten living life. These Aeromonas infections increasingly recognized as a serious worldwide public health concern. Nevertheless, the current pathogen detection method which based on molecular culture and PCR techniques are fundamentally slow and time consuming. Consequently, the discoveries have created awareness in development of effective pathogen detecting tool to control the related quality and prevent further infections. In recent time, several researchers have attempted to develop rapid detecting tool. The main features of detecting tool based on its ease of use, simplicity, low cost, superb sensitivity and specificity will be directed to quickly becoming tool of choice for pathogenic variants. Despite advanced engineering, there is still need for an accurate and rapid pathogen detection tool. Even today, the crucial decisions related to the presence of microorganisms have to be made before the results of microbiological tests are available. The entire system must be detected quickly to avoid human suffering due to illness and losses due to product withdrawals. Hence, a rapid system is required to detect "on the spot" to replace the common method which takes days to solve. A part from that, a simple and easy method or device is also required to identify microorganisms in a hassle free way. A "straight-forward" miniaturized system should be proposed to replace the traditional method which involved complex instrumentations as well as high cost. Moreover, a convenient system is also essential for recognition of targeted microorganism in order to solve problems effectively.

In recent times, variety of smart sensor has been developed as a detecting tool which was capable to deliver low detection limits within hours for a wide range of samples. However, the necessities of a simple microbial detector which can provide a sensitivity of 10^2-10^3 cell/m*l* with a rapid analysis time are still desirable. The sensitivity level test is mainly to investigate the responsive of the device for low detection limit. Besides, previous study specified that the accuracy of detecting microbe was efficient in optical measurement techniques (Ferreira et al., 2001). Thus, fiber optic was used widely in several measurement parts since fiber optic sensors provide a highly sensitive and low cost detection tool. For instance, Daniel (2003), proposed evanescent wave fiber optic biosensors to identify particulate matter in minutes directly from complex matrix samples using robust antibody-based assays. Ferreira et al, (2001) conducted research about Intensity modulated fiber optic sensor based on response of light wave intensity when interacted with bacteria that to generate signals. The fiber optic sensor recognized bacteria concentration up to 10^3 cells/*ml* in less than four hours. Although detection limit of the sensor was successful, yet the time taken to rectify the problem was quite longer compared to other types of fiber optic sensor. As the need for lower detection limit, high sensitivity level plus rapid system is still needed. Cell concentration is vital to determine the percentage of contamination (i.e. food), quality (i.e. water), percentage of infection (i.e. disease) and others. Along with concentration of cells, the known transmission rate of cells could resolve the further infection by some preventive way.

1.3 Aim and Objectives of the Research

The aim of this research is to develop fiber optic biosensor to detect microorganism in a particular sample. The studies consist of the following objectives:

i. To determine the optical absorption spectrum of *Aeromonas hydrophila* using fiber optic flow cell and fiber optic microchannel.

ii. To compare the performance of the fiber optic flow cell and fabricated microchannel for detection of microorganism.

1.4 Scope of Research

This thesis presented an approach to detect and identify the targeted microorganism in particular sample using optical technique. In order to develop an optical microbial detection system, suitable platform, optimum wavelength, optical characteristic of the sample, safety aspect and appropriate source of transmitter as well as detector were determined based on preliminary study using fiber optic flow cell.

The scope of this research paper consists of design and fabrication of the microchannel based on fiber optic flow cell, preparing various samples, runs the experiment in order to apply as a multi-functional fiber optic biosensor and finally validate the entire results obtained from fabricated microchannel by theoretical value and calculated value. An approach related to optical characterization of microorganism was evaluated by using fiber optic flow cell. Consequently, the microbial detection system is designed based on lab-on-chip with optical technique for measurement. The proposed fiber optic biosensor was fabricated and tested based on Beer-Lambert law of absorption which the final output delivered the detecting region of particular cell plus number of cell detected in sample. An appropriate light source and detector was used according to selected wavelength for this research. Besides, fiber optic was chosen as medium to transmit and receive output signals as it presented low attenuation while measurement thus the efficiency of overall results will remain consistent.

The design and fabrication of the microchannel for the biosensor was carried out by using photolithography method which will be explained further in chapter three. Besides, fiber optics were aligned and fixed permanently on the biosensor to direct the light transmission to the samples as well as detection medium of photodetector. The main targeted bacteria which need to be detected and identified for this research included *A.hydrophila* and *E.coli.* However, few other samples were used for the comparison and justification of the results obtained. Preparing samples were discussed in details in chapter three. For this study, three types of experimental work conducted including transmission measurement, absorbance measurement of sample to identify peak absorbance region, and test the sensitivity level with lower detection limit. All these experimental work were carried out by using fiber optic flow cell as well as fiber optic microchannel for results justification.

The total setup of experiment was consisting fiber optic microchannel and fiber optic flow cell, light source, photodetector and samples to test. The different peak absorbance spectra of each sample at their region of interest lead to the identification of the targeted microorganism. Then, all the results will be justified by comparison with photospectrometer, fiber optic flow cell, theoretical values and calculated values. Finally, the obtained results with saved spectrum will be shown in the form of graphical values and table for comparison as well as validation purpose.

1.5 Thesis Organization

The method proposed in this research investigated the difference involve in biosensor technology particularly in detection and identification of targeted microorganisms. The paper also had a brief overview of the important features of the proposed methodology and results obtained with uncertainties. This outline of this thesis was divided into five chapters and presented with a view to allow the readers at different levels to quickly allocate areas of their interest.

The first chapter included introduction which presented the background information of the study plus development and techniques of the study. Problems which lead to development of this research and the necessary objectives in order to achieve the aim of this research has described here.

The following chapter referred to literature review of the subjects of interest. It presented a brief history of the lab-on-chip technology, biosensor information and also optical detection system in biosensor for particular applications. It follows by the measurement techniques of the biosensor for optical detection method and its concepts were discussed. It also enclosed the previous works done for biosensors with optical detection and the optimization needed for more efficient outputs. Then, the optical properties of biological cells with related measurement techniques were introduced. The review of optical properties of microorganism also delivered brief details of the theoretical basis of the output signal processing techniques used for measurements in this research.

The third chapter described detail study of the microchannel (fiber optic flow cell) used for preliminary work for this research, design and fabrication of microfluidic fiber optic biosensor, methods involved in sample preparation as well as the measurement techniques needed to carry out the experimental work. The analysis of the fiber optic flow, wavelength selection, light source and detector used for further detection and process were discussed. Similarly, the material used to design the biosensor and methodology involved to fabricate it with fiber optic also added in this chapter. It followed by the selection of an optimum wavelength based on fiber optic flow cell measurement, related light source and detector were discussed.

The next chapter explained the measurement results using fiber optic flow cell and developed fiber optic biosensor. The detection regions for samples were selected based on the maximum absorption at particular region by the biological samples used for this research. Besides, the enumeration of cells were measured by the number photons detected for each sample. The uncertainty during measurements and errors of results explained further in discussion section. In addition, the justification of the results and discussion of every experimental work, challenges, computing platform used for experiments also discussed here.

And the final chapter comprised conclusion drawn from this experimental work, contribution of this research and recommendations plus suggestions of several ideas for related future work.

Lastly, the complete experimental set up and some other related graphical illustration were included in the final parts of several appendices.

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