Design and implementation of a Low Cost Prototype of an Automated Bioreactor

Bilal J. Karaki
Palestine Polytechnic University
Hebron, Palestine
E-mail: bilal_j_karaki@student.ppu.edu

Saleh O. Al-Takrouri
Palestine Polytechnic University
Hebron, Palestine
E-mail: saleh@ppu.edu

***Abstract*— This project aims to build a prototype of a automated bioreactor (fermentation system) which is used to grow cells in a large scale with high efficiency. The main functions of the bioreactor are: to keep the temperature and acidity of the solution in the bioreactor almost constant, to mix the micro-organisms with a specific speed, to make aeration if needed, and to decrease pressure and residue during the fermentation process. Moreover, this project is essential to Palestine Polytechnic University since it will provide a chance to B.Eng and B.Sc students to gain practical skills to compliment their education. Also, the project aims at providing a chance to researchers who are working at the laboratories of Biotechnology Research Center at Palestine Polytechnic University to improve the quality of their research. Moreover, the project increases the benefits and reduces the time which is consumed through enzymes production.**

I. Introduction

Fermentation systems play an important role in the science of biology, and they have a great effect in many other fields. A fermenter is a system used to grow cells in a large scale with high efficiency under controlled environment [1].

The automatic fermenter is essential in the fields of chemical and biological sciences [2]. This importance motivated us to work in this field. We will design a system to do the work faster and more reliable than the manual procedure. Moreover we improved the ability to perform the required processes without interference by the human operator.

In this project, the attention is directed towards the field of fermintation systems which are used to produce enzymes. In particular, we are proposing a design based on industrial electronics to build a prototype of a bioreactor.

This project is preceded by the work achieved by Chu for protein production by large-scale cell bioreactor [3]. Protein products originally proposed by large-scale bioreactor (e.g. [4], [5], [6], and [7]).

A well-known applications of the fermentation processes by bioreactors appear in a treatment of the quality of the enzymes. However, many researchers are concerned about continuous production systems for enzyme production such as Takeshita *et al* [8].

We constructed a prototype of a laboratory fermenter that has a volume of five liters. The system will be able to measure and control the vital parameters of the biological culture such as temperature, acidity concentration, pressure and dissolved oxygen.

This project is a combination of electrical and mechanical components that act together to control biological conditions in order to provide a safe environment for micro-organisms to grow cells in a large scale with high efficiency. The basic structure of an automatic fermenter is shown in Figure 1.

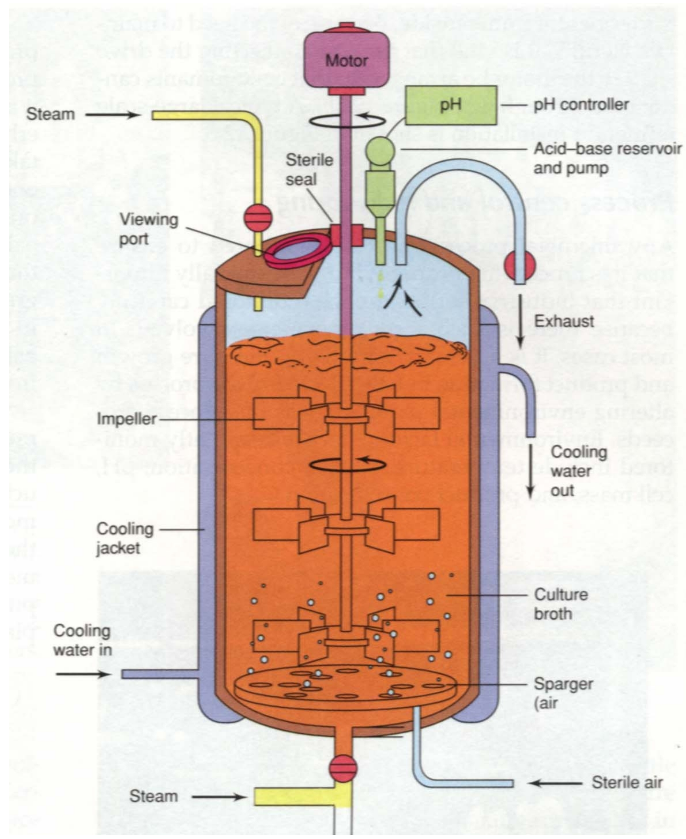

Fig. 1. Automated fermenter [9]

II. Objectives of the Project

This project aims to achieve the following objectives:

1) To design the hardware and software of a bioreactor system according to specifications as required by BioTRU.
2) To implement a prototype of the bioreactor according to the previous objective.
3) To provide an efficient way to local production of enzymes which are needed in biology science.

III. Overview of automated control system of bioreactor

The control system of the bioreactor takes the information from the user and makes a comparison between the actual values and desired values in order to reduces the error to obtain better values.

The fermentation process can be improved by using a programmable logic controller (PLC). It is very essential in this field to construct an automated fermentation system in order to increase the quality of research, and make their job easier.

We will provide a treatment of important features to this system, in order to enhance the ability of the fermentation process.

The aim of the project is to prevent undesirable changes in the parameters of the solution which contains the micro-organisms. Such changes occur due to the surrounding area or due to chemical reactions in the media.

Usually the solution of organisms must have constant parameters (such as temperature and pressure) which are specified by the user. And some of the features included in the proposed fermenter system are advancements in automatic control, high resolution measurements for all parameter, and system reliability.

The parameters that may change are temperature, acidity concentration, dissolved oxygen, stirrer speed and pressure. This means that the fermenter system can be divided into five control systems.

1) Thermal System description: The temperature of the solution in the fermenter is very important parameter, this importance refers to the efficiency of the products which depends on bioreactor temperature. If the temperature of the bioreactor depart from desired value the efficiency will decreased.

If the temperature of the solution in the fermenter system deviates from the desired value, this deviation must be prevented using cold or hot water pass through tube surrounds the vessel. and the thermal system will stop if the temperature error smaller than allowed error to increases electrical efficiency.

The challenge is how the system makes the decision to start or stop the pumps, the two set point control method is used for tracking the desired value of temperature [10]. The two set point is used to adjust value with smallest allowed temperature error.

according to the application of the two set point usually used when the system tracking one variable in opposite direction, for example in this project the temperature of the system may be increased or decreased, therefore the two set point is essential to maintain constant temperature for the bioreactor.

2) Acidity concentration: The pH is one of the most commonly measured chemical values and it is used in many fields, such as health care, environmental control, pharmaceutical industry and others [11]. The pH can be defined as the negative logarithm of the hydrogen ion concentration as described by equation (1).

$$pH = -Log(a_H) \tag{1}$$

Where a_H is a measure of the acidity concentration of hydrogen ions in units of mol/L.

Acidity concentration can be measured using pH unit. pH measurement is very important because a chemical process such as speed of reaction can be determined from measurement of pH [12].

3) Dissolved oxygen: Dissolved oxygen (D.O) is an important parameter of fermentation. In the solution of micro-organisms, the dissolved oxygen concentration is needed and necessary for aerobic micro-organism [13].

To provide dissolved oxygen to micro-organism, aeration is needed. Aeration task needs a vial containing pure oxygen gas, where the micro-organism are supplied with oxygen when the dissolved oxygen is decreased.

4) Agitation system: An agitation system is an essential part of fermenter, especially large scale fermenter. The agitation system is used to mix the contents of the fermenter.

Mixing of micro-organism can be achieved in many different techniques, the most preferred technique in fermenter system is accomplished by an agitation system consists of motor, impellers with 4 to 6 blades and a gear.

Impellers are auxiliary parts of the agitation system, usually available for mixing applications. The main purpose of mixing is to remove the carbon dioxide that is produced due to the fermentation process and aeration.

5) Pulse Width Modulation: Pulse Width Modulation (PMW) is a method used to change the average power that is supplied to the motor of the agitator. PMW is the most popular method which is used in agitation systems to control the mixing speed. A varied time periodic wave of Pulse width modulation (PWM) is used to change average voltage.

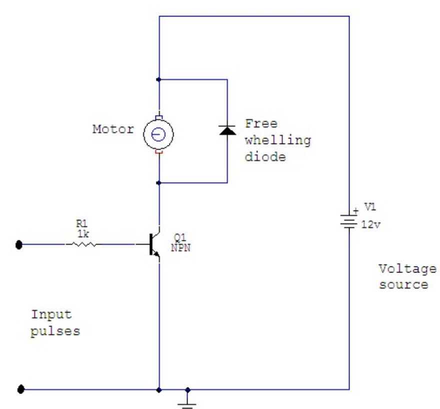

Fig. 2. PWM Control Circuit

When the transistor wich is shown in the control circuit in Figure 2 is turned on for a time T_{on}, the maximum voltage appears on the motor, and when the transistor is turned off for a time T_{off}, the voltage across the motor is zero as shown in Figure 3.

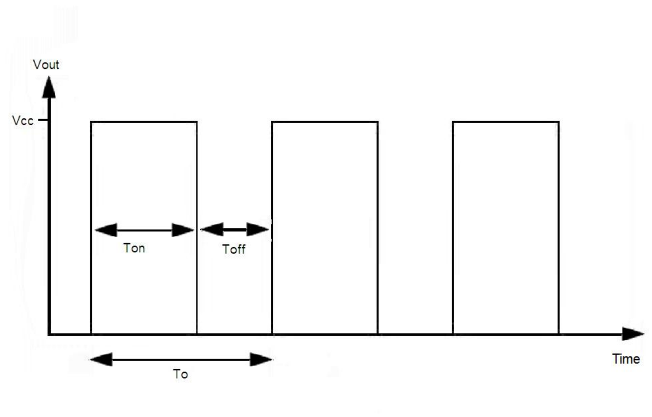

Fig. 3. Pulse Width Modulation Wave

The average output voltage is determine by equation 2

$$V_{av} = \frac{1}{T_0}\int_0^{T_0}(V_{cc})dt = \frac{1}{T_{on}+T_{off}}\int_0^{T_{on}}(V_{cc})dt = \frac{T_{on}}{T_{on}+T_{off}}V_{cc} \quad (2)$$

Where V_{av} is the average output voltage, T_{on} is the on time period and T_{off} is the off period time.

There are mainly two ways to generate a pulse width [14]:

- PWM with constant frequency and variable on and off time:
- PWM with variable frequency and constant on time.

The preferred method is the first type because a change in the frequency affects the stability of the motor specially at low frequencies; at low frequencies the DC motor starts and stops for long time periods, which causes unstable rotation.

6) Fermenter vessels: Fermenter vessels must be non-toxic to the micro-organism used during the fermentation process, and should be easy to stir, reliable, cheap, and have a window on the wall for visual monitoring [15].

There are two types of laboratory scale fermenter: shaker flasks and flat bed bottles [16]:

- A shaker flask is a conical vessel that is made from glass and is available in different sizes. The typical volume of this type is 250 mL.
- A flat bed bottle has a flat bottom, similar to a bottle horizontally laid. Flat bed bottles are suitable in small volume fermenter.

The large scale fermenter is the most commonly used [17]. It has a cylindrical vessel with a motor to stir the content of the tank. Large scale fermenters (stirred tank fermenters) are used in research to produce enzymes, medicine, etc. Stirred tanks of the large scale fermenters are manufactured from glass or stainless steel 316.

7) Pressure and residue: During the of growing micro-organisms, the produced residue causes an increase in pressure in the vessel. Residue production is the result of the chemical and biological reactions which occurs in the vessel.

Another factor that causes increase in pressure in the fermenter vessel, is the aeration of micro-organisms. Therefore, there is a need for an exhaust with valve to out all the residue and decrease the pressure of the vessel. The maximum pressure must be specified by the user.

IV. Design of the bioreactor prototype

This section shows methods for designing the fermentation system using PLC. The design procedures to implement the fermenter will be described in this chapter, also this chapter contains of all description of devices and all stages which are needed to implement the fermenter.

The fermenter control system will include sensors, actuators, a motor, switches and wires. However, all of which should be designed with electronic circuits to connect them with control unit PLC.

V. Electronic circuits and electronic devices

A. Power supply

The fermenter which will implemented in this project should be properly supplied with a constant level DC voltage, DC voltage is required for control unit and the motor of agitation system, the motor operates at 12v DC voltage. The power supply used in our project is required to meet the following specifications:

- High voltage regulation.
- High current rating.

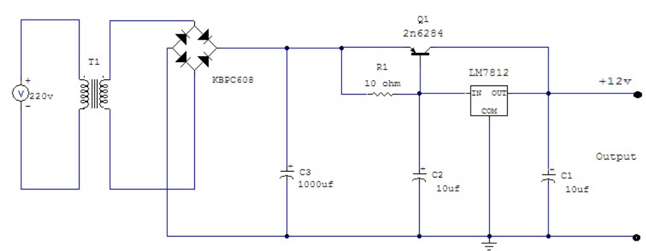

Fig. 4. Power supply circuit

The circuit in Figure 4 shows the construction of the power supply which is used to produce 12 volts DC from $220v$ AC. The circuit produces 12 volts DC using IC LM7812, but the input must be greater than 12 volts. Therefore, the output voltage of the transformer is 16v rms voltage, so its output voltage calculated as specified in equations (3) and equals to $21.2v$.

$$V_{p(rec)} = V_{rms}\sqrt{2} - 2V_{diode} = 16\sqrt{2}v - 1.4v = 21.2v \quad (3)$$

Where $V_{p(rec)}$ is the peak to peak out put voltage after rectifier stage, V_{rms} is the rms value of the output voltage of the transformer and V_{diode} is the forward voltage of the diode usually equals $0.7v$ for silicon diode.

Ripple voltage which is appearers on the capacitor must be bounded between 12 and $21.2v$, this bounded region denoted by $V_{r(pp)}$ and equals to $9.2v$. Now we can calculate the capacitance value of the capacitor by equation 4.

$$C = \frac{P_m V_{p(rec)}}{2fV_o^2 V_{r(pp)}} = 3000\mu F \quad (4)$$

Where $V_{p(rec)}$ is the peak to peak out put voltage after rectifier stage, V_o is the out put voltage of the IC regulator and equals $12v$, f is the frequency of the rectified wave and ranges from 100 to 120 Hz,$V_{r(pp)}$is the peak to peak ripple voltage andp_m is the maximum power needed to the DC motor and equals to 50 watt.

Therefore the capacitance of the capacitor filter is greater than 3000-μF, and its rated voltage greater than 22-volts voltage due to $V_{p(rec)}$ value. And the regulator is LM7812.

The rated current of IC LM7812 is 1A, to obtain higher current from the IC a PNP bipolar junction transistor (D45H2A) can be used. For more details about the PNP transistor and the IC regulator you can see appendix A.

B. pH meter

We used a pH meter with analog output to adapt its output signal with PLC. We will use a pH meter because the produced voltage of the pH electrode is very small and the electrode has very large internal resistance therefore its conditioning circuit is complex and can not give higher accuracy than the meter.

The pH meter that will be used in this project must have the following characteristics:

- Analog output voltage representing pH value.
- Automatic temperature compensation.
- Temperature measurement range from 0 to 100 C.
- Frequency rejection range from 50 Hz to 60 Hz.
- 0 to 14.00 pH reading.
- Less than 0.05 pH resolution.

C. Motor

In this project the motor placed at the top of the tank because the top entry stirrer model is easily manipulated during manufacturing and it is more robust and more reliable than the bottom entry stirrer model.

The stirrer speed ranges from 50 to 200 rpm, and the incremental speed equals 25 rpm [18].

A permanent magnet DC motor used in the agitation system in this project because it has advantages over either Ac motor types and other types of DC motors [19]:

1) Hige mechanical energy that is produced versus its small size and weight.
2) High efficiency that can reach to 90/100.
3) Cheap relative to other motor control systems such as servo motor.
4) Quick starting, stopping and accelerating.
5) High initial torque.

D. Pressure sensor

The pressure sensor made of semiconductor crystal, its resistance depend on the value of pressure with linear relation.

To convert the change of resistance of the pressure sensor into a change in voltage we will connect the sensor with fixed

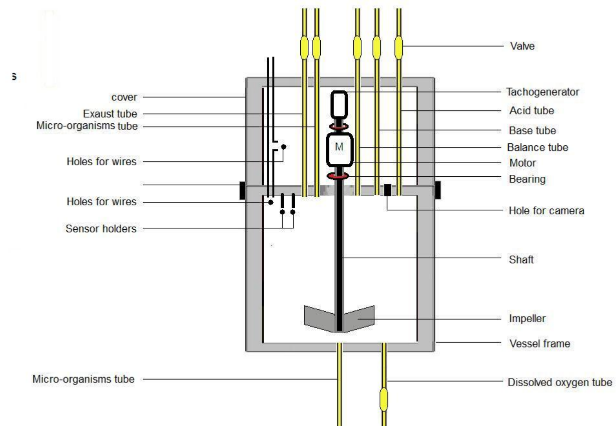

Fig. 5. Structure of the fermenter

resistance and DC voltage source as shown in Figure 6. This circuit make a voltage divider between two resistance and the output will taken from pressure sensor resistance.

commercially name of the pressure sensor which will used is MPVZ5010GW7U.

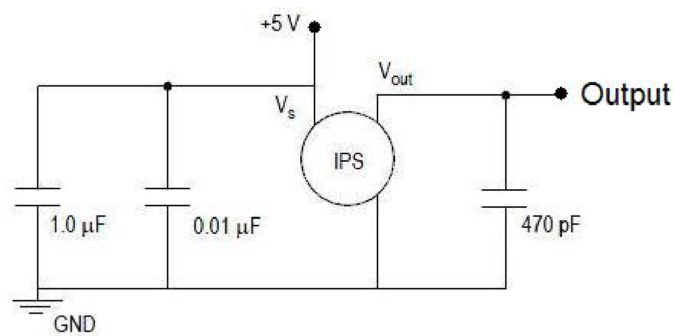

Fig. 6. Pressure sensor circuit

VI. Mechanical components

A. Cover of the fermenter

The micro-organisms must isolated from the outside world, therefore, there is a cover to isolate the vessel which is contains the micro-organism.

The cover of the fermenter contains the motor, digital camera and tachogenerator, and their wires will pass through the cover to the outside world .

Tubes which pass through the cover are:

- Base tube: Used to transfer base substance from base container to the fermenter.
- Acid Tube: Used to transfer acid substance from acid container to the fermenter.

- Exhaust tube: Used to reduce the pressure of the vessel of the fermenter.
- Wires tube: Used to passes the wires of motor, tachogenerator, pH electrode, temperature sensor and the camera from the cover into the control unit.
- Balance tube: Used to balance the pressure between the vessel of the fermenter and the containers of the base-acid containers as you can see it in 7.

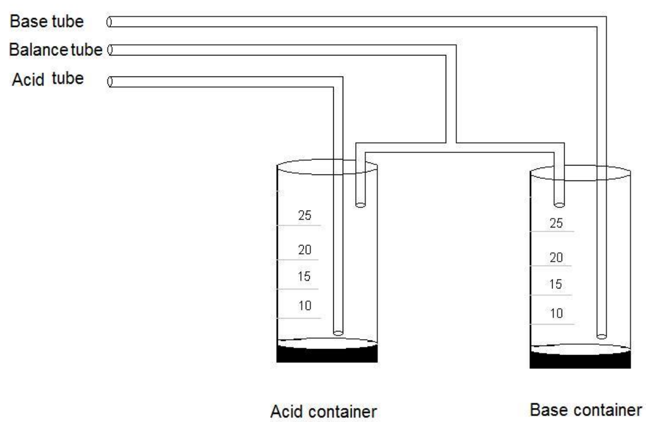

Fig. 7. Base-acid containers

B. *Cover switches*

We used limit switches for detecting the proper placement of the vessel cover. This is used for protecting impellers of the bioreactor. Using four normally open switches connected in series. When the cover is placed properly all the switches are closed and the bioreactor is allowed run. If one of the switches is open the system will not work because the cover is placed wrongly or it is opened.

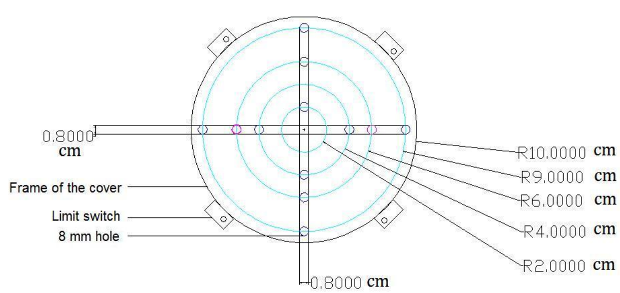

Fig. 8. Bottom view of the cover

It is possible to design this system which have switches inside the bioreactor, but the micro-organisms may be in contact with these switches. Therefor in this system, the switches are placed on the wall of the fermenter as shown in Figure 8.

C. *Structure of the tank*

The vessel (or tank) will be made of stainless-steel 316 which is the same material used for manufacturing food processing appliances. the vessel has a cylindrical shape to facilitate the mixing task. The vessel will be a pressure cooker with 20 cm radius and 20 cm hight.

VII. VALVES

Valves are control devices of air or fluid ways using internal coils, in this fermenter the valves are used for the following systems:

- The heating-cooling system: Used to obtain desired temperature for the micro-organism.
- The filling-vacume system: Used to fill or to vacume the micro-organism from the vessel.
- The base-acid system: To add base or acid to the micro-organism.
- The exhaust system: To deceases pressure and vacume the residue which causes by the micro-organism.

In this project we used two different types of valves. The first type of valves has one input and only one output, in other words this valve has two position. And the second type of the valves has five input-output with three positions. Figure 9 shows the symbol of the previous two types of the valves.

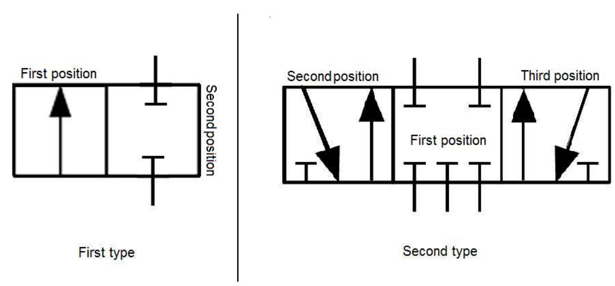

Fig. 9. Types of valves

The first type is used for filling-vacume, base-acid and exhaust system. And the second type is used just for heating-cooling system.

VIII. THERMAL SYSTEM DESIGN

The thermal control system requires to the second type of the valves is mentioned previously. Figure 10 shows the design specifications of the thermal system.

The thermal control system consists of a pump, valve, heater and tubes. The pump is used to transfer cold or hot water using the tubes to the vessel of the fermenter.

In the thermal system there are three principle states depend on the temperature of the micro-organisms:

- If the temperature of the micro-organisms equals to the required temperature value, then the pump turned off and the valve works in the first position.

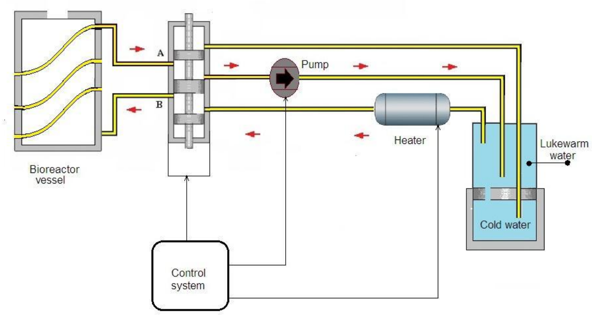

Fig. 10. Thermal system.

- If the temperature of the micro-organisms is less than the required temperature value, then the pump turned on and the valve works in the second position to connect point A to the pump and point B to the tube which is connected to the heater as shown in the Figure 10.
- If the temperature of the micro-organisms is greater than the required temperature value, then the pump turned on and the valve works in the third position to connect point A to the pump and point B to the tube which is connected to the cold.

In the last two states the pump take the cold or hot water and returned them into their water container.

IX. Conclusions

In this pregraduation project, we have successfully designed a fermentation system to various applications for enzyme production in BioTRU.

The PLC is the main control unit in our proposed project, which is used to control a set of parameters through subsystems temperature, acidity, dissolved oxygen and pressure. The PLC also used to interface with the computer.

Thermal control system designed with two position control system to increase its stability. pH control system uses a pH meter to measure concentration of the solution of the fermenter and the pH meter connected to the PLC. Dissolved oxygen controlled by the time as specified by the user. Also the pressure of the fermenter reaches to value that specified by the user.

X. Future work

Not only this project provides Automated control system to the fermentation process, but also this project opens the way for further development in the future. For example, a dissolved oxygen meter it can also be used rather than timing method, can be used a dissolved oxygen meter as pH meter is used in the pH control system.

Moreover, this proposed project can be treated by others using increase the volume of the vessel to be very large scale fermenter in order to use it in industrial applications.

Acknowledgment

I would like to thank my supervisor Dr. Saleh O. Al-Takrouri for his continuous guidance and support. Also I would like to acknowledge many colleagues and teachers who assisted, advised, and supported my project over the months. I am grateful to many persons who shared their knowledge and experiences, especially Dr. Fawzi Al Razim and Ms. Asmaa Al Tamimi.

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