

**DESIGN & DEVELOPMENT OF MICROORGANISM
ACTIVITY MONITORING TRANSDUCER USING
ULTRASONIC SENSORS**

AINI SALWA BINTI HASAN NUDIN

UNIVERSITI MALAYSIA PERLIS

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ACTIVITY MONITORING TRANSDUCER USING
ULTRASONIC SENSORS**

by

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A thesis submitted in fulfillment of the requirements for the degree of
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LIST OF SYMBOLS

$^{\circ}\text{C}$	degree Celsius
Ω	ohm
%	percentage
μF	micro-Farad
cm	centimeter
dBr	relative decibel
Hz	Hertz
$\text{k}\Omega$	kilo-ohm
kHz	kilo-Hertz
$\text{M}\Omega$	mega-ohm
MHz	mega-Hertz
ml	milliliter
mm	millimeter
ns	nano second
V	volt
V_{ref}	reference voltage
V_{RMS}	root mean square voltage

LIST OF ABBREVIATIONS

A.Niger	Aspergillus Niger
AC	Alternating Current
Ag/AgCl	Silver/Silver Chloride
BJT	Bipolar Junction Transistor
CAD	Computer-Aided Design

DC	Direct Current
DRC	Design Rule Check
E.Coli	Escherichia Coli
LED	Light Emitting Diode
NaCl	Sodium Chloride
PCB	Printed Board Circuit
PDA	Potato Dextrose Agar
PDMS	Polydimethylsiloxane
PQC	Piezoelectric Quartz Crystal
Redox	Reduction Oxidation
S.Cerevisiae	Saccharomyces Cerevisiae
S.Officinarum	Saccharum Officinarum
SAW	Surface Acoustic Wave

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Rekabentuk dan Pembangunan Transduser Untuk Pemantauan Aktiviti Mikroorganisma Menggunakan Penderia Ultrasonik

ABSTRAK

Tesis ini telah dijalankan untuk mengkaji perkembangan aktiviti mikroorganisma yang dipantau oleh transduser dengan menggunakan penderia ultrasonik sebagai komponen utama bagi mengesan isyarat yang dihasilkan oleh aktiviti mikroorganisma. Terdapat 3 sampel yang telah digunakan untuk mengkaji transduser iaitu *Saccharum Officinarum* yang dicampur dengan air paip, dan, *Saccharomyces Cerevisiae* dan *Aspergillus Niger* yang dicampur dengan air suling. Ujikaji ini membuktikan bahawa ketiga-tiga sampel ini memberi isyarat voltan yang berbeza. Hal ini sekaligus membuktikan bahawa transduser mampu mengesan perubahan votan berdasarkan perubahan kepekatan dan jumlah sel di dalam sampel yang di uji. Kaedah membangunkan transduser ini dilakukan terbahagi kepada dua peringkat. Pada peringkat pertama, komponen elektrik yang mampu menghasilkan isyarat yang bersesuaian telah dipilih dan di uji. Lakaran litar telah dilakukan dengan menggabungkan semua komponen elektrik yang mempunyai fungsi tertentu berdasarkan spesifikasi yang telah diberikan oleh pembekal komponen tersebut. Pada peringkat ini, perisian Rekabentuk Grafik Berkomputer (CAD) telah digunakan untuk melakar Papan Litar Bercetak (PCB). Pada peringkat kedua, sebuah bekas khas telah dihasilkan bagi meletakkan sampel dan penderia yang digunakan. Untuk menghasilkan bekas ini, bahan yang bercirikan ringan, mempunyai sifat seperti kaca untuk proses pemantauan, tahan suhu dan mempunyai kandungan kimia yang stabil telah dipilih sebagai bahan utama bagi menghasilkan bekas tersebut. Perisian Rekabentuk Grafik Berkomputer telah digunakan bagi merangka bentuk bekas tersebut. Kegunaan transduser telah diuji menggunakan cecair bakteria pseudo dan mikroorganisma yang sebenar. Keputusan yang diperolehi menunjukkan bahawa isyarat yang telah dihasilkan oleh transduser bergantung kepada kepekatan cecair yang dikaji. Semasa mikroorganisma diuji, kaedah pengiraan sel telah dilakukan bagi mengkaji hubungan di antara isyarat magnitud dan jumlah sel. Penambahan dan pengurang sel telah digunakan sebagai penanda untuk aktiviti mikrob. Hasil eksperimen ini membuktikan bahawa penggunaan penderia ultrasonik bagi proses pemantauan mikroorganisma mempunyai potensi untuk di selidik dengan lebih mendalam dan boleh dibangunkan untuk menghasilkan sistem tinjauan pantas bagi sesetengah industri.

Design & Development of Microorganism Activity Monitoring Transducer Using Ultrasonic Sensors

ABSTRACT

This thesis presents the design and development of a microorganism activity monitoring transducer by using ultrasonic sensors as the main part in detecting the signal of microorganism activity. Three types of samples which are *Saccharum Officinatum* in tap water, *Saccharomyces Cerevisiae* and *Aspergillus Niger* in distilled water are used in the experiments and are found to give different voltage signals. This indicates that the transducer is able to detect changes of concentrations and the number of cells in the samples tested. The proposed design consists of two parts. In the first part, the selection of electronic components that is able to produce an appropriate output signal has been made and tested. The design of the circuit is done by combining the application circuit for each component based on the detail that is supplied by each component manufacturer. In this stage, a Computer-Aided Design (CAD) software is used to design a Printed Circuit Board (PCB). The second part is the design of a container that is used to place the sample and also the sensor. A material with properties such as glass, light weight, temperature resistant and chemically stable has been chosen as the main material to build the container. A CAD software is used to design the shape of the container and fabricate it. The functionality of the transducer is tested by using pseudo bacteria liquids, real microorganisms and the results obtained shows that the output signal of the transducer is dependent on the density and concentration of the tested liquid. During the testing of the microorganisms, a proposed method of cell counting is done to show the relationship between the signal magnitude and the number of cells. The increment or decrement of live cells has been used as indicator of microbe activity. The experiment results show that the use of ultrasonic sensors for microorganism monitoring has a potential to be further investigated and developed towards a reliable and fast monitoring system required in certain industries.

CHAPTER 1

INTRODUCTION

1.1 Overview

Intelligent microorganism activity monitoring systems are one of the important requirements in industries nowadays. This system has been further improved for microbe activity monitoring by observing the growth rate of the microbes. The design and development of such systems focus on the usage of various sensors in monitoring the activity of microbes.

This research aims to improve these monitoring systems especially in terms of response time, complexity, cost, accuracy and its efficiency. With the rapid growth in the nation's industrial sector, a system which is easy to handle and installed in an existing facility is truly required.

Such systems are useful in industries that are exposed to high humidity levels like food and paint industries or most industries that use liquid as their main compound in their products. In addition, this system will not be only limited to the industrial sector, but it can also be used in the military sector. It is possible that in the future, bioweapons may contain harmful microbes that can affect humans and the environment. It is hoped that these types of systems can help in detecting the level of contamination in vast areas.

1.2 Problem Statement

There are quite a number of existing microorganism activity monitoring systems in the market (Chang et al. 2007)(Yang & Bashir, 2008). However, from this list, there seems to be no system that uses the common ultrasonic sensor as its main sensors to detect microbe activity. Thus, the main scope of this research is to design and develop a fully functional biosensor for a microbe activity monitoring system and the system must be intelligent to differentiate signals in order to identify all activity of microbes in terms of live and dead phases of the microbes.

For the microorganism activity monitoring system to be intelligent, it needs to interact with the user in order to learn the conditions of the tested medium and also connect to other devices. Hence, we have to make an interface which includes software and hardware. However, this research will only concentrate on interfacing the output signal which is shown on an indicator based on the medium tested.

1.3 Objectives

© There are two objectives that need to be achieved in this project. The objectives are:

- 1) To design and test a transducer which is able to monitor pseudo bacteria and microorganism activities.
- 2) To design a suitable and safe container for the placement of microorganisms and ultrasonic sensors.

1.4 Methodology

In this section a general methodology adopted in this research is provided. Details of these approaches are given in chapters 2, 3, 4 and 5. In general, this project can be divided into several sections as shown in Figure 1.1.

First, a complete and detailed literature review has been conducted to obtain a general understanding of the problem and also to classify the problem of detecting microorganism activity using suitable sensors.

The circuits have been designed by using a CAD software. In this project, two types of circuits have been designed which are the transmitter circuit and the receiver circuit. In doing this, suitable electronic components must be chosen. After all components have been chosen, the circuits are then fabricated. After fabricating the board, all electronic components are soldered onto it. Both receiver and transmitter circuit will then be tested. If any error occurs, circuit connections and the component on the circuits will be rechecked.

When the circuits have been completed with no error, the project is continued by designing a suitable container to place the circuits as well as sensor by using the SolidWork software. The container has been designed based on the diameter of the steel pipe that is available in the local market. Then, the container will be fabricated. The circuit then will be assembled with the container to produce a transducer. The circuits which are integrated into the container will be tested with pseudo bacteria and microorganism samples.

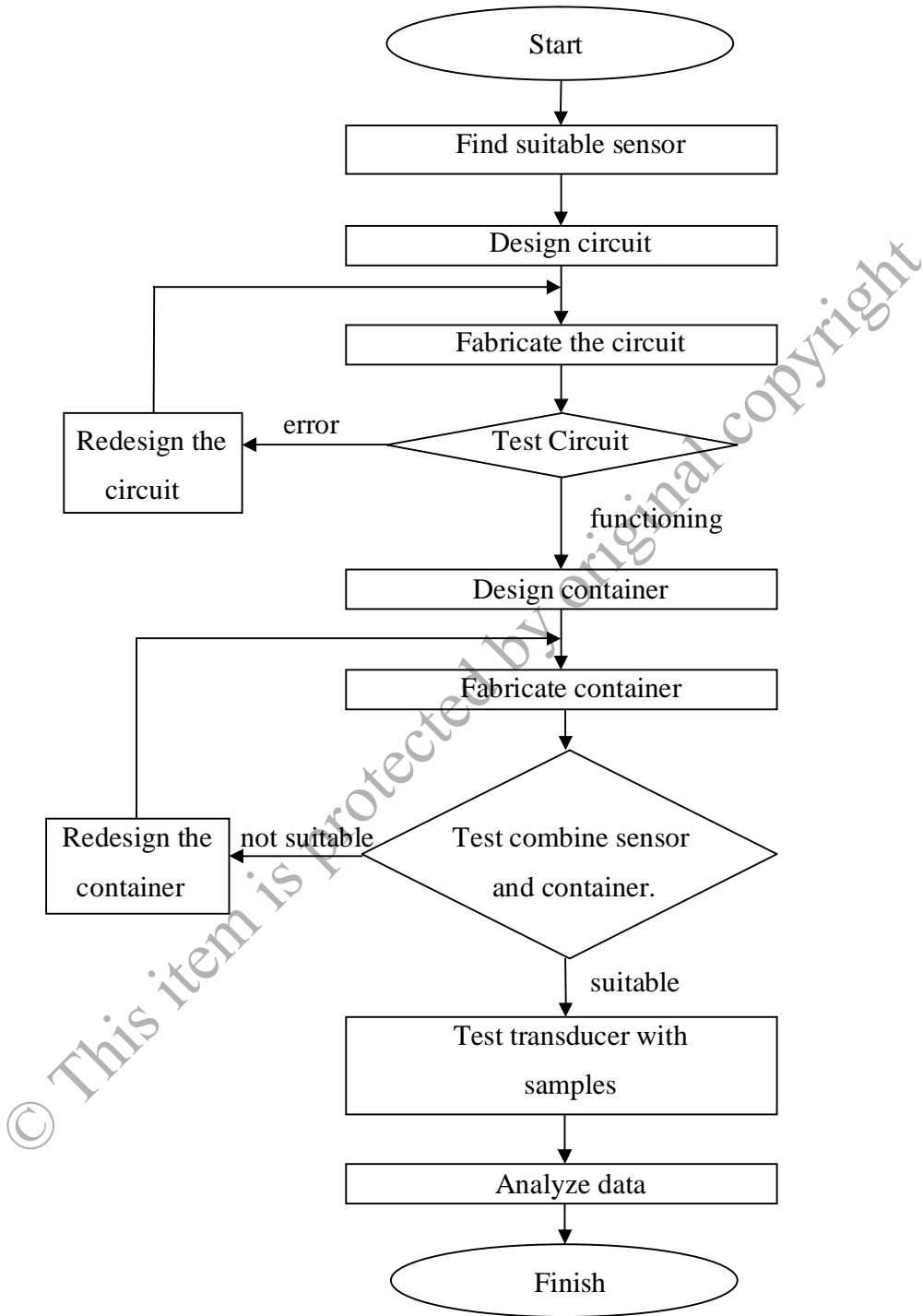


Figure 1.1: Flow chart of the activity done in this project.

1.5 Project Expectations

The main contribution of this project is the design and development of a microorganism activity monitoring system. This project includes the hardware design for the system using ultrasonic sensors. Suitable electronic components are chosen in order to make the transducer indicator show the current state of microbes in the tested medium.

The design application is developed using the Computerized Aided Design (CAD) to achieve fast results in designing both the Printed Circuit Board (PCB) and container. This transducer can be readily used by attaching the sensor at selected sections of pipes in industries if it is to be used online, or just simply put a tested medium in the readymade container of the transducer for offline testing.

1.6 Report Organization

There are five chapters in this report. Each of these chapters discusses different aspects of the project. The first chapter will briefly present the project background, problem statement, objective, methodology of the project and project expectations. This chapter gives an idea to the readers to obtain a general overview of the project that has been implemented.

Chapter 2 presents the literature review of the project which includes the understanding of microorganism in terms of life cycle and types, methods used in microorganism monitoring systems, previous works on detecting microorganism, and the usage of ultrasonic sensors in industries.

There are three main parts in Chapter 3. The first part discusses about the design of the circuits, which are the transmitter and receiver circuits. The second part is about circuit fabrication while the third part describes the design and fabrication of the container.

Chapter 4 discusses all the results that has been obtained by the transducer during the experiments. The samples that are used in the experiment are pseudo bacteria liquids and liquids with microorganisms.

The last chapter which is Chapter 5 will conclude this project by presenting the summary, recommendation for future work, commercialization of the transducer and also the recommendations to improve the transducer design and performance.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter presents a brief review on the various sensors used to monitor microorganism activity. We begin with an introduction to microorganism which is also known as microbes. Following this, various sensors used to detect microorganism activity will be presented. Next, we focus on the application of ultrasound sensor in various industries, concentrating on any process related to microorganism activity detection. Finally, we summarize the contents of this chapter by presenting a comparative tabulation of the performance of these sensors.

2.2 Microorganism

Microorganism also known as microbe, is a microscopic or submicroscopic organism that is too small to be seen by the naked eye and it has many types and shapes. Microbes can be classified into five types which are fungus, protozoa, algae, bacteria and archea. Even though viruses cannot be seen by the unaided human eye, it is still not considered as a microorganism because it is actually a non-living cell. The size of a microorganism is typically a few micrometers in length with a wide range of shapes, ranging from spheres to rods and spirals (Biology-online, 2008).

Microorganism lives in every habitat of our Earth; it grows in soil, acidic hot springs, radioactive waste, water and in the Earth's deep crust, as well as in organic matter and the live bodies of plant and animals (Fredricson et al., 2002). Microorganisms are able to carry out their life process of growth, energy regeneration and reproduction independently and are very useful in our lives especially for decomposing any dead animals and plant for the benefit of our ecosystem. It is also considered beneficial because it helps in the Earth's element cycles such as carbon cycle and nitrogen cycle. Microbes exhibit great diversity; some grow and multiply by only using energy obtained from sulphur, ammonia, hydrogen and iron. They also obtain carbon for cell synthesis from carbon dioxide.

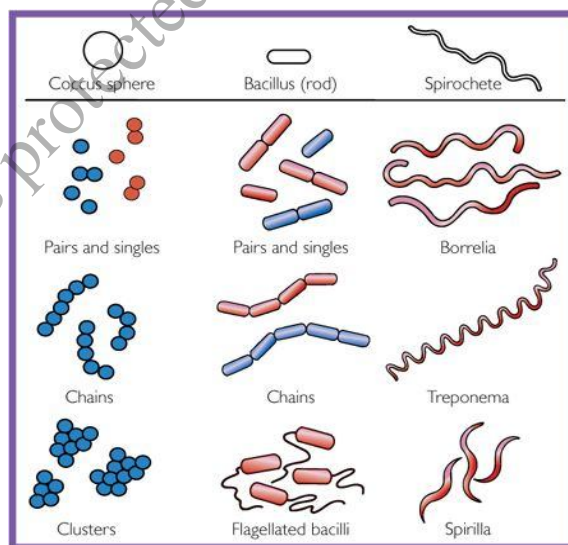


Figure 2.1: Microorganism shapes (Stepp & Woods, 1998).

Microorganism grows by binary fission, where the microbe splits into two identical cells as shown in Figure 2.1. Given a suitable condition, this microorganism can reproduce

quickly (Shelly John et al., 2002). In an environment that has a lot of oxygen, bacteria aerobically respire and break down sugar to produce carbon dioxide gas and water. In conditions where there is lack of oxygen, bacteria respire anaerobically and produce ethanol and carbon (Holloway, 2010). This type of microbe has a different life cycle from many other types of microbe because it is involved with the asexual and sexual reproduction.

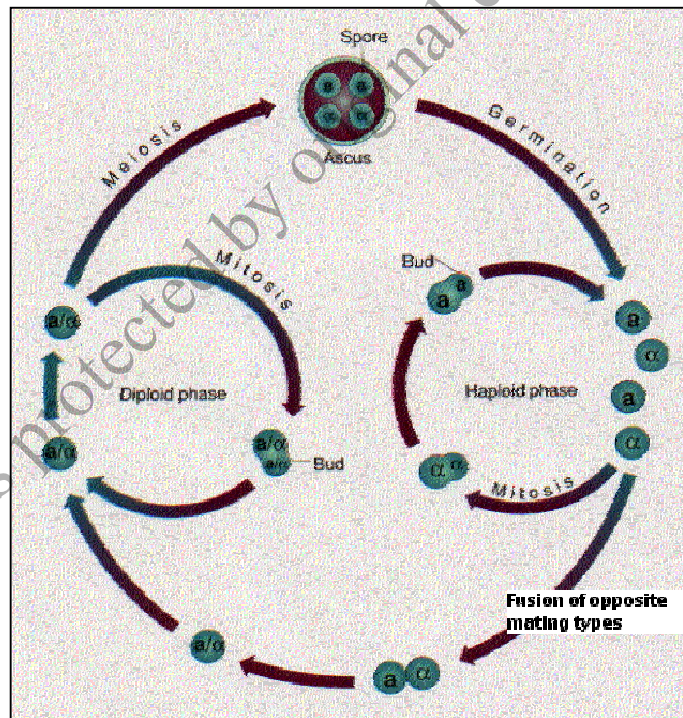


Figure 2.2: Reproduction phase of *Saccharomyces Cerevisiae* (Kimball, 2011).

By referring to Figure 2.2, germination is a process where the inactive and non-dividing spore begins to grow. This process happens when there are plenty of nutrients present. When the spore grows, the cell can undergo budding or mating process that depends on environment factors. Proliferation or budding are reproduced through an

asexual process where the haploid mother cell produce two daughter cells that are smaller than the original cell. Budding will not happen in a condition that lacks nutrients. Mating can exist as two types of haploid cells labeled as a and α which are efficient mate together to form a diploid cell with one nucleus. To promote mating, cells produce pheromones which then stop the budding process and inhibit cell growth to ensure each cell has one copy of each chromosome resulting in the diploid product of mating. The diploid cells formed by mating can undergo meiosis which is a sexual reproduction process where one cell is divided into four cells that are known as spore. This process occurs when the cells starve for nitrogen and carbon. The individual spores or haploid cells are released when the ascus wall degrades.

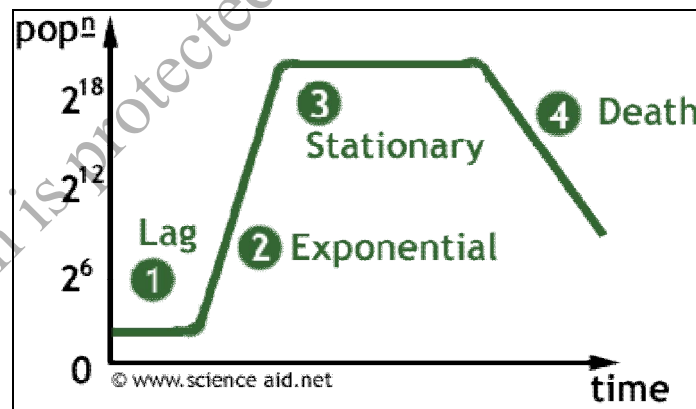


Figure 2.3: Microbe growth pattern (Claydon, 2008).

Most microorganisms have four identical growth phases. However, the time taken for each phase for any type of bacteria is different from each other. Based on Figure 2.3, the first phase of a microorganism life cycle is called the lagging phase. In this phase, the microbe is alive and active. However, the microbe is not increasing in population. The

second phase is called the exponential phase where the microbe growth increases based on the nutrient supply and the space where the population of microbe lives. In this phase, the sum of active microbes is more than the sum of dead microbes. The third phase is the stationary phase where there is no change in microbe population. This is due to the population of microbe that has reached the maximum limit where the environment can support. The final phase is the death phase where the population of dead microbes is more than the sum of live microbes in their population (US National Institute of Health, 2009). This phase shows that the activity of microbe increases as time increases (Ahmet et al., 2008).

Microorganisms are an essential part of the digestive process of animals and insects. Septic systems and most waste water treatment facilities are designed to allow the work of microbes to naturally break down the harmful components (Shelly John et al., 2002). These microorganisms have also been used widely in food industries especially for brewing, winemaking, baking and pickling. It has also been used to control the fermentation process in the production of cultured dairy products (Ishige et al., 2005).

2.3 Monitoring Microorganism Growth

In this research, we are focusing on two types of microbes which are bacteria and fungus that are widely used for experimental purpose and also in food production. There are seven methods discussed related to bacteria monitoring while only two methods are discussed for fungus monitoring. It should be noted that most of the methods used for bacteria monitoring can also be used for yeast monitoring.