Characterization Of Quartz Crystal Microbalance Sensor For Detection Of Bacteria Inactivation After Plasma Treatment

by

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LIST OF SYMBOLS

°C	Degrees Celsius
%	Percentage
μg	Microgram
μL	Microliter
mL	Millimeters
g	Gram
ng	Nanogram
V	Volts
mV	Millivolts
Δm	Change in mass per unit area
f_0	Resonance frequency
Δf	Change of the resonance frequency
ρ_q	Density of quartz
μ_q	Shear modulus of quartz
A	Area
A	Ampere
F	Frequency
Hz	Hertz
MHz	Megahertz
s	seconds (s)
cm	centimeter

MΩ	Megaohm
pF	Picofarad
KΩ	kiloohm
R_{lim}	Limit resistor
C_X	Changeable capacitor value
R_1	Bias or feedback resistor 1
R_2	Bias or feedback resistor 2
C_1	Load capacitor 1
C_2	Load capacitor 2
v/v	Volume per volume

LIST OF ABBREVIATIONS

ATTC	American Type Culture Collection
Au	Gold
BSA	Bovine Serum Albumin
CFU	Colony forming unit
CMOS	Complementary Metal-Oxide Semiconductor
DC	Direct Current
DNA	Deoxyribonucleic acid
EDC	1-ethyl-3-(3-dimethylaminoprppyl) carbodiimide
GPIB	General purpose interface bus
GUI	Graphic User Interface
He	Helium
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
IC	Integrated Circuit
LOD	Limit of detection
MHDA	16-Mercaptohrxadecanoic acid
NHS	N-hydroxysuccinimide
NaOH	Sodium hydroxide
QCM	Quartz Crystal Microbalance
QMB	Quartz Microbalance

SAM	Self-Assemble Monolayer
SD	Standard deviation
SEM	Scanning Electron Microscopy
ssDNA	Single-stranded DNA
TEM	Transmission Electron Microscopy
TTL	Transistor-transistor Logic
VOC	Volatile Organic Compound

Pencirian Penderia Quartz Crystal Microbalance untuk Ketidakaktifan Bakteria Selepas Rawatan Plasma

ABSTRAK

Tesis ini membentangkan kajian tindakbalas frekuensi untuk mengesan bakteria aktif dengan menggunakan pengesan *Quartz Crystal Microbalance*. Ciri-ciri tindakbalas frekuensi terhadap pengesanan bakteria dibincangkan. Bakteria *Escherichia coli* dengan kepekatan $x10^8$ *Colony Forming Unit per milliliter* di bunuh dengan menggunakan alat pensterilan iaitu tekanan atmosfera jet plasma. Alat pengesan telah dibina untuk memantau perubahan frekuensi bakteria dalam keadaan yang berbeza dengan menggunakan 9 MHz kuarza kristal. Untuk mengesan sampel bakteria, permukaan pengesan telah disediakan dengan menggunakan teknik *Self-Assemble Monolayer* bersama-sama antibodi *Escherichia coli* sebagai *bioreceptor* untuk pengecaman antigen. Pengesan *Quartz Crystal Microbalance* dengan elektrod emas dipasang dalam litar pengayun dan didedahkan pada suhu bilik. Litar *Transistor-transistor Logic* telah digunakan kerana ia dapat memacu Pengesan *Quartz Crystal Microbalance* di udara dan air. Hanya sebelah kristal di dedahkan kepada sampel cecair dengan menggunakan ukuran statik. Tindakbalas frekuensi diperhatikan untuk menyelidik tingkah laku bakteria *Escherichia coli* pada permukaan pengesan. Keputusan menunjukkan bahawa bakteria selepas dinyahaktif mempunyai perubahan frekuensi yang lebih tinggi daripada bakteria sebelum rawatan plasma. Analisis bacaan frekuensi yang dibentangkan menunjukkan bakteria yang dibunuh alat atmosfera jet plasma mempunyai penambahan berat memandangkan tindakbalas frekuensi adalah berbeza. Situasi ini berpunca daripada beberapa faktor seperti kehadiran molekul asing dalam sampel bakteria tidak aktif, jenis bioreseptor, kekasaran permukaan dan keadaan permukaan elektrod. Analisis juga merangkumi kesan piezoelektrik, keadaan ujikaji dan ciri-ciri kekasaran permukaan. Daripada 10 alat pengesan yang digunakan dalam ujikaji, purata menunjukkan 8 daripada 10 alat telah berjaya mengesan bakteria. Pengesanan juga bergantung pada keadaan permukaan elektrod. Selain itu, sistem ini mempunyai keupayaan untuk mengesan keadaan bakteria aktif dan tidak aktif dalam air.

Characterization of Quartz Crystal Microbalance Sensor for Detection of Bacteria Inactivation after Plasma Treatment

ABSTRACT

This thesis presents a study of frequency response for detection of inactive bacteria using Quartz Crystal Microbalance sensor. The characteristics of frequency response towards detection of bacteria were discussed. The *Escherichia coli* bacteria with concentration $x10^8$ colony forming unit per milliliter were inactivated using new sterilization device which is atmospheric pressure plasma jet. A sensor device was constructed to monitor frequency response of bacteria in different condition by using 9 MHz quartz crystal. For detecting the bacteria sample, the sensor surface was prepared using Self-Assemble Monolayer technique together with *Escherichia coli* antibody as bioreceptor for antigen recognition. Quartz Crystal Microbalance sensor with gold electrodes were mounted in oscillator circuit and exposed to room temperature. The Transistor-transistor Logic oscillator circuit was utilized since it was able to drive the Quartz Crystal Microbalance sensor in air and liquid. Only one side of the crystal were exposed to the liquid sample by using static measurement. The measured of frequency response were observed in order to investigated the interfacial behavior of bacteria *Escherichia coli* on surface of sensor. The results show that the bacteria after inactivation have higher frequency shifting than bacteria before plasma treatment. The difference in frequency responses showed that bacteria inactivated by atmospheric plasma device have increment of mass. These situations are caused by several factors such as unspecific molecule presence inside inactive bacteria sample, types of bioreceptor, surface roughness and condition of electrode surface. The analysis also includes piezoelectric effects, experimental conditions and the characteristic of surface roughness. From average result of 10 sensors device utilized in the experiment, 8 were successful in bacteria detection. The detection also depends on condition of electrode surface. Moreover, this system has the ability to detect bacteria active and inactive in liquid situation.

CHAPTER 1

INTRODUCTION

1.1 Overview

Piezoelectric oscillator devices are often used in electric circuits as frequency standard clocks in electronic components, communication systems, and time bases (Kurosawa *et al*., 2006; Satoh *et al*., 2011). A Quartz Crystal Microbalance (QCM) sensor is piezoelectric device that has been used for detection purpose in various applications since 1956 (Salt, David 1986). Over the past decades, QCM sensors have been developed for the detection of small molecules, volatile organic compounds, proteins, microbes and many others substances (Mohammed *et al*., 2008). The example of QCM sensor application such as microbiological detection (Wilson & Baietto, 2011; Tome *et al*., 2012; Yan *et al*., 2012), environmental monitoring (Kurosawa *et al*., 2006), and food quality assurance (Ricci *et al*., 2007). This sensor type is attractive to researchers because it is a versatile tool, offering high sensitivity and selectivity, real-time measurement, a high temperature coefficient and the ability to detect molecule as small as nano-size (Janshoff *et al*., 2000). In sensor applications, QCM sensors have been used as transducer devices influenced by stiffness, variation of surface mass, density, and viscosity. These factors are then transferred to the driver circuit as deviations in the frequency oscillation (Satoh *et al*., 2011).

The bacteria *Escherichia coli* (*E.coli*) were chosen as the sample bacteria for the purpose. The *E.coli* bacteria are particularly found in our digestive tract. An infection with this bacteria species can lead to death or serious illness. Several research project have been conducted for the detection of *E.coli* using QCM biosensors (Su & Li, 2004; Mao *et al*., 2006; Wang *et al*., 2007; Kon *et al*., 2007; Liu *et al*., 2007; Wu *et al*., 2007; Wang *et al*., 2008; Guo *et al*., 2012; Farka *et al*., 2014; Thanh Ngo *et al*., 2014). However, the majority of the above mentioned researchers focus more on application analysis in real samples, and only deal with standard recognition, which identifies the presence of bacteria in the samples.

In this research, frequency response from bacteria detection was investigated using QCM sensor. QCM sensors have been previously used to study bacteria, but this is the first time that QCM sensors were used to study the characteristics of bacteria inactivated by plasma. An atmospheric plasma jet device was used for inactivation. practical uses for atmospheric plasma devices include surface modification treatments, sterilization processes, and wound healing (Korachi & Aslan, 2013). Plasma treatment is effective with microbes since it possesses reactive species that are able to damage the structure of microbes (Stoffels *et al*., 2008; Park *et al*., 2012). This makes plasma a tool for the purpose of sterilization, and presents a challenge when it comes to developing a system used to detect inactive bacteria.

In order to build a sensor able to recognize the inactive bacteria, a proper biological probe is required, to detect the target sample. There are several methods used in the QCM coating process namely, polymer coating (e.g.: glow discharge, dipping, spin coating, and electrochemical deposition), Langmunir and Langmunir-Blodgett films and chemical modification (e.g.: self-assembly monolayer can be used in solution or as a vapour). The self-assembly monolayer (SAM) technique, together with the antigen-antibody principle, were used as coating methods on the electrode surface. A carboxylic acid and an amide group were used as the first monolayer on the electrode surface, for the purpose of coating. This method was used by Wang *et al*. (2008) and Thanh Ngo *et al*. (2014) to detect the *E.coli* bacteria. Anti-*Escherichia coli* antibodies were immobilized on the surface of gold electrodes to catch the *E. coli* bacteria. The SAM method is the simplest way to provide a reproducible, well-ordered structure that is suitable for modification with a bioreceptor.

Other than that, due to interest in biosensor development, researchers tend to focus on detection and on improving surface modification. The circuit interface used to drive the sensor, however has been neglected (Erbahar *et al*., 2014). Currently, researchers only use commercial instruments in order to obtained accurate measurements such as Q-Sense (Antosiewicz *et al*., 2015), Maxtek Research QCM (Hiatt & Cliffel, 2012), the QCM-D E4 system (Jachimska *et al*., 2016), and many more. All these researchers discuss detection mass changes and surface modification analysis. In this work, the abilities of oscillator circuits to detect bacteria, are described and compared. The results of the oscillator circuits are observed between the gas phase measurement and the liquid phase measurement.

Oscillators have its own ability to generate frequency resonance. The quartz crystal is a highly precise and stable oscillator, however it becomes unstable when immersed in liquid (Janshoff *et al*., 2000). In order to have stable frequency resonance that operates in a liquid, a suitable oscillator circuit needs to be selected, in order to avoid the measurement miscalculation. A Pierce, a Transistor-transistor Logic (TTL), and a Colpitts circuit oscillator are utilized at the circuit interface for the QCM sensor.

This oscillator circuit meets the local frequency standards for electronic devices in all applied fields.

Since the QCM sensor analysis was carried out in the liquid phase, a typical method is utilized, by applying a droplet of liquid to the electrode's surface. Only one side of the electrode is required, in order to reduce the instability of frequency resonance due to the viscosity and the density of the liquid (Janshoff *et al*., 2000). Using only one side of the electrode can also eliminate the influence of conductivity and the dielectric constant, as well as reduce liquid damping, and help the crystal reach stable oscillating conditions. Measurements taken in a liquid can be affected by several factors such as environment (liquid temperature), liquid damping, and the rigidity of the coating process (Vaughan & Guilbault, 2007). The measurements are done using static mode, so liquid viscosity can be ignored. In this experiment, factors are such as temperature and environment are kept at constant temperature.

1.2 Problem Statement

Atmospheric pressure plasma jet device is widely used in medical purposed especially in microbe inactivation. This device is preferred in biomedical application because it was non-thermal devices (generate low temperature plasma gas) which suitable for the treatment of heat-sensitive materials such as cells, tissues and medical equipment (Laroussi, 2009; Korachi & Aslan 2013). Effectiveness of plasma treatment on microbe can be evaluated using two methods, namely, the microorganism culturing method and the microscopy analysis. The microorganism culturing method is preferred by researchers, since it is inexpensive and effective way to identify and monitor the growth of bacteria after plasma treatment. The culture technique is also commonly applied in hospital pathology to identify the microbe and select the suitable medicine for treatment. However, studying the growth of the microbes using the culture method requires a safe environment for the researchers. The culturing process requires laboratory environment, with appropriate techniques in order to avoid the contamination of the samples. The samples need to be incubated for about 18 hours to observe the growth of the sample, which is time-consuming. Rapid results and accurate data are important in medical diagnosis. Culturing the microbes also requires knowledgeable and trained staff to handle the task.

The second method, which is microscopy analysis, uses various techniques, including traditional microscopy, Scanning Electron Microscopy (SEM) (Lackmann *et al*. 2013; Blumhagen *et al*. 2014; Chang & Chen, 2016) and Transmission Electron Microscopy (TEM) (Hong *et al*., 2009; Park *et al.*, 2014; Abdel *et al*., 2016) which have also been utilized in studies to analyze the microbes after plasma treatment. However, these devices require technical personnel to operate it, complicated procedure and longer time for image analysis. Most importantly, some of these techniques, such as SEM and TEM, introduce costs since they are very expensive. Therefore, to overcome these two methods of analysis of bacteria, this project uses QCM as biosensor device to characterized the bacteria after inactivated by plasma device

1.3 Objective

1. To develop a QCM sensor as a tool for the detection of microorganisms in a liquid.