THE DESIGN OF A REGULATED LOW TEMPERATURE ENVIRONMENT FOR STORING BIOLOGICAL SAMPLES

By

Monica Widjaya

A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE

Department of Mechanical Engineering

2002

ABSTRACT

THE DESIGN OF A REGULATED LOW TEMPERATURE ENVIRONMENT FOR STORING BIOLOGICAL SAMPLES

By

Monica Widjaya

The purpose of this research is to design, fabricate, and test a thermally controlled environment (0°C to -10°C) for holding biological samples in a low temperature incubator. In regards to this design, twelve boxes made with various materials are built to hold the biological samples and stabilize the temperature. It is important to choose materials that can provide temperature uniformity within the samples. In addition, a temperature controller system is used to regulate the temperature of the biological samples between 0°C to -10°C. During the process of designing the twelve boxes, a mathematical modeling is used to attempt to improve the design. This mathematical modeling is used to change the thickness of the walls within the box in order to optimize the design.

After optimizing the design of the box, the twelve boxes are tested during conditions of automatic defrost, opening the incubator door, and opening the incubator door and lid of the box at the same time. The results show that the biological sample in these twelve boxes are able to maintain a regulated set temperature between 0°C to -10°C during the automatic defrost condition. In addition, the biological samples are able to maintain a steady temperature for at least three minutes during the opening incubator door, and opening the incubator door and lid of the box at the same time. These results meet the requirements that were requested by the Center of Microbial Ecology.

ACKNOWLEDGMENTS

I would like to give my sincere gratitude to my advisor, Dr. John J. McGrath, for providing this opportunity as well as his encouragement and understanding. Along with my advisor, I would like to thank Dr. Craig W. Somerton and Dr. Ahmed Naguib for serving on my advisory committee. Furthermore, I would like to give my special appreciation to Theron J. Hicks for building the controller temperature system. Also, I would like to give my extreme gratitude to the Microbial Ecology Department in Michigan State University for their support. Finally, I would like to thank my family as well as my friends for their support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

The purpose of this research is to design, build, and test a thermally controlled environment (0°C to -10°C) that can maintain a regulated temperature for holding biological samples in a low temperature incubator. This thermally controlled system consists of twelve boxes that were built to hold the biological samples and stabilize the sample temperature. Also, a temperature controller system is used to regulate the temperature inside the boxes. These twelve boxes are tested during conditions of automatic defrost, opening the incubator door, and opening the incubator door and lid of the box at the same time. The results give confidence that for the conditions mentioned this thermally controlled system can maintain a regulated temperature in the required amount of time given by the Center of Microbial Ecology. A detailed description of this system is explained in Chapter 2.

Before explaining a description of the thermally controlled system, a literature search is given to explain about the background of the microorganisms. The background explains the different types of microorganisms. It provides geographic locations where they are formed, and it gives a definition of how microorganisms are created and how they survive. In addition, Section 1.1 also describes previous research that has been completed by biologists. Section 1.2 explains why this research is unique and that biologists are interested in this research because it provides information to show the importance of studying the behavior of microorganisms in a low temperature environment.

1.1 Literature Search

Microorganisms such as microbial, microalgae, cyanobacteria, and microbacteria have been found viable during a geologically significant time (more than three to four millions years). These viable microorganisms are found throughout the cold regions of the earth such as the North Eurasia, North America, and Antarctica [1].

In Antarctica, microorganisms inhabit the ice on land, lakes, and sea. This microhabitat occurred when water interfaces with land and the cold winter air at temperatures below the freezing point of water (-30°C). As a result of the temperature below freezing, it caused a great osmotic stress as ice freezes and leaves microsites of concentrated salt solutions at temperature well below freezing. As a result, a new microhabitat is then created. During the winter time, the surface of ice itself on land, lakes, and sea is lacking water to support a microhabitat. Although, even without water, it is proven that a viable microorganism is detected and anhydrobiotically preserved deep within the ice sheet and permafrost [2].

In addition to the ice on land, lakes, and sea, microorganisms can also grow and survive in the cold deserts of continental Antarctica. The desert surface biota has endured a long period of dehydration, hypersalinity, transient and daily freeze-thaw cycles, and prolonged low temperatures during winter. Yet, it is evident that the low temperature and the limitation of water accessibility help microorganisms to regulate their survival and growth in this region [2].

In North Eurasia, microorganisms are often found in the permafrost region of Northeastern Siberia. In this region, freezing conditions have existed here for over three million years. Not only organic residues, but also large numbers of viable bacteria have been preserved for three million years in ice [3].

Due to the existence of microorganisms and their capability to survive and grow in the permafrost regions, biologists have conducted expeditions in North Eurasia to obtain samples of permafrost soils and sediments. There are several procedures to be performed before the study of the permafrost soils began. First, permafrost sediments were detected. After the sediments are detected, the frozen sediments cores were obtained by using a slow rotary drill so that the sediments cores will not melt. These cores were immediately placed in frozen storage. This is because these sediments have been continuously frozen since they were deposited, therefore it is important to keep them frozen. Afterward, these samples cores were sent to the laboratory for the study of microbial activities in different ranges of temperatures [4]. The results of this study are explained in the next paragraph.

Viable microorganisms have been discovered from buried Siberian permafrost soils and sediments, where their age is ranging from seven thousand to two million years. These samples were analyzed for viable populations of anaerobic bacteria and their metabolic end products. The samples were taken from the tundra zone of Kolyma-Indigirkan Lowland, where permafrost occurs throughout the area to depths of 600-800 meters. Evidence showed that buried permafrost in this region has remained continuously frozen since its deposition. The current average temperature of the sediments ranges from -9°C to -12°C. The results of the study showed that anaerobic bacteria maintain their viability at a temperature range of -7°C to -12°C for very long periods of time, from several thousand to two million years. These long time survivals may require some adaptive mechanisms in order to endure these stresses. It is also concluded that the long-term conservation of authigenic sulfides and the activity of sulfate-reducing bacteria at 4°C leads to the theory that these minerals as well as methane were formed within the permafrost [4].

Other samples of permafrost soil have also been taken from a different depth (0.5-3.8 meters) during expeditions to Northern Siberia. These permafrost soil samples were buried in frozen soil for 2-5 million years where the temperature is around -10°C. Viable aerobic heterotrophic microorganisms have been found in these permafrost soil samples. The morphology and physiology of these microorganisms were identified and studied. During this study, the following characteristics were studied: cell shape, motility, ability to sporulate and form colonies, range of growth temperature, and capacity to grow in media of different salinity. In order to determine the optimal growth temperature, the microorganisms were incubated at 0°C, 4°C, 10°C, 15°C, 20°C, 25°C, and 30°C. The study showed that not all the cells have the same shape. Some cells have a small shape, some have a shape of a rod, and others have irregular shapes of rods. As for the growth temperature, all cells are growing in the temperature range of 0-30°C. Their optimum growth temperature is higher than 15°C, mainly 25°C [5].

Recently in the Siberian permafrost soils, viable microorganisms at a different depth are studied. These permafrost soils have a temperature range between 9°C to -13°C and were taken from a depth of 55 meters. Thirty isolates from different permafrost samples representing aerobic bacteria are being investigated for their cold adaptation. The results show all cells have a capability to grow at or below 0°C. But, the lowest growth temperature has not yet been determined. During their growth at low temperature, the cells developed a very peculiar layer. At temperature growth between 0°C, 10°C, and 20°C, the cells strain showed significant changes in their total fatty acid composition. These feature changes might be because of adaptations to cold temperatures, which are currently proposed as part of a unifying adaptation strategy for microorganisms [6].

All these studies mentioned in the previous paragraphs show that Siberian permafrost microorganisms have a capability to grow at or above 0°C and develop different strategies to maintain their viability and activity at low temperatures. Moreover, during the repeated warming/cooling and freezing/thawing, cells have an ability to protect themselves by adaptating to this environment. From preliminary studies, biologists were able to identify and study the microorganism's activities and adaptations during temperature growth at or above 0°C. But, the activities and adaptations at temperature growth below 0°C have not yet studied by biologists [6].

Currently, a proposal is being made to study the Siberian permafrost microorganism adaptations and activities at growth temperatures between 0°C and -10°C [6]. As mentioned previously, permafrost soil samples need to be kept frozen. To fulfill this requirement, samples are kept in a low temperature incubator. In order to study the microorganism activities at different low temperature growth conditions, not only do the samples need to be kept in a low temperature incubator, but they also need to be kept in a thermally controlled environment to regulate the temperature. Therefore, a thermally controlled environment needs to be designed and fabricated to function inside the incubator in order to accomplish microorganism studies.

1.2 Why this Research is Unique

This research is unique because by designing and manufacturing the controlled environment boxes, it would help biologists to conduct a study of the growth activities and adaptations for the Siberian permafrost samples at temperatures between -0°C and -10°C. By studying their growth activities and adaptations in a lower temperature environment, it will help answer these questions: what are the forms of the vital activity preserved by cells? Are microorganisms able to periodically change from cryoanabiosis to the inhibited or retarded state, but still have a vital activity and propagation? What quantitative, morphological and genetic changes are caused by the adaptation of ancient microbial complexes? In the future, the answers of these questions will help to answer the keystone problem: How long can life be preserved? Today nobody can solve this basic question by modeling, experiments or calculations [3].

According to the evidence taken by NASA missions seeking signs of past life on Mars, it showed that there was once water flowing in Mars, which indicated that Mars once had a warmer temperature and a thicker atmosphere. These conditions would have supported the evolution of life at an early time. Since the Mars environment is so similar to Earth, then viable cells in the Earth permafrost could be considered as an analogue of potential habitants of Martian frozen progressions. If viable Earth permafrost microorganisms could live in a Martian environment then the next question is: Could Earth organic materials survive 3.5 billion years if it were buried and frozen at Martian temperatures (-70°C)? Even more intriguing, could microorganisms survive? And if they did not survive, would their cell structure remain intact? By studying the activity and adaptation of the Siberian biological samples, a conclusion can be made whether there microorganisms can survive on Mars. If there are activities and adaptations of Siberian organic material during temperatures growth at 0ºC and -10ºC, then it may be possible for the Siberian organic material to survive at Martian temperatures and for a longer period time [3].

Chapter 2

DESCRIPTION OF THE SYSTEM

This chapter describes and clarifies the main objective of the system that controls the temperature of the biological samples. Before describing the system, it is important to first understand the behavior of the incubator and define the problems of the incubator that can affect the sample temperature. Furthermore, it is important to define the problems associated with the conditions of automatic defrost, opening the incubator door, and opening the incubator door at the same time. Then understanding these problems can help in the design procedure to build a temperature controlled system that can fulfill the design requirements that are needed. The end of this chapter also provides a brief description of the test procedure used to acquire the data needed to verify the functionality of the system.

2.1 Objective

The main objective of this research is to keep the biological sample temperature stable during the conditions of automatic defrost, opening the incubator door, and opening the incubator door and lid of the box at the same time. Before designing a temperature controlled environment for holding the biological samples, the specifications for the environment of the biological samples need to be defined. The temperature of the samples needs to be controlled and regulated at set temperatures between 0°C and -10°C. Also, the biological sample temperature needs to be maintained at a constant temperature for a long period of time during their incubation. Moreover, the temperature of the biological samples must be the same as the temperature controlled environment. The maximum temperature error that is allowed is ±1°C.

In the process of designing and building a thermally controlled environment the first step is to establish the correct living conditions for the biological sample environment. There are several steps for designing and building the biological sample environment. First, a low temperature box was built based on the specifications from the previous paragraph and the incubator characteristics. This box is then tested and studied in the low temperature incubator to study the performance of the box and determine if the sample temperature can be regulated. The results of the box were favorable, but did not yet fully comply with the specifications. In order to optimize the box performance, an experimental characterization of the system is conducted. Then, the results of the experimental characterization are modeled with computing software called Mathematica. Once the mathematical modeling is verified with respect to the experimental data, the modeling will be used to develop optimal designs for the box. After the design of the box is established, then twelve boxes are built and tested based on the optimized design. The test procedure is given at the end of this chapter.

2.2 Behavior of the Incubator

Before designing a thermally controlled environment for the biological samples, it is important to understand the incubator itself. An incubator is designed to keep conditions favorable for development or testing. In this research, the incubator (Model Number BOD 50 ABA, manufactured by the General Signal Laboratory Equipment) is a refrigerator that has 2 temperature ranges, which are an incubator mode (0°C to 60°C) and a freezer mode (-20°C range). This low temperature incubator has an automatic defrost feature for removing the excessive ice on the cooling coil. The incubator also consists of four shelves, where the temperature differences of each shelf during automatic defrost is between 0.5°C-1°C. The graph of the temperature differences for each shelf can be seen in Figure 1 on the next page.

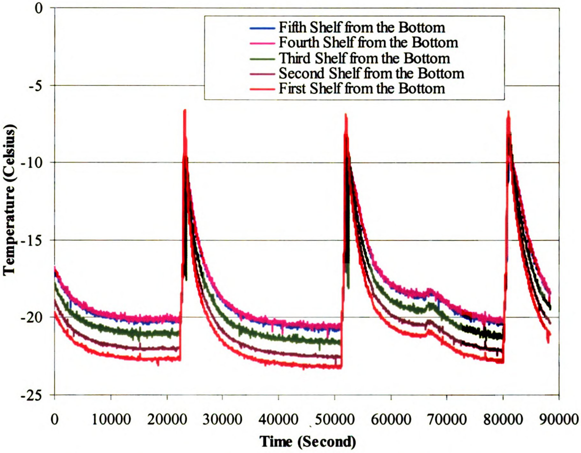


Figure 1 - Temperature Difference between each Shelf of the Incubator

As can be seen in Figure 1, the temperature is warmer from the bottom to the top shelf. This is because the cooling coil is located near the bottom shelf, which makes the cold air flow from the bottom shelf to the top shelf. Due to the small temperature difference, it can be concluded that the temperature inside the incubator is almost uniform.

During the freezer mode, the temperature of each shelf inside the incubator is in the region of -21°C for approximately 5 hours, then the automatic defrost feature turns on. Once the automatic defrost is on, the temperature of each shelf is heated to approximately -7°C, thus allowing the ice on the cooling coil to be defrosted. It takes approximately 15 minutes for the temperature to change from -21°C to the defrost temperature of -7°C. After the temperature reaches -7°C, then the automatic defrost feature turns off and the temperature in the incubator is cooled back to its original setting, which is -21°C.

2.2.1 *Defining the Problem*

After discussing the behavior of the low temperature incubator, the next step is to define the problem within the incubator that needs to be considered in order to design the thermally controlled environment for the biological samples. The first problem concerns keeping the temperature of the biological samples at a constant temperature when the automatic defrost turns on. During automatic defrost, the temperature in the incubator warms to approximately -7°C causing the temperature of the biological sample to tend to warm considerably. Furthermore, the temperature changes to -7°C within approximately 15 minutes, which is a severe change in temperature and could cause harm to the living biological samples. The second problem concerns regulating the temperature of the biological samples regardless of what the temperature is inside the incubator. For example, the temperature of the samples shall be able to regulate at a set temperature between 0°C to -10°C even if the incubator temperature is at -12°C. Other problems include: regulating the sample temperature inside the box when the incubator door is opened, or when the incubator door is opened and the lid of the box is opened at the same time.

2.2.2 *How to Solve the Problem*

For this research, a box using a temperature controller was already designed to hold biological samples in the low temperature incubator. But, it was found that the result of this box did not fulfill the design specification. As a result, a design optimization was done based on the original design to comply with the design specification. Using the optimized design, twelve boxes were fabricated based on the fact that only a total of twelve boxes can fit inside the incubator. Furthermore, the Center of Microbial Ecology requested that nine of the boxes to be filled with cyro vials and three of the boxes to be filled with petri dishes. Figure 2 illustrates the twelve boxes inside the incubator.

Figure 2 - Twelve Boxes inside the Incubator

The fabrication process is important because it can affect the performance of the box. For example, the walls of the box must fit together tightly with a minimum amount of air leakage. If there is air coming into the box through the leakage, this can affect the temperature performance of the sample. Table 1 shows the dimensions of each material of the box.

Table 1 - Dimensions of Each Material of the Box

Material	Dimensions		
	Length	Width	Depth
Styrofoam Box	27.1 cm	19.8 cm	8cm
Aluminum Box	22.5 cm	14.5 cm	5cm
Styrofoam Sample Holder	21.8 cm	13.8 cm	2.5 cm

Because this box is already designed, there are constraints for optimizing the design of the box. For example, the materials for the box have already been chosen. The materials used are styrofoam and aluminum. To optimize the design of the box, the styrofoam sample holder is modified. Also, the arrangement of the samples is changed. The modification to the different sample holders and change in arrangement in the sample is explained in Chapter 3. Shown below in Figures 3, 4 and 5 are pictures illustrating the original design and the optimized design of the box using different sample holders. Figures 6 and 7 also show the sample holder for the petri dishes and the cryo vials for a new design optimization.

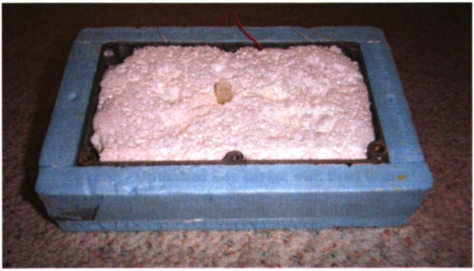


Figure 3 - The Box with the Original Design

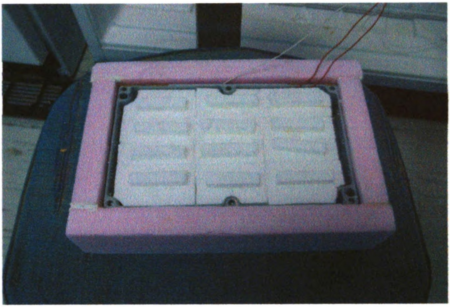


Figure 4 – Optimized Box Design with Cyro Vials

Figure 5 - Optimized Box Design with Petri Dishes

Figure 6 - Styrofoam Sample Holder for Cyro Vials

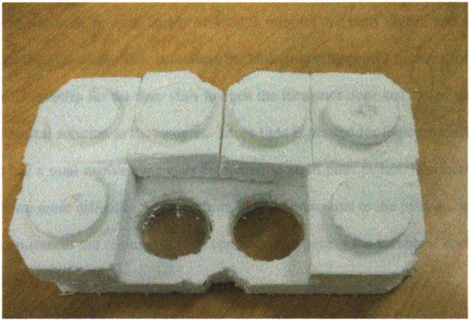


Figure 7 - Styrofoam Sample Holder for Petri Dishes

The box that has already been designed must keep the sample temperature steady during automatic defrost condition, opening the incubator door, and opening the incubator door and top of the box at the same time. During the automatic defrost condition, the warmest incubator temperature is at -7°C. The box made with styrofoam and aluminum materials is built to damp this temperature as it is warming during the automatic defrost conditioning. Obviously, if the samples are placed inside the incubator without using this box, the sample temperature will have the same approximate temperature as the incubator. By placing the sample inside the box that is made from styrofoam and aluminum materials, the sample temperature would not be influenced by the warm incubator temperature (-7°C). This is because the materials damp the outside temperature and keep the sample temperature colder than -7°C. Therefore, building a box with styrofoam and aluminum materials can help to solve this problem.

During opening of the incubator door condition, the biological samples temperature needs to be kept steady at least 3 minutes for petri dishes and at least 5 minutes for the cyro vials. This is because the biologists only need 3 minutes for the petri dishes and 5 minutes for the cyro vials to open the incubator door and top of the box for adding chemical solution to the samples. More time is needed for the cyro vials because each box has a total twelve cyro vials compared to eight petri dishes. Furthermore, the cyro vials are more difficult to remove from the box compared to the petri dishes. These same circumstances are required for the condition of opening the incubator door and top of the box at the same time.

Even though the box damps the incubator temperature, the problem is not completely solved. This is because the sample temperature is still not regulated even though the sample temperature stays colder than -7°C. Looking at Figure 8, the warmest the sample temperature can reach during an auto defrost condition is -12°C, but the temperature still needs to be regulated.

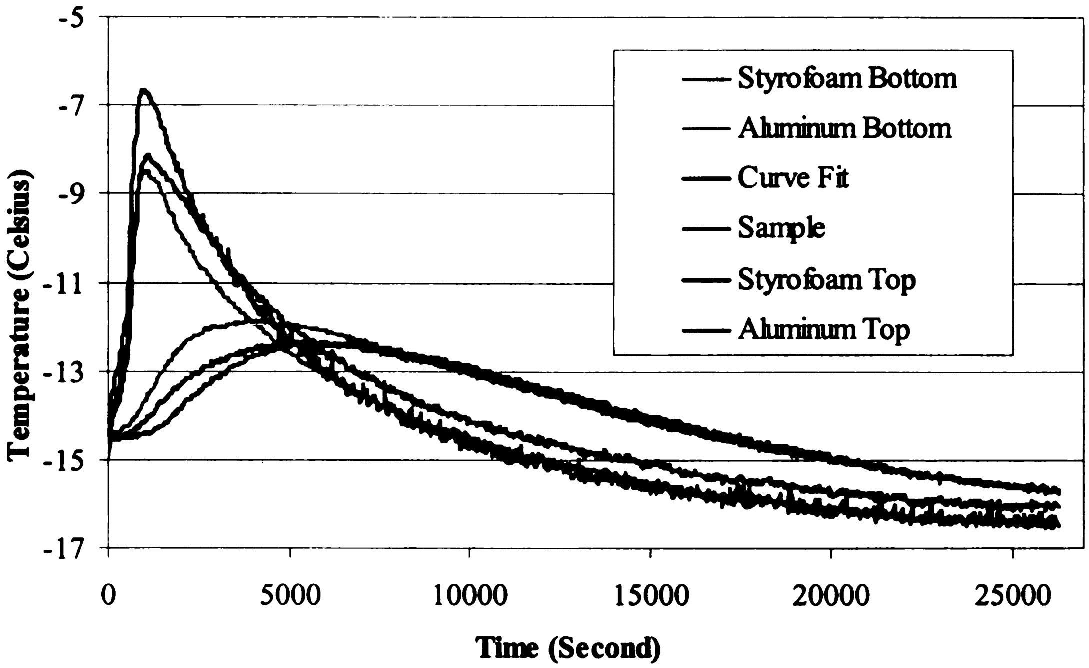


Figure 8. Original Design Box Temperature and Sample Temperature

To solve this issue, a temperature controller is used to regulate the sample temperature. For example, the temperature controller could regulate the sample temperature at -9°C instead of its previous warmest temperature of -12°C during the automatic defrost condition. The temperature controller would still be enabled to regulate the sample temperature at -9°C even though the incubator temperature goes down to -6°C. This is because the warmest sample temperature during the automatic defrost condition is -12°C, meaning the temperature controller would need to be enabled in order to regulate the sample temperature down to -9°C. This controller can be used to regulate the biological sample temperature at a different set temperature, which is also a solution of the second problem mentioned in the previous section. For example, if the incubator temperature is -25°C and the biological samples need to be set to a temperature of -5°C, then the temperature controller will turn on and generate enough heat for the sample to reach -5°C. This condition is known as the transient state. Once -5°C is reached, the temperature controller will enter a steady state condition and will regulate the temperature at -5°C.

The temperature controller system consists of a closed-loop electrical control circuit, plastic heater, and thermocouples within the aluminum box that senses the temperature of the samples and provides feedback to the electronic controller. The electronic controller was built by Theron Hicks. Figure 9 shows the picture of the temperature controller system.

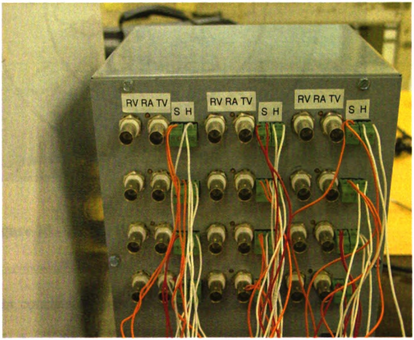


Figure 9 - Temperature Controller System

As can be seen in Figure 9, the labels on the temperature controller consist of a reference voltage (RV), the potentiometer (RA), the temperature voltage (TV), the sensor temperature (S), and the voltage to the plastic heater (H). There are a total of twelve channels in the temperature controller system because there are a total of twelve boxes that were built to fit inside the incubator. Figure 10 shows the plastic heater attached to the bottom side of the aluminum box. The heater output (H) from the temperature controller system provides a voltage across the two wires that are attached to the plastic heater shown in Figure 10.

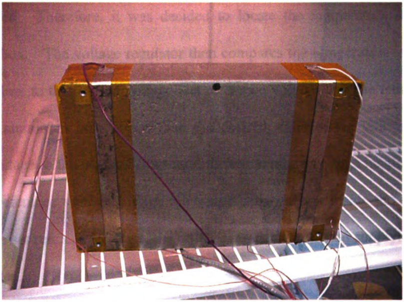


Figure 10 - Plastic Heater Attached to the Aluminum Box

This plastic heater consists of resistance on both sides. One side has infinite resistance and the other side has a finite resistance value. The plastic heater is connected to the regulated output voltage of the temperature controller. When supplying a voltage through the resistance of the plastic heater, the resistance becomes heated, thus generating heat for biological samples. In order to have good heat generation for the biological samples, the plastic heater is placed underneath the aluminum box. The voltage regulator also has a reference voltage, which can be adjusted by the user for changing the temperature at the plastic heater. After the user sets the desired voltage, the temperature sensor gives the feedback to the voltage regulator. The temperature sensor needs to be placed in a location where the temperature needs to be monitored and regulated. The perfect location for this sensor is inside one of the cyro vials. But, the sensor cannot be placed inside a cyro vial because the microorganisms will be contaminated. Therefore, it was decided to locate the temperature sensor inside the aluminum box. The voltage regulator then compares the temperature voltage inside the aluminum box to the desired voltage (set reference voltage). If the temperature voltage inside the aluminum box is less than the desired voltage, the voltage regulator then supplies voltage to the plastic heater until the temperature voltage inside the box is equal to the set reference voltage. When the temperature voltage inside the aluminum box is equal to the set reference voltage, the voltage regulator will stop supplying voltage to the plastic heater.

2.3 *The Testing Procedure for Data Collection*

The temperature performance of all twelve boxes is tested in different conditions, such as automatic defrost condition, opening the incubator door, and opening the incubator door and lid of the box at the same time. First, the boxes are filled with the samples. Then, the boxes are placed inside the incubator for 1 day, so that the temperature inside the incubator becomes steady. After the temperature is uniform everywhere inside the box, the box is tested for the conditions that were mentioned. The boxes are tested with the temperature controller enabled to determine the performance of the box. The test results for the twelve boxes and the conditions mentioned are shown in Chapter 5. The tables showing the procedures for operating the temperature controller and operating the Data Acquisition software can be seen in the Appendix.

Chapter 3

DESIGN

After understanding the theory of heat transfer, the next step is to design a box that can provide the most efficient performance for controlling the low temperature environment. The objective of the box is to maintain a constant temperature inside the box even though the temperature outside the box is varying. In order to comply with this objective, the styrofoam and aluminum materials are used to meet the requirements needed in a low temperature environment. The explanation about the types of materials used and the reason why these materials are used can be found in Section 3.1. Once the box is fabricated with the styrofoam and aluminum materials, the next objective in the design process is attempting to enhance the performance for the box. This can be done by generating a model from the experimental test results of the box. The model can be achieved using two methods, which are using a finite element method or mathematical modeling method. The finite element method analysis uses commercial software called FLUENT to perform a numerical analysis to find the temperature distribution inside the box. The mathematical modeling uses an analytical solution for finding the temperature distribution inside the box. In this research, the finite method analysis only models the steady state condition for determining the temperature distribution inside the box. Due to lack of knowledge in C programming, the boundary conditions of the box during the transient state of the incubator can not be defined. As a result, the finite method analysis was not considered in this research because the transient state of the incubator causes the most harm to the biological sample. Section 3.2 explains the finite analysis in more detail. Section 3.3 explains the mathematical modeling. The design optimization of the box can be found in Section 3.4.

3.1 Explanation of the Styrofoam and Aluminum Materials

The first step of designing a thermally controlled low temperature environment is finding the correct materials for the box used for storing the biological samples. This is because it can help stabilize the incubator temperature even during automatic defrosting conditions. It is important that the material used can create an insulated environment inside the box and damp the incubator temperature during automatic defrost conditions so that the temperature of the biological samples can be steady at a uniform temperature. According to the heat transfer theory, the best material used for insulation systems is a material that has a low thermal conductivity, a high heat capacity, and a low density. The ratio of a low thermal conductivity and a high heat capacity will provide a low thermal diffusivity. This low thermal diffusivity is beneficial for damping the incubator temperature during automatic defrost conditions. The next paragraph explains more detail about insulation materials.

As mentioned in heat transfer theory, insulation materials are composed of a multilayer of small hollow spaces. These hollow spaces are designed to restrict the motion of air and to trap the air. As a result, the restriction of air motion in the void space will reduce the effective thermal conductivity of the system material. If the performance of the insulation material is enhanced, then there is less thermal conductivity in the material. The insulation materials also have a high heat capacity and a low thermal diffusivity. According to the heat transfer theory, a material that has a low heat capacity is not an acceptable material to store a thermal energy. As for thermal diffusivity, a material that has a small thermal diffusivity (α) will respond longer to reach a new equilibrium condition compare to a large thermal diffusivity. These properties match the characteristics of insulation materials. According to Chapter 1, a box was designed using styrofoam and aluminum materials. Styrofoam was used as the first layer of the box because it has a low thermal diffusivity, which helps to damp the incubator temperature during an automatic defrost condition. The thermal diffusivity of styrofoam and aluminum is described as following,

$$\alpha = \frac{k}{\rho Cp} = \frac{0.029 \text{ W/m} \cdot \text{K}}{50 \text{ kg/m}^3 * 1000 \text{ J/kg} \cdot \text{K}} = 5.8\text{E} - 7 \text{ m}^2\text{/s} \tag{1}$$

Moreover, styrofoam is easily available, simple to manufacture, and inexpensive compared to the other insulation materials.

Since the biological samples are put in the box, it is important that the box has a uniform temperature distribution. This will ensure a uniform temperature within the biological samples. In order to have a uniform temperature, the material needs to have a Biot number less than 0.1. When a Biot number of the material is less than 0.1, then the conduction resistance within the solid material is much less than the convection resistance across the fluid boundary layer. Therefore, the major temperature gradient occurs through the fluid layer at the boundary, assuring that the temperature gradient in the material is small. As a result, there exists a uniform temperature distribution in the solid material when the Biot number is less than 0.1.

Next, it is necessary to determine the Biot number for styrofoam. Equation (2) shows the Biot number for styrofoam.

$$Bi = \frac{hL}{k} = \frac{3*0.026}{0.029} = 2.7 > 0.1 \qquad (2)$$

According to the Equation (2), the Biot number for the styforoam is greater than 0.1, which means that the styrofoam is not a good material to have a uniform temperature distribution. To solve this problem, the box needs to have another layer of material, which has a Biot number less than 0.1. From Chapter 1, the second material that was used was aluminum. The Biot number of aluminum is shown below:

$$Bi = \frac{hL}{k} = \frac{3*0.0035}{170} = 6.2E-5 > 0.1 \qquad (3)$$

By having a Biot number less than 0.1, the aluminum can improve the uniform temperature distribution for the box. Besides having a uniform temperature distribution, aluminum also has another advantage. Aluminum has a large thermal conductivity, so aluminum is a good conductor. As mentioned in Chapter 1, a heater will be placed underneath the aluminum layer of the box for heating the biological samples when the temperature becomes too cold. Since aluminum is a good conductor, the heat will easily diffuse through the aluminum layer of the box and heat the biological samples. Using the temperature controller that was explained in Chapter 1, the heat can be regulated.

3.2 Finite Element Method Modeling

Using a software package named FLUENT, a numerical method analysis is used to model the temperature distribution inside the box when the temperature controller is enabled and disabled during the steady state condition. As mentioned previously, the transient state cannot be defined due to lack of knowledge in C programming. The software package FLUENT 5.2 is a commercial program that is used to solve and compute fluid flow and heat transfer in a complex 2D or 3D geometry. For this modeling, the box is modeled in 2D geometry and only half of the box is modeled due to symmetry of the box. Only 2D geometry is used because of lack of knowledge using FLUENT in 3D applications. During the transient state, the boundary condition for the box is the incubator temperature dependant upon time. As for a steady state condition, the boundary condition of the box does not depend upon time because the incubator temperature is not changing.

The modeling is able to illustrate the temperature distribution inside the box when the temperature controller is enabled and disabled during the steady state. The results of this modeling are then compared with the experimental results. The result shows that the temperature difference between the experimental and the finite element method are close, which is below 1^{o}C.

3.2.1 *Finite Element Method Modeling with Temperature Controller Disabled*

Figure 11a shows the comparison between the experimental and the finite element method results during the steady state condition for styrofoam bottom.

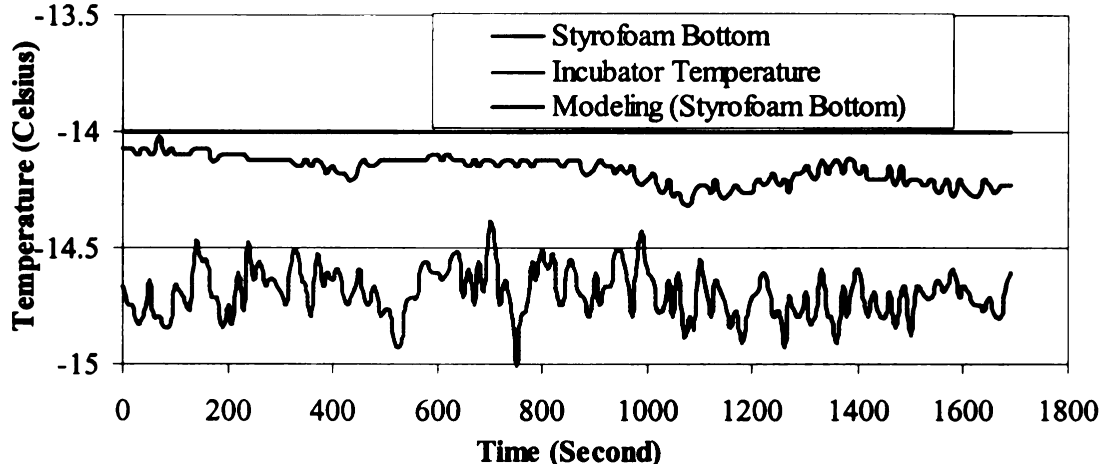


Figure 11a - Comparison between Measurement and Modeling for Styrofoam (Temperature Controller Disabled)

For the styrofoam bottom, the temperature difference between the modeling and experimental result is about 0.1°C. Figure 11b is the comparison between the modeling and the experimental result for the aluminum bottom.

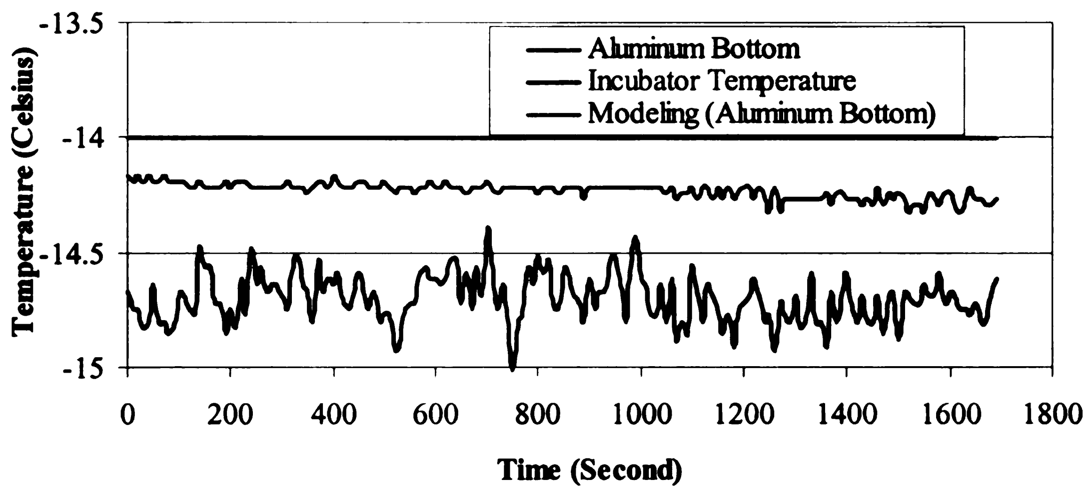


Figure 11b - Comparison between Measurement and Modeling for Aluminum (Temperature Controller Disabled)

The temperature difference between the experimental and modeling result for the aluminum bottom is about 0.2°C. Figure 11c is the comparison between experimental and modeling results for the sample.

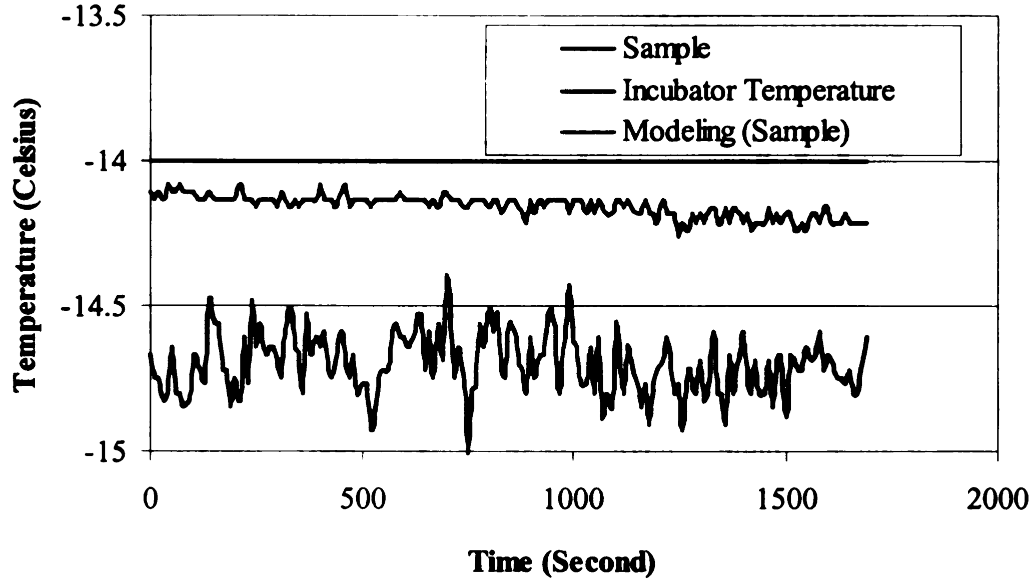


Figure 11c - Comparison between Measurement and Modeling for Sample (Temperature Controller Disabled)

From Figure 11c it can be seen that the temperature difference between the modeling and experimental result is about 0.1°C.

Looking at Figures 11a, 11b, and 11c for the styrofoam bottom, aluminum bottom, and samples, the results for the two cases are very close, which is a temperature difference below 1°C. This temperature difference is acceptable.

3.2.2 *Finite Element Method Modeling with Temperature Controller Enabled*

Figure 12a shows the comparison between the experiment and modeling when temperature controller enabled at -6.7°C for styrofoam.

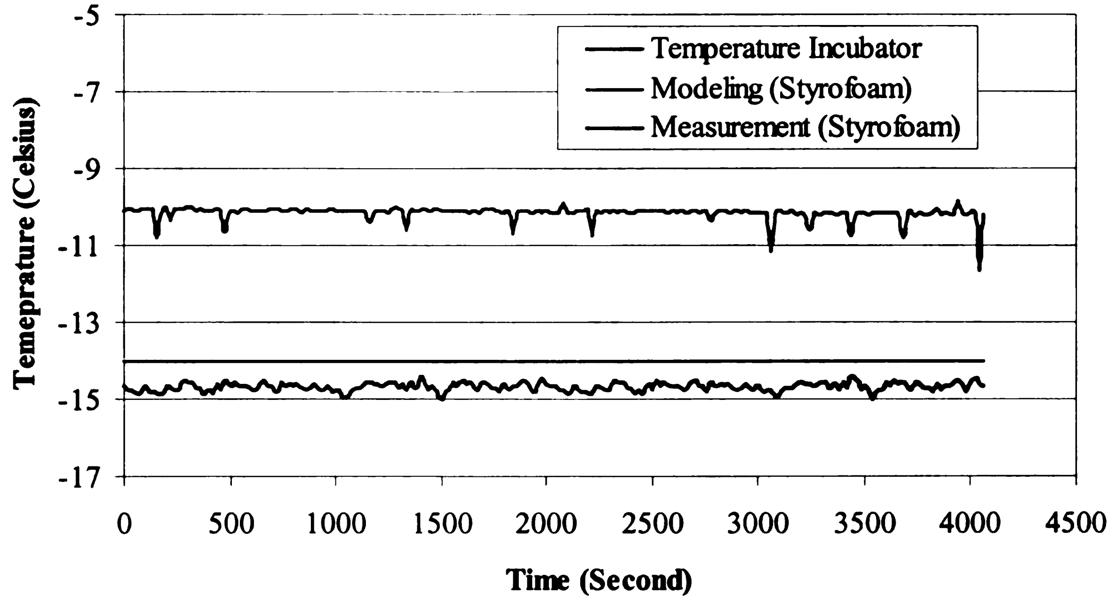


Figure 12a - Comparison between Measurement and Modeling for Styrofoam (Temperature Controller Enabled)

Looking at Figure 12a, the temperature difference between the measurement and modeling for styrofoam is about 4°C. Figure 12b is the comparison between the measurement and modeling for aluminum bottom. The temperature difference between the measurement and modeling is about 0.1°C.

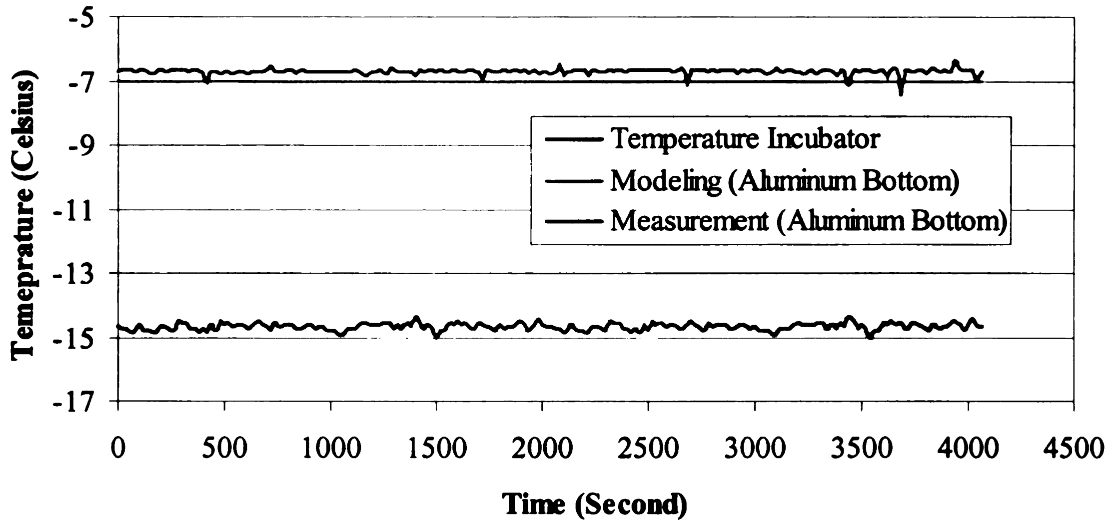


Figure 12b - Comparison between Measurement and Modeling for Aluminum (Temperature Controller Enabled)

Figure 12c shows the comparison between the modeling and measurement for the sample. As it can be seen in the graph, the temperature difference is about 1.6°C.

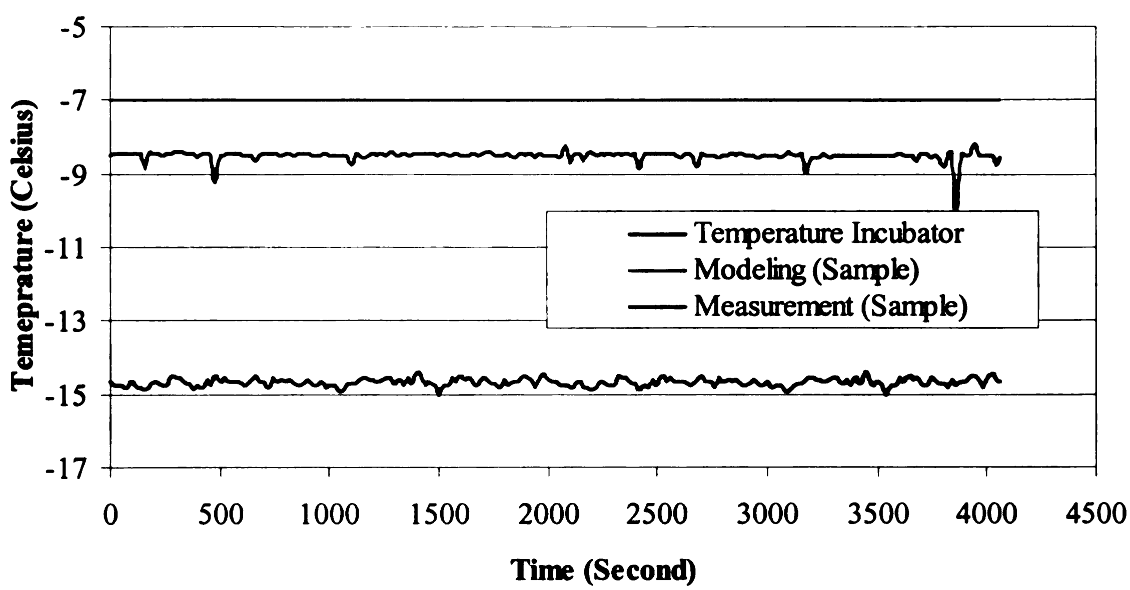


Figure 12c - Comparison between Measurement and Modeling for Sample (Temperature Controller Enabled)

Since the temperature difference between measurement and modeling for the styrofoam bottom and sample is above 1°C, therefore these modeling results are not acceptable. This error may occur because the natural convection coefficient (h) that was used in FLUENT is not correct, or the error may occur because 2D analysis was used instead of 3D analysis. In addition, the error may be caused due to lack of knowledge using the FLUENT software package.

3.3 Mathematical Modeling

Even though the box is three-dimensional, it was determined that one-dimensional analysis was acceptable to help improve the design of the box. This means that the x, y, or the z-axis needs to be used for this analysis. For this analysis, mathematical modeling is used to solve and plot first order differential equations using the theory of transient conduction and plane wall steady state conduction analysis. The first order differential equation used in this analysis represents the temperature distribution through each material of the box. This first order differential equation is represents the equivalent thermal circuit for the composite wall of the box. The resistance of each material is divided by two since the thermocouple measurement of the temperature is in the middle of each material. After plotting the mathematical results of the temperature distribution through the x, y, and z-axis, they need to be matched with the experimental results. The plots show that the z-axis best matches the experimental results, meaning that heat transfers through the z-axis faster than the x or y axis. Therefore, the z-axis is used in the mathematical modeling for optimizing the design. The next sections explain the modeling of the system without the temperature controller and with the temperature controller.

3.3.1 *Mathematical Modeling with Temperature Controller Disabled*

It is useful to first analyze the temperature characteristics of the box with the electronic temperature controller disabled. This is because the results provide a benchmark describing a passive system. The first condition that needs to be modeled is the changes in the internal temperature of the box during an automatic defrost condition. The second condition to be modeled analyzes the change in the internal temperature of the box when opening the refrigerator door. The last condition modeled is analyzing the internal temperature of the box when opening the refrigerator door and opening the top of the box at the same time.

As shown in Figure 13, the box is three-dimensional meaning that temperature gradients may exist along all three coordinates. To simplify the heat transfer analysis, the modeling deals with one-dimensional heat transfer through the box. To understand a more accurate flow of heat transfer through the box, a three-dimensional analysis should be considered in future research. The coordinate direction used in the modeling is explained in the next paragraphs. Note that in Figure 13 the box is drawn only half its original size. This is because the compositions of materials within the box are easier to visualize.

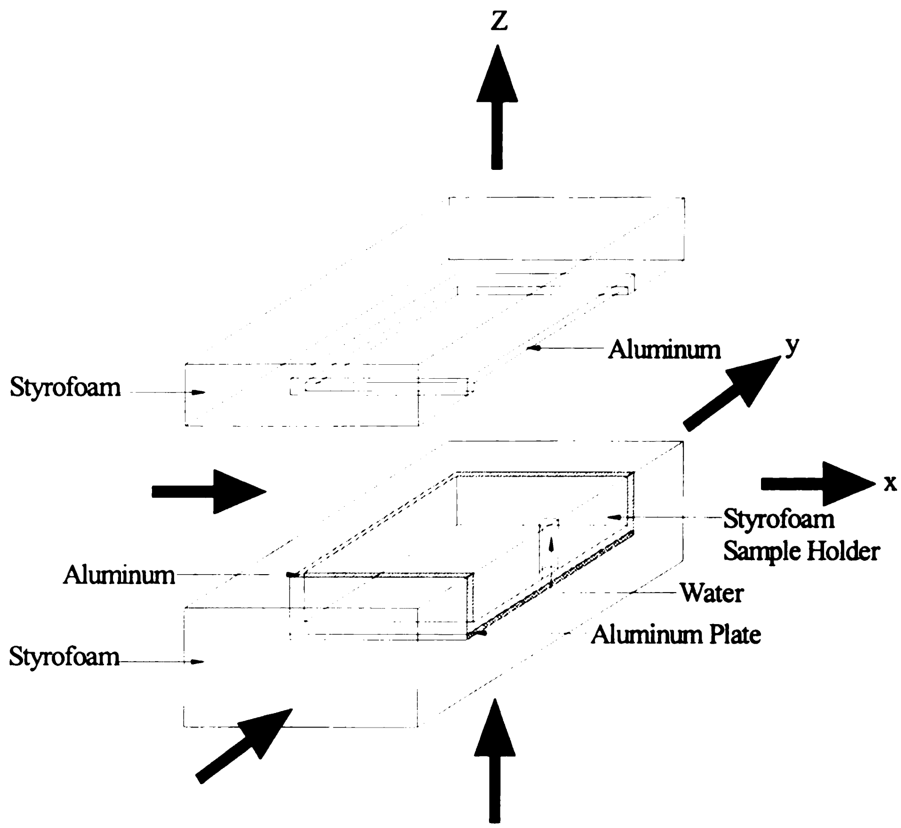
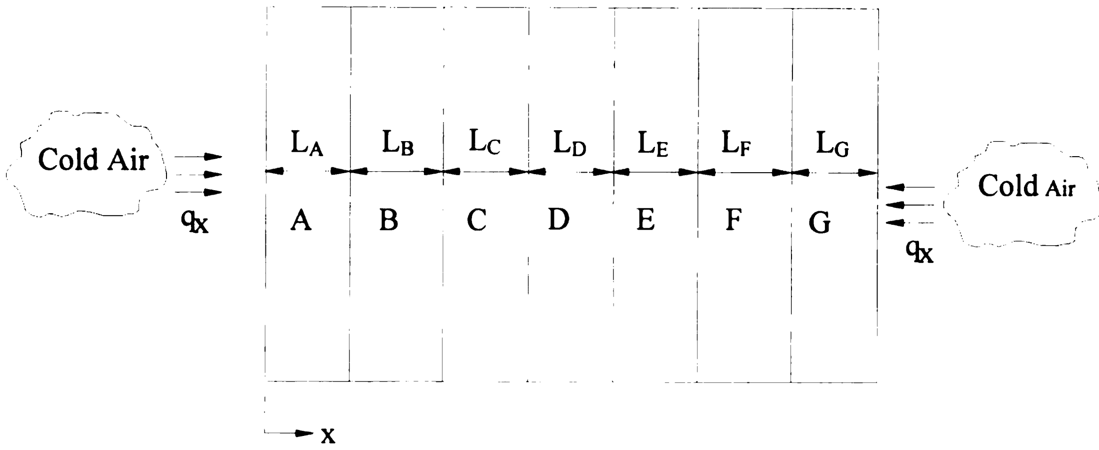


Figure 13 - Box with Three Coordinate Directions

First, heat transfer in the x-direction is considered. Figure 14 illustrates the heat transfer in the x-direction of the composite wall of the box. It can be seen from Figure 14 that heat transfers through each material of the box. An electrical circuit can be generated to represent the composite wall in the x-direction. This representation is shown in Figure 15.



A = G: Styrofoam
B = F: Aluminum
C = E: Styrofoam Sample Holder
D : Sample
L : Thickness

Figure 14 - Composite Wall for the Box in x-direction

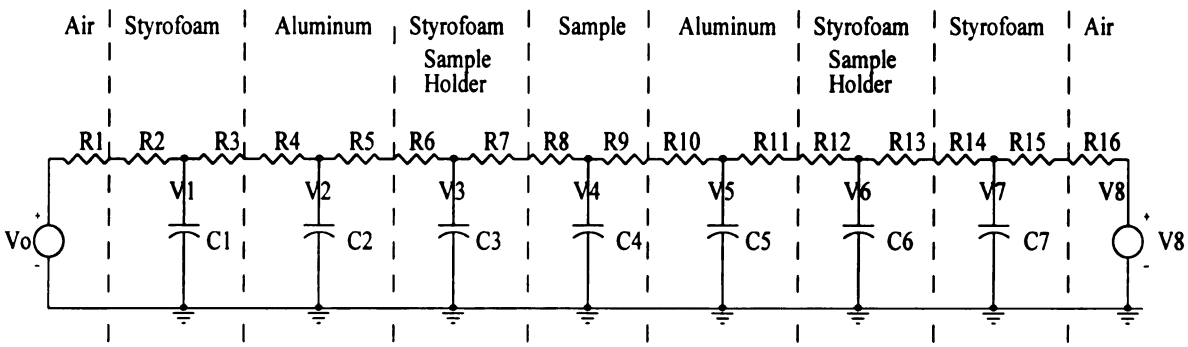


Figure 15 - Electrical Circuit Representation for Composite Wall in x-direction

It is important to define the area and thickness of each material in order to optimize the design of the box. It can be seen from Figure 15 that the area and thickness of each material of the box can be represented as resistance and capacitance parameters. Using node voltage analysis, equations can be generated from the electrical circuit. The Equations (4) – (10) are shown below,

$$\frac{V_o - V_1}{R_1 + R_2} = V_1^{'} C_1 + \frac{V_1 - V_2}{R_3 + R_4} \tag{4}$$

$$\frac{V_1 - V_2}{R_3 + R_4} = V_2^{'} C_2 + \frac{V_2 - V_3}{R_5 + R_6} \tag{5}$$

$$\frac{V_2 - V_3}{R_5 + R_6} = V_3^{'} C_3 + \frac{V_3 - V_4}{R_7 + R_8} \tag{6}$$

$$\frac{V_3 - V_4}{R_7 + R_8} = V_4^{'} C_4 + \frac{V_4 - V_5}{R_9 + R_{10}} \tag{7}$$

$$\frac{V_4 - V_5}{R_9 + R_{10}} = V_5^{'} C_5 + \frac{V_5 - V_6}{R_{11} + R_{12}} \tag{8}$$

$$\frac{V_5 - V_6}{R_{11} + R_{12}} = V_6^{'} C_6 + \frac{V_6 - V_7}{R_{13} + R_{14}} \tag{9}$$

$$\frac{V_6 - V_7}{R_{13} + R_{14}} = V_7^{'} C_7 + \frac{V_7 - V_8}{R_{15} + R_{16}} \tag{10}$$

Where,

$V_o = V_8$ = Voltage source = Forcing function

$V_1 = V_5$ = Styrofoam temperature/voltage

$V_2 = V_6$ = Aluminum temperature/voltage

$V_3 = V_7$ = Styrofoam sample holder temperature/voltage

V_4 = Sample temperature/voltage

$C_1 = C_5 = \rho*Cp*A*L$ =Styrofoam capacitance

$C_2 = C_6$ = Aluminum capacitance

$C_3 = C_7$ = Styrofoam sample holder capacitance

C_4 = Sample capacitance

$R_1 = R_{16} = 1/(h*A)$ =Air resistance

$R_2 = R_3 = R_{10} = R_{11} = L/(2*K*A)$=Styrofoam resistance

$R_4 = R_5 = R_{12} = R_{13}$ = Aluminum resistance

$R_6 = R_7 = R_{14} = R_{15}$ = Styrofoam sample holder resistance

$R_8 = R_9$ = Water resistance

Equations (4) - (10) that are generated contain a voltage at each node of the electrical circuit. Each node voltage represents temperature in each material. Adjusting the resistance and capacitance values in the equations corresponds to adjusting the area and the thickness of the box. Adjusting the resistance and capacitance values would change the voltage or temperature at each node of the circuit. If the changes in values benefit the design of the box, then changes can be implemented in adjusting the area and thickness of the wall of the box. After calculating the temperature in each material, the modeling results are then compared with the experimental results. The next paragraph explains how the supply voltage, V_o, is generated for the modeling.

In order to find the voltage or temperature in the equations, the supply voltage, V_o, must be calculated. This voltage is the voltage source representing the temperature outside the box. Furthermore, V_o is the temperature of any shelf in the incubator at a given period of time. The voltage source, V_o, can be obtained by finding the equation of the temperature incubator graph during the automatic defrost condition. This equation is found by entering the graph of the automatic defrost response into the software Tablecurve 2D using curve fitting method. Once the equation for V_o is found, Equations (4) – (10) are then implemented in Mathematica software to find the voltage of each node for each material. In the heat transfer analogy, the node voltage for each material is equal to the temperature in each material.

Figures 16a, 16b, 16c, and 16d show the comparison between the mathematical modeling results and the temperature measurement results for the x-direction. For the incubator temperature, V_o, the result of the curve fit during automatic defrost conditions can be seen in Figure 16e. These figures referred to the plane wall composite in Figure 14.

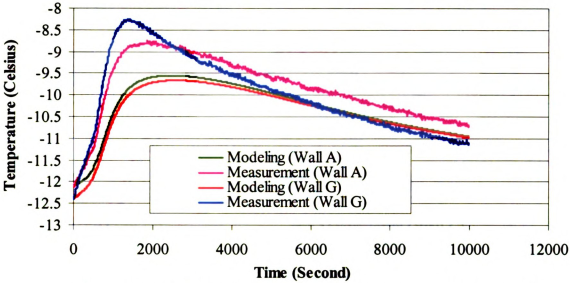


Figure 16a - Styrofoam Modeling vs. Measurement during Automatic Defrost Condition (Non Heater- x Direction)

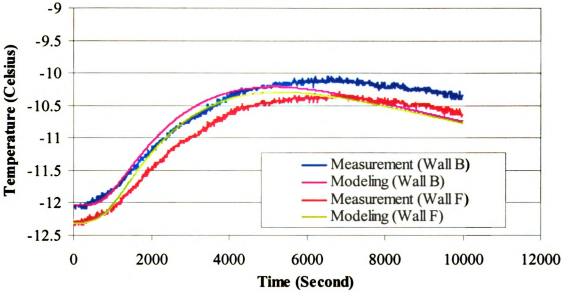


Figure 16b - Aluminum Modeling vs. Measurement during Automatic Defrost Condition (Non Heater - x Direction)

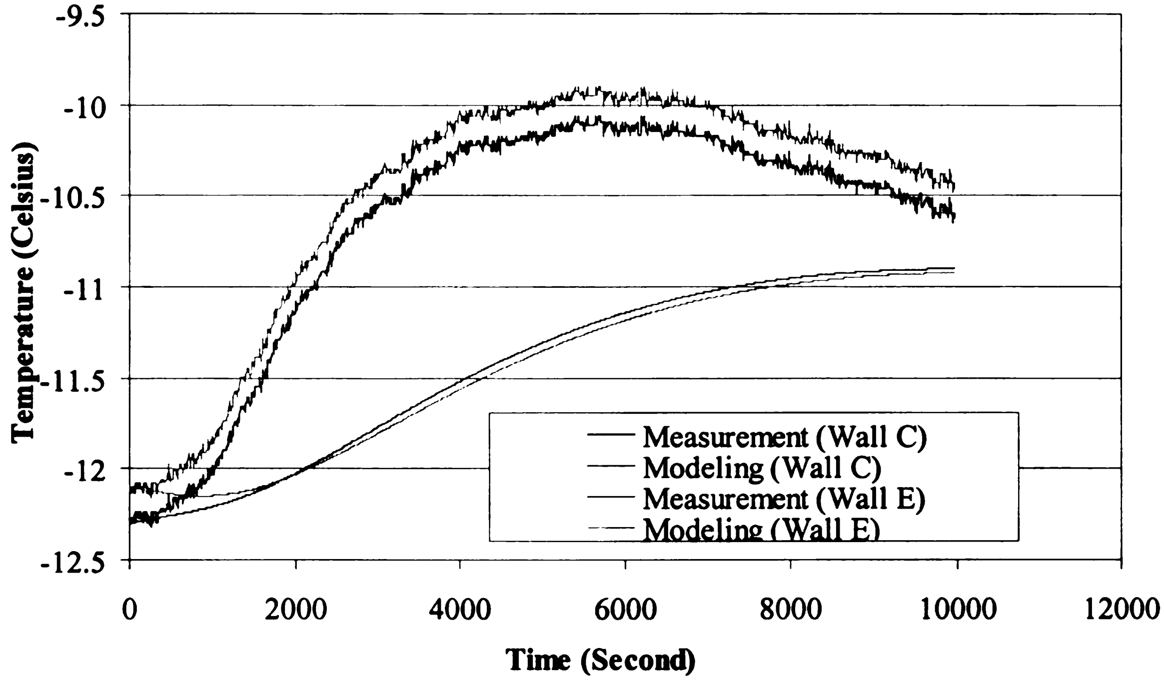


Figure 16c - Styrofoam Sample Holder Measurement vs. Modeling during Automatic Defrost (Non Heater - x Direction)

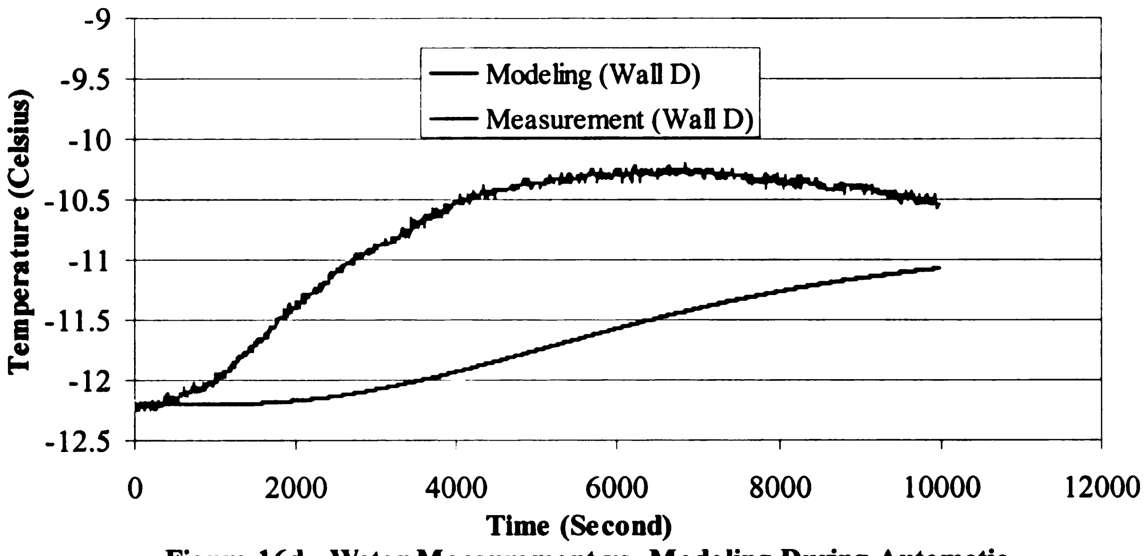


Figure 16d - Water Measurement vs. Modeling During Automatic Defrost (Non Heater - x Direction)

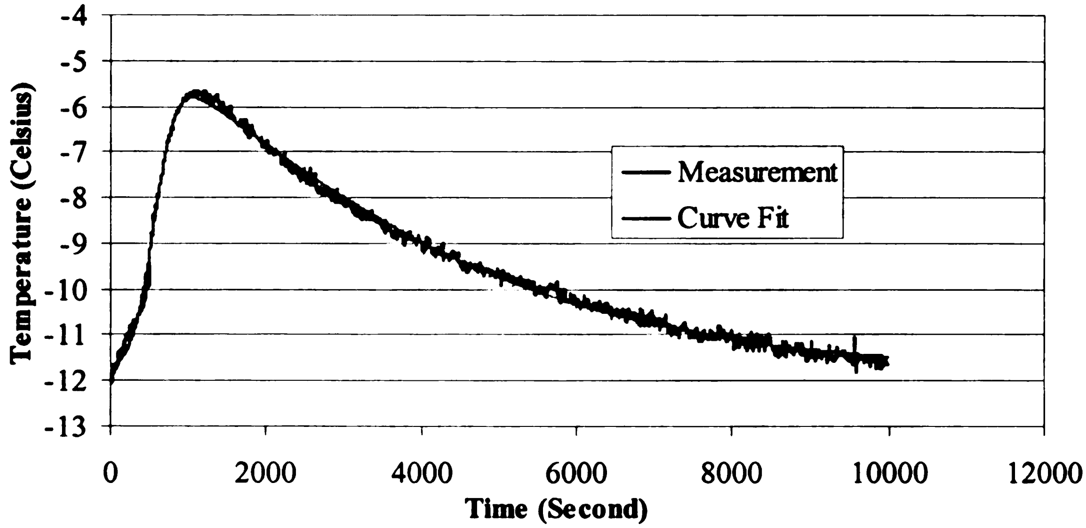


Figure 16e - Incubator Temperature during Automatic Defrost (Non Heater - x Direction)

Figures 16a and 16b show that the temperature measurement results and the mathematical modeling results for the styrofoam and aluminum boxes in the x-direction are similar. But, the temperature of the measurement results and the mathematical modeling results are not similar for the sample and styrofoam sample holder. Since these two results are not close, then it was decided that the x-direction shall not be used for the mathematical modeling.

Next, heat transfer in the y-direction is analyzed. The composite wall shown in Figure 14 for heat transfer flow and the equivalent electrical circuit shown in Figure 15 are the same for the y-direction as in x-direction. As a result, the same mathematical modeling procedure in the x-direction is now performed in the y-direction. Then the mathematical modeling results for each material in the y-direction are compared to the experimental results in the y-direction. This comparison can be seen in Figures 17a, 17b, 17c, and 17d. These figures referred to the plane wall composite in Figure 14.

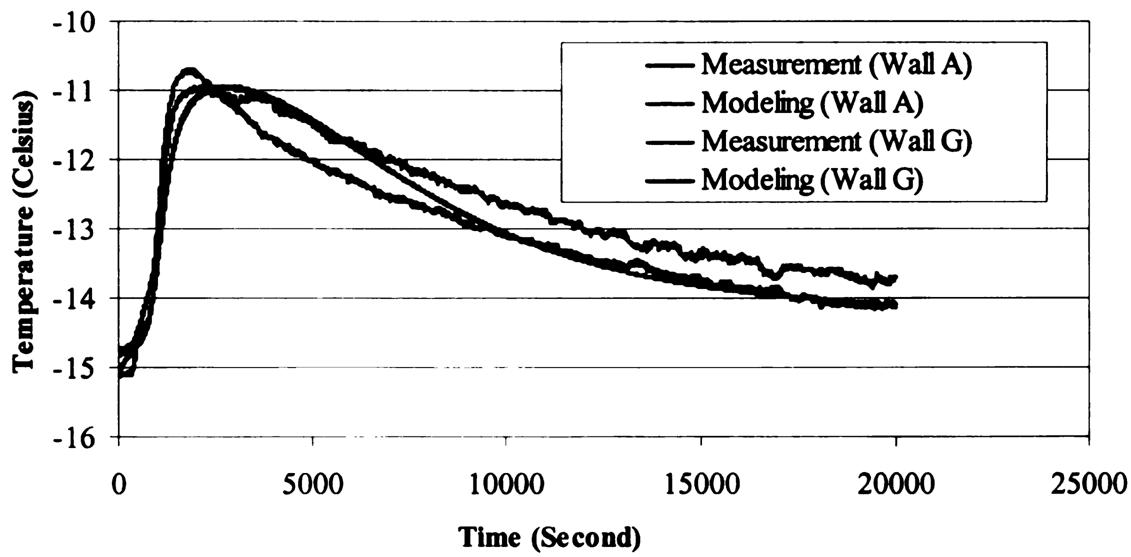


Figure 17a - Styrofoam Measurement vs. Modeling during Automatic Defrost (Non Heater- y Direction)

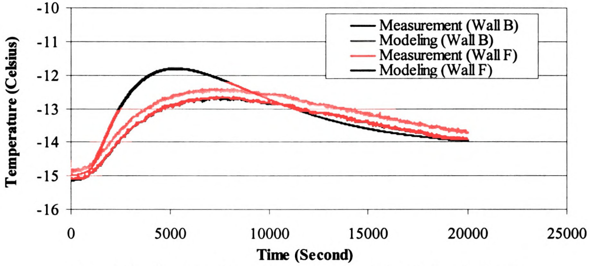


Figure 17b - Aluminum Measurement vs. Modeling during Automatic Defrost (Non Heater - y Direction)

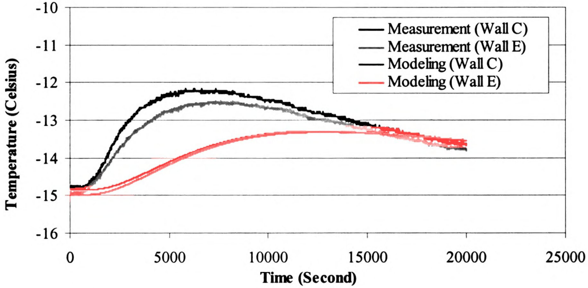


Figure 17c - Styrofoam Sample Holder Measurement vs. Modeling during Automatic Defrost (Non Heater - y Direction)

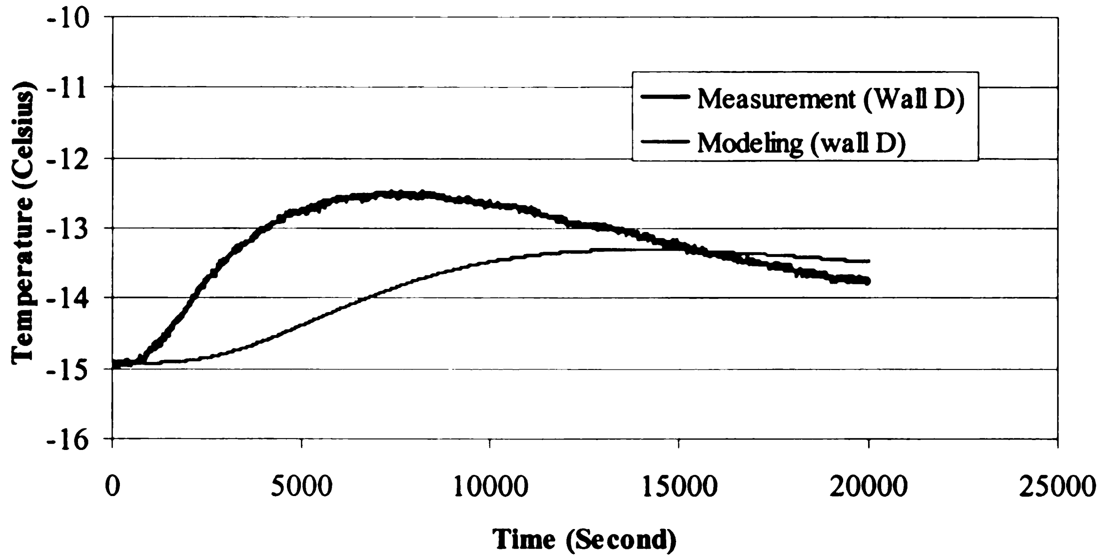


Figure 17d - Sample Measurement vs. Modeling during Automatic Defrost (Non Heater - y Direction)

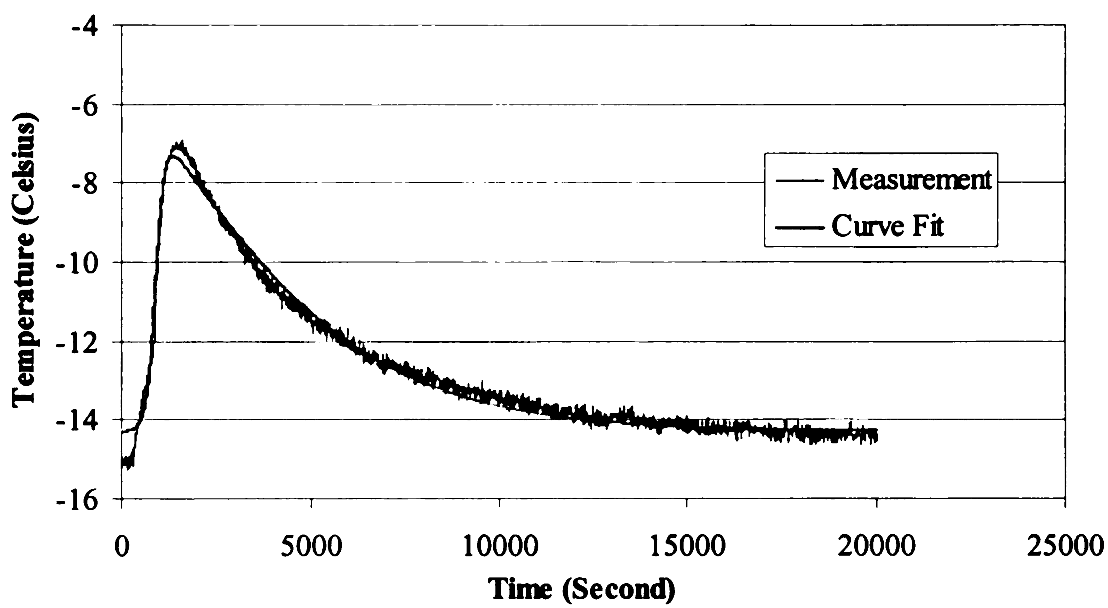


Figure 17e - Incubator Temperature during Automatic Defrost (Non Heater - y Direction)

Just like the results in the x-direction, the temperature measurement results and mathematical modeling results are not similar. As a result, heat transfer direction in the y-direction is not used. Finally, heat transfer in the z-direction is analyzed. Figure 18 illustrates the composite wall for the box in the z-direction.

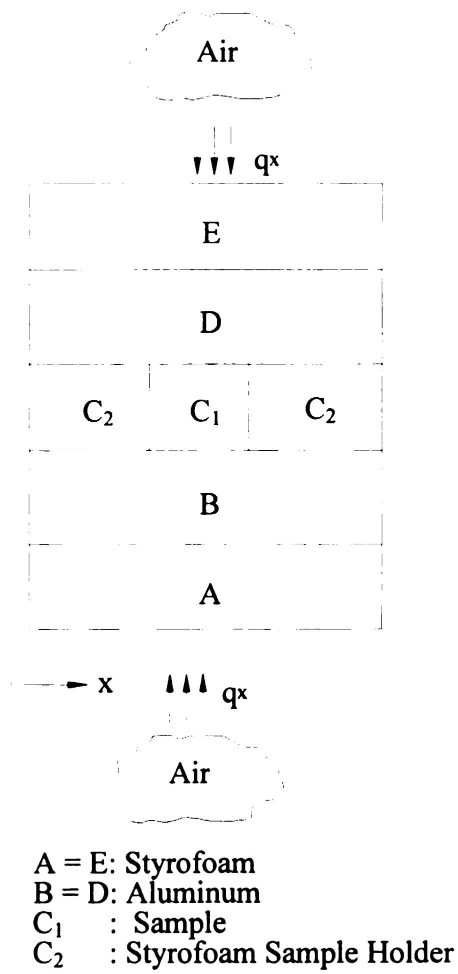


Figure 18 - Composite Wall for Box in z-direction (1)

Looking at Figure 18, the heat is first transferred through the styrofoam (walls A and E) and secondly through the aluminum (walls B and D). The heat reaches the styrofoam sample holder (wall C_2) and the sample (C_1) at the same time. Keep in mind, heat is also transferred from the styrofoam sample holder (C_2) to the sample (C_1) due to the heat transfer in x-direction. But this condition is ignored because heat first reaches the sample in the z-direction even before it is transferred from the sample holder through the x-direction. The reason why is because in the z-direction there is less thermal resistance compared to the x-direction due to the styrofoam sample holder. The new composite wall for the box in the z-direction is depicted in Figure 19.

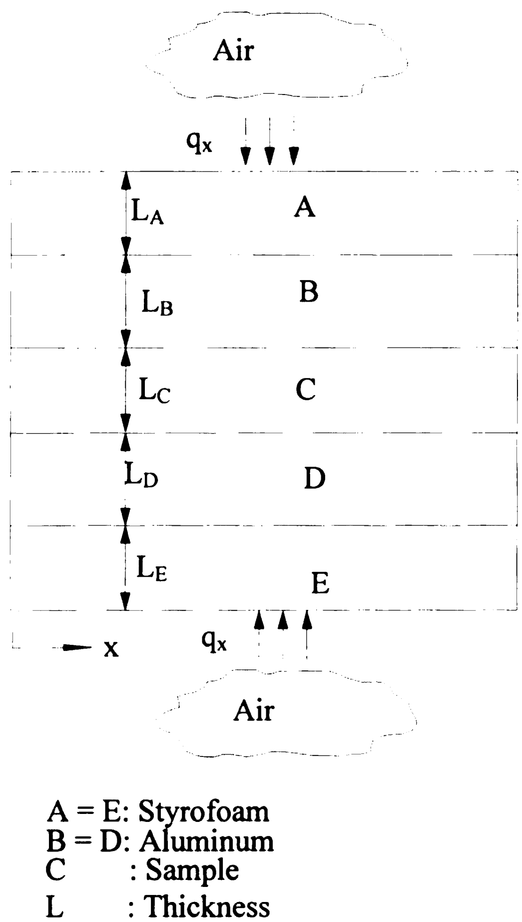


Figure 19 - Composite Wall for Box in z-direction (2)

Based on Figure 19, the electrical circuit for the plane wall is shown in Figure 20.

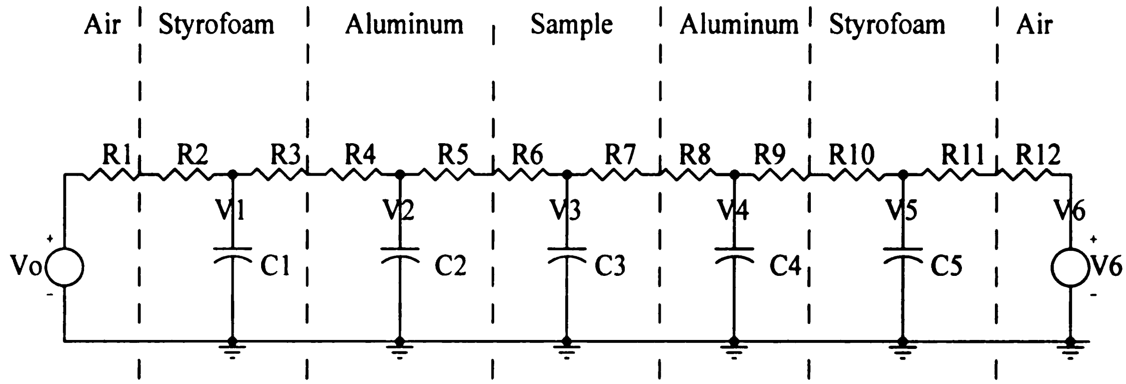


Figure 20 - Electrical Circuit Representation for z-direction

The equations of this electrical circuit are described as the following:

$$\frac{V_o - V_1}{R_1 + R_2} = V_1^{'} C_1 + \frac{V_1 - V_2}{R_3 + R_4} \tag{11}$$

$$\frac{V_1 - V_2}{R_3 + R_4} = V_2^{'} C_2 + \frac{V_2 - V_3}{R_5 + R_6} \tag{12}$$

$$\frac{V_2 - V_3}{R_5 + R_6} = V_3^{'} C_3 + \frac{V_3 - V_4}{R_7 + R_8} \tag{13}$$

$$\frac{V_3 - V_4}{R_7 + R_8} = V_4^{'} C_4 + \frac{V_4 - V_5}{R_9 + R_{10}} \tag{14}$$

$$\frac{V_4 - V_5}{R_9 + R_{10}} = V_5^{'} C_5 + \frac{V_5 - V_6}{R_{11} + R_{12}} \tag{15}$$

Where,

$V_o = V_6$ = Voltage source = Forcing function

$V_1 = V_5$ = Styrofoam temperature/voltage

$V_2 = V_4$ = Aluminum temperature/voltage

V_3 = Sample temperature/voltage

$C_1 = C_5$ = Styrofoam capacitance

$C_2 = C_4$ = Aluminum capacitance

C_3 = Sample capacitance

$R_1 = R_{12}$ = Air resistance

$R_2 = R_3 = R_{10} = R_{11}$ = Styrofoam resistance

$R_4 = R_5 = R_9 = R_8$ = Aluminum resistance

$R_6 = R_7$ = Water resistance

The comparison of the modeling results and the temperature measurement results are depicted in Figures 21a, 21b, and 21c located. Figures 21a, 21b, 21c, and 21d show that the modeling results and measurement results are similar. These figures referred to the plane wall composite in Figure 19.

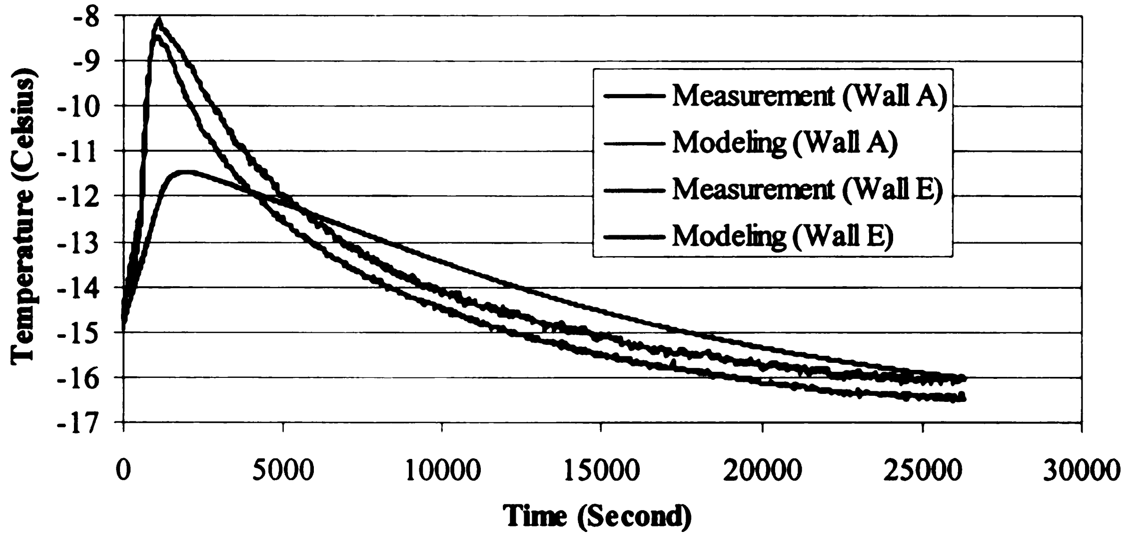


Figure 21a - Styrofoam Measurement vs. Modeling during Automatic Defrost (Non Heater - z Direction)

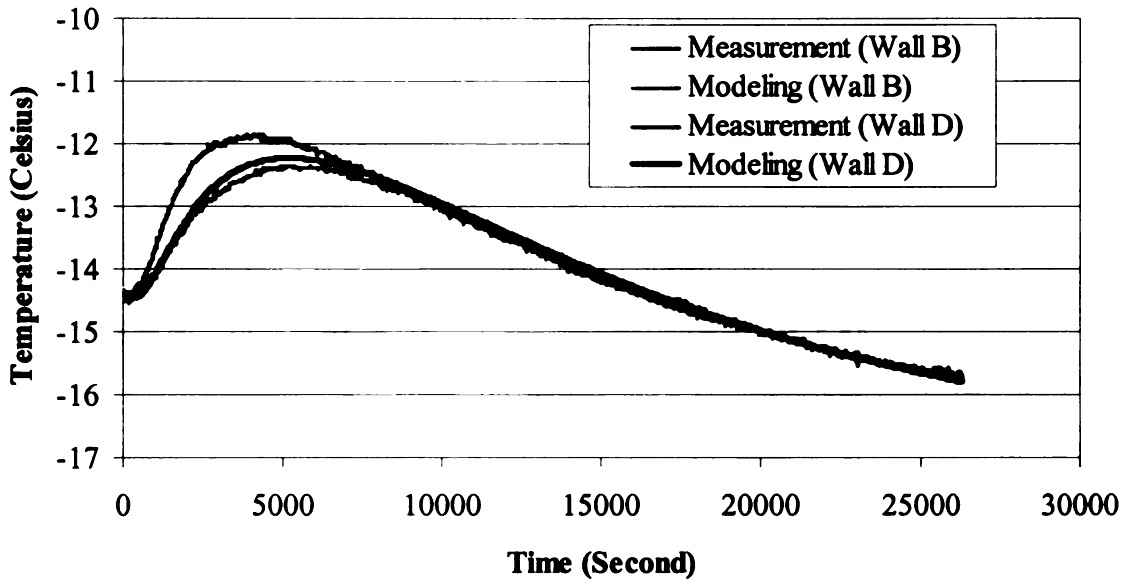


Figure 21b - Aluminum Measurement vs. Modeling during Automatic Defrost (Non Heater - z Direction)

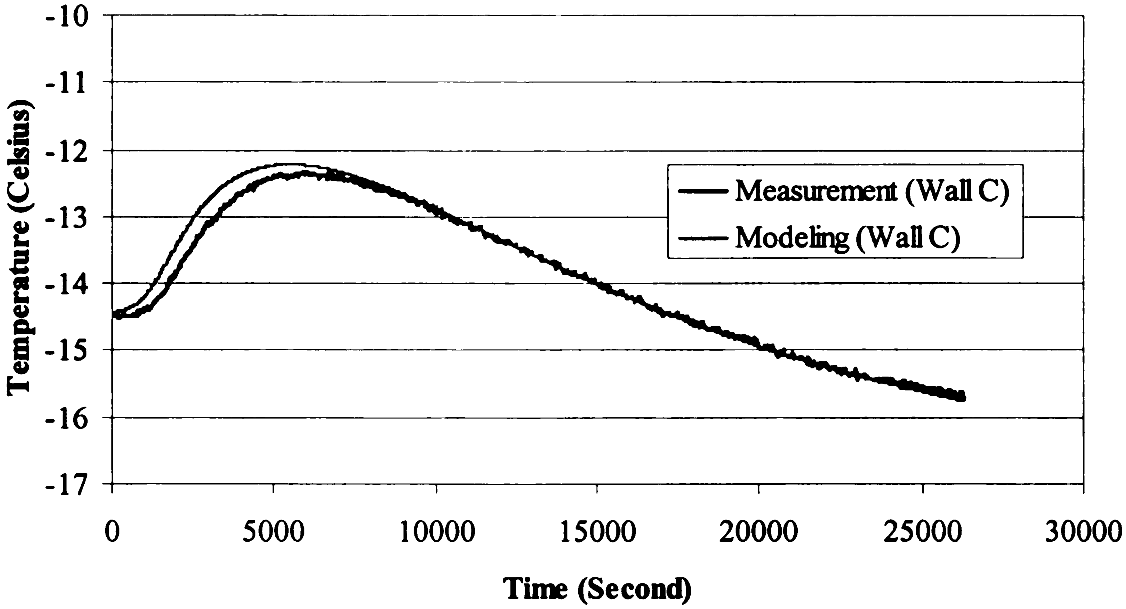


Figure 21c - Sample Measurement vs. Modeling during Automatic Defrost (Non Heater- z Direction)

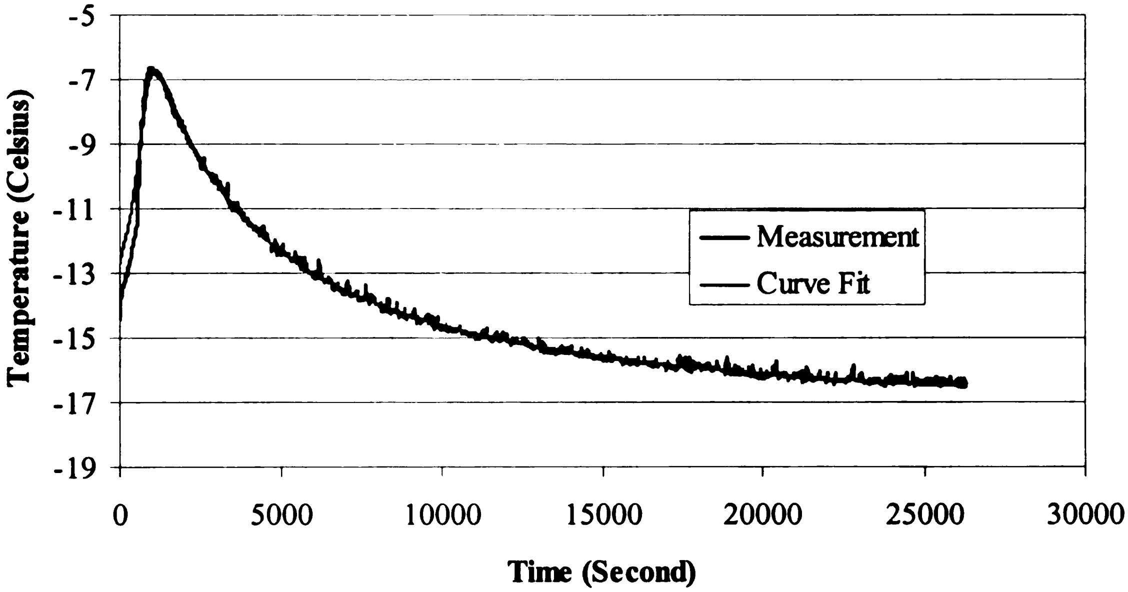


Figure 21d - Incubator Temperature during Automatic Defrost Measurement (Non Heater - z Direction)

Figure 21a shows that the temperature difference for the styrofoam between modeling results and measurement results are around 3°C at 1980 seconds and the temperature difference is become smaller as time is increasing. The temperature difference for aluminum is about 1.43°C at 4200 seconds and similar to the styrofoam, the temperature difference becomes less as time increases. As for the sample, its temperature difference is about 0.16°C at t = 500 second and the temperature difference decreases as time increases. Since the temperature difference for each material is relatively small and the line graph between the modeling results and the measurement results for each material are similar, this mathematical modeling can be used to model the box in the z-direction.

The second condition to be modeled is analyzing the change in the internal temperature of the box when opening the incubator door. The same procedure for modeling the automatic defrost condition can be used in this condition also. The only difference is the supply voltage, V_o. This supply voltage is solved using the same procedure explained for the x-direction analysis. Once the supply voltage V_o is found, then the voltages at each node of the material are determined. These node voltages represent the temperature of each material. Since the only difference is the supply voltage (V_o) or in another words, the incubator temperature, then the same composite wall and electrical circuit analogy from Figures 19 and 20 are used for the condition when the incubator door is opened. Figures 22a, 22b, and 22c show the temperature difference between the measurement and mathematical modeling results for styrofoam, aluminum, and the sample during opening the incubator door. These figures referred to the plane wall composite in Figure 19.

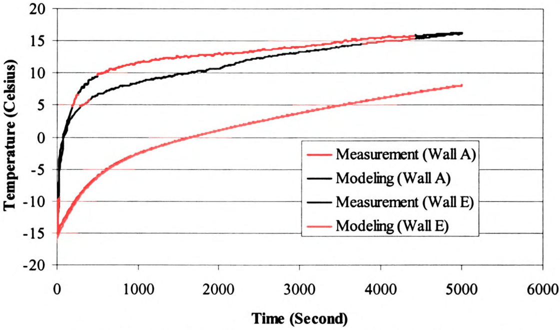


Figure 22a - Styrofoam Measurement vs. Modeling during Opening Door Incubator (Non Heater)

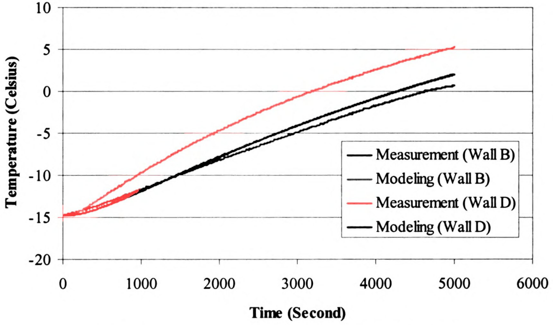


Figure 22b - Aluminum Measurement vs. Modeling during Opening Incubator Door (Non Heater)

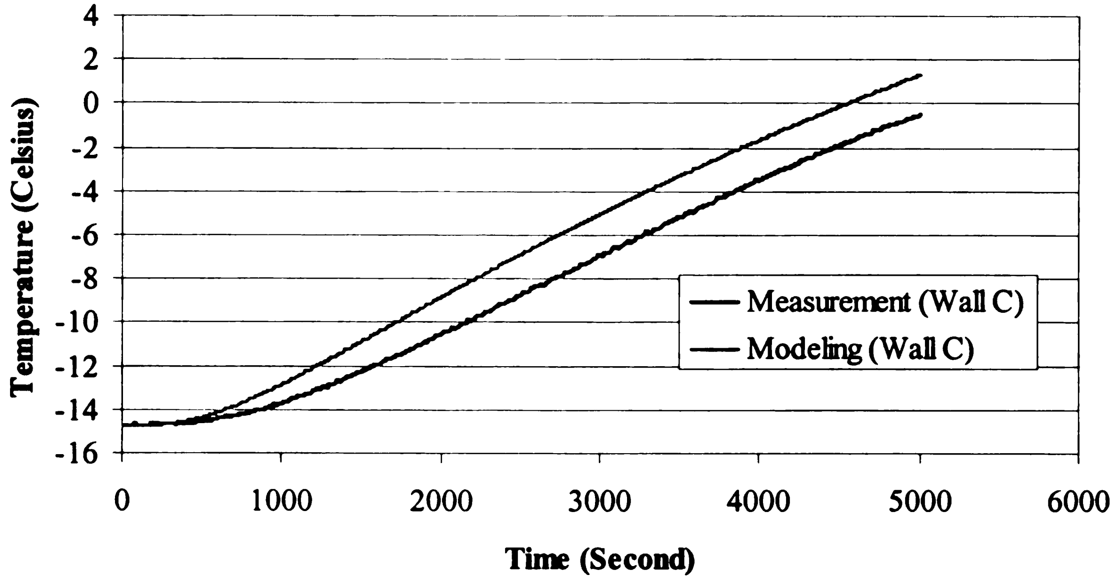


Figure 22c - Sample Measurement vs. Modeling during Opening Incubator Door (Non Heater)

In Figures 22a, 22b, and 22c the temperature difference for styrofoam, aluminum, and the sample becomes smaller as time increases. But, for this research, the temperature of the sample needs to be steady for at least 3 minutes for petri dishes and at last 5 minutes for the cyro vials when the incubator door is opened and when the incubator door and the top of the box are opened at the same time. For the styrofoam, the maximum temperature difference is about 12°C for 600 second. The maximum temperature difference for aluminum shows that the aluminum bottom is around 0.4°C and the aluminum top is around 1.5°C. As for the sample, the temperature difference is about 1°C. Looking at Figures 18b and 18c the temperature difference for the aluminum and the sample are acceptable and the graphs between the measurement and modeling results for the aluminum and the sample are similar. But, Figure 22a shows that the temperature difference between the measurement and modeling result is not acceptable for the styrofoam. It was determined that the temperature difference is large because the placement of the thermocouple while conducting the experiment is not precisely in the middle of the styrofoam wall.

The final condition is opening the incubator door and the lid of the box at the same time. Since the lid of the box is opened, therefore the layer of aluminum and the layer of styrofoam are eliminated from the electrical circuit and composite wall. Figures 23 and 24 show the composite wall and electrical circuit for this model with the layer of aluminum and styrofoam removed.

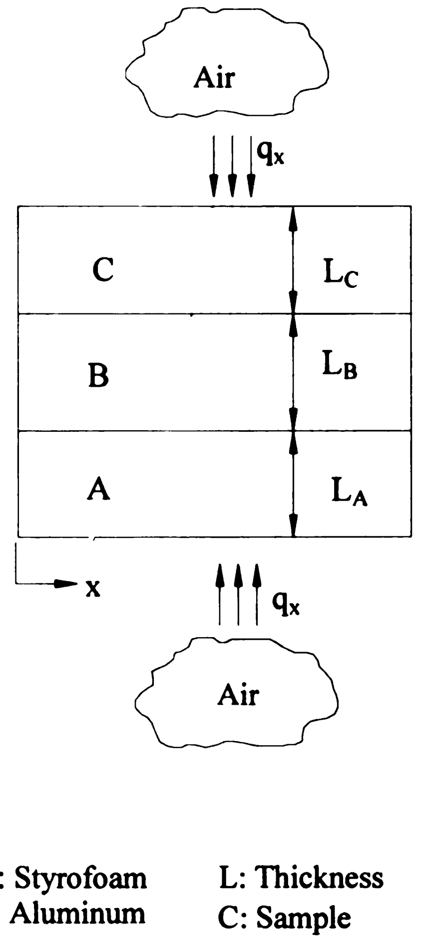


Figure 23 - Composite Wall when Top of the Box is Opened

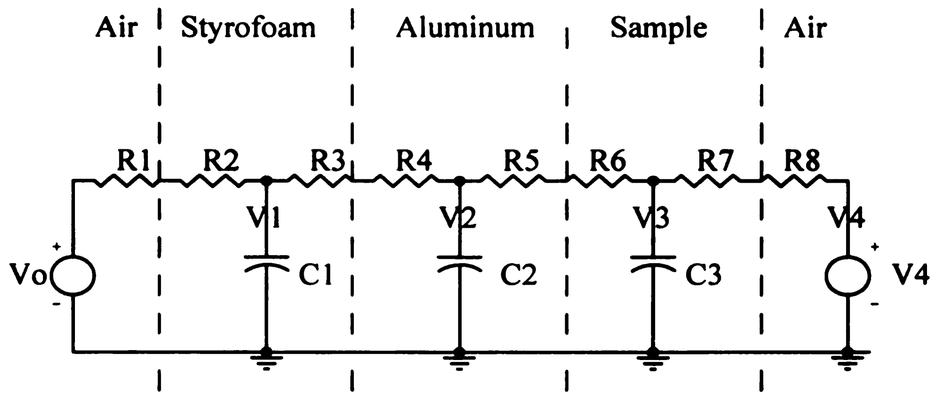


Figure 24 - Electrical Circuit when Top of the Box is Opened

The equations are described as follows:

$$\frac{V_o - V_1}{R_1 + R_2} = V_1^{'} C_1 + \frac{V_1 - V_2}{R_3 + R_4} \tag{16}$$

$$\frac{V_1 - V_2}{R_3 + R_4} = V_2^{'} C_2 + \frac{V_2 - V_3}{R_5 + R_6} \tag{17}$$

$$\frac{V_2 - V_3}{R_5 + R_6} = V_3^{'} C_3 \tag{18}$$

Where,

$V_o = V_4$ = Voltage source = Forcing function

V_1 = Styrofoam temperature/voltage

V_2 = Aluminum temperature/voltage

V_3 = Sample temperature/voltage

C_1 = Styrofoam capacitance

C_2 = Aluminum capacitance

C_3 = Sample capacitance

$R_1 = R_8 =$ Air resistance

$R_2 = R_3 =$ Styrofoam resistance

$R_4 = R_5 =$ Aluminum resistance

$R_6 = R_7 =$ Water resistance

The comparison between mathematical modeling results and temperature measurement results are shown in Figures 25a, 25b, and 25c during the opening incubator door and top of the box at the same time. These figures referred to the plane wall composite in Figure 23.

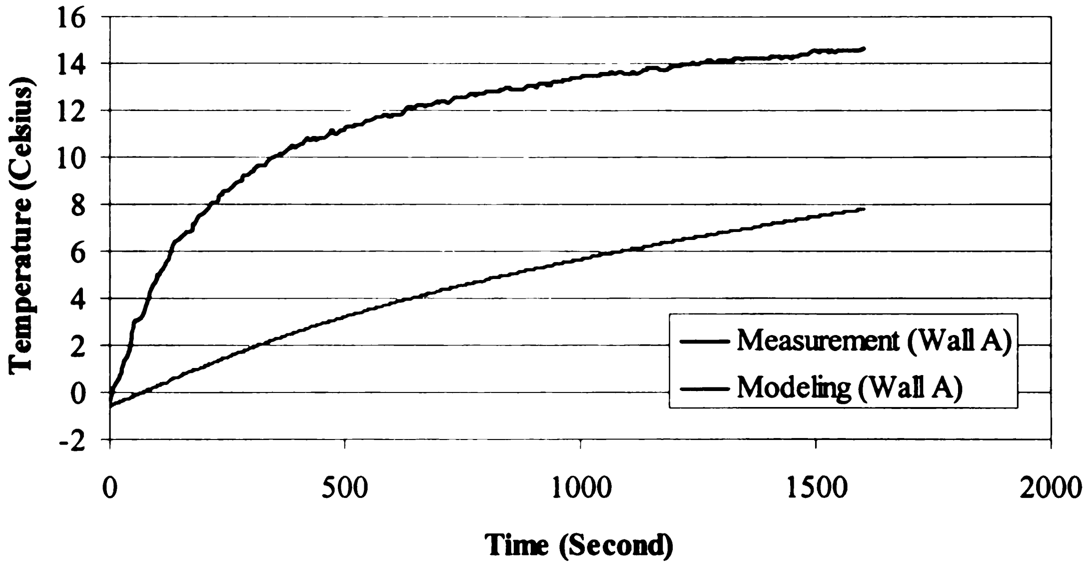


Figure 25a - Styrofoam Measurement vs. Modeling during Opening Incubator Door and Top of the Box (Non Heater)

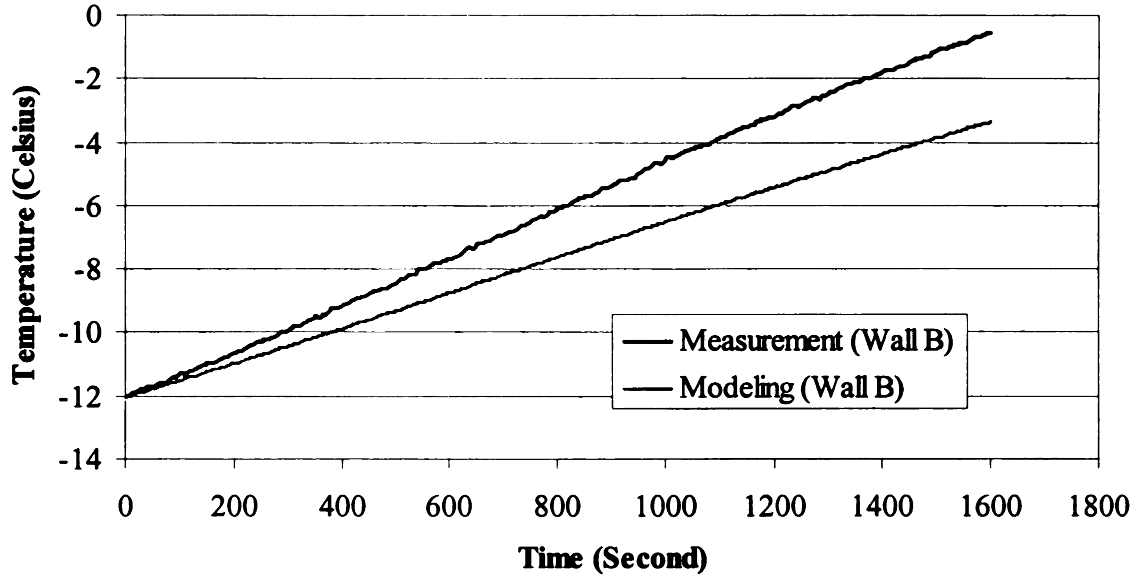


Figure 25b - Aluminum Measurement vs. Modeling during Opening Incubator Door and Top of the Box (Non Heater)

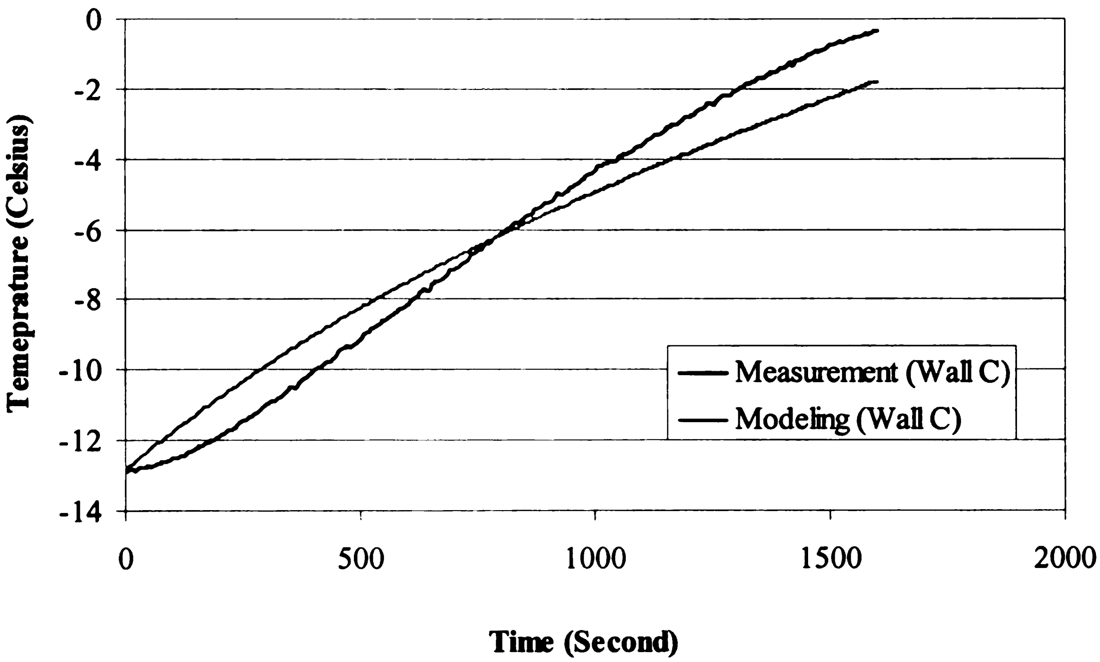


Figure 25c - Sample Measurement vs. Modeling during Opening Incubator Door and Top of the Box (Non Heater)

As can be seen, the graph for the styrofoam bottom and sample are not similar for the mathematical modeling results and measurement results. As for the aluminum bottom, the graph for the mathematical results and measurement results are similar. Looking at Figures 25a and 25b, the styrofoam and aluminum temperature difference is larger as the time increases.

The temperature difference when the automatic defrost is enabled and when the incubator door is opened may occur because the mathematical modeling describes one-dimensional heat transfer analysis instead of the three-dimensional heat transfer analysis. This means the heat transfer in the x and y-direction may affect the performance of the box but is not taken in to account since the modeling is one-dimensional using the z-direction. In addition, the error may be caused because of the location of the thermocouples in each material. The thermocouples may not be placed in the exact middle position of each material as specified by the mathematical modeling. As a result, this may cause an error in the temperature measurement results for each material. The software code of the mathematical modeling in the heat transfer in x, y, and z-direction with the conditions explained can be seen in the Appendix. The next section explains the heat transfer analogy when the temperature controller is enabled.

3.3.2 *Mathematical Modeling with Temperature Controller is Enabled*

This section explains the modeling and performance of the box while the temperature controller is enabled. As mentioned in Section 3.2, the temperature controller regulates the temperature inside the box and keeps the temperature of the samples steady. The modeling of the box with the temperature controller enabled uses the same assumptions as mentioned in the previous modeling explained in Section 3.3.1. This means the z-direction of the box is used for modeling the heat transfer analogy. While using the temperature controller, heat is generated inside the box to warm up the samples. The heater inside the box only works when the temperature of the box is colder than the set temperature. When the temperature inside the box has not yet reached the set temperature, the heater will generate the heat until the temperature reaches the set temperature. After the set temperature is reached, then the heater will turn off and stop generating heat. When the temperature inside the box is below the set temperature, the heater will turn on and generate the heat again. Thus, this temperature controller system functions as a regulated system known as "bang-bang" controller.

While modeling this temperature controller system, one issue is how to model the box when the heat is generated inside the box. In order to solve this problem, the heat transfer rate produced by the heater needs to be found and analyzed. The heat transfer rate can be found by calculating the output power of the heater. Looking at Equation (19), the value of the voltage and resistance can be found by measuring the output voltage and resistance of the controller system. The calculation of heat transfer rate is described as following:

$$P = \frac{V^2}{R} = \frac{24^2}{53} = 10.87\,Watts \tag{19}$$

After finding the heat transfer rate, a logic statement is imbedded in the mathematical modeling corresponding to how the heater works. Using an arbitrary set temperature between 0°C and -10°C, the logic statement is described as following:

$$\begin{aligned} &Q/:Q[Y\_/;Y<-6.7]=10.87; \\ &Q/:Q[Y\_/;Y\geq -6.7]=0 \end{aligned} \tag{20}$$

Where,

Y = set temperature in the aluminum

Equation (20) states that if the aluminum temperature is less than -6.7°C, then the heater will turn on and generate heat with the amount of 10.87 Watts to the inside the box. On the other hand, if the sensor temperature is greater than -6.7°C, then the heater will turn off and discontinue generating the heat. The sensor temperature monitors the temperature of the aluminum box. The next paragraph will explain the mathematical modeling when the temperature controller heater is regulating the temperature inside the box during the automatic defrost condition.

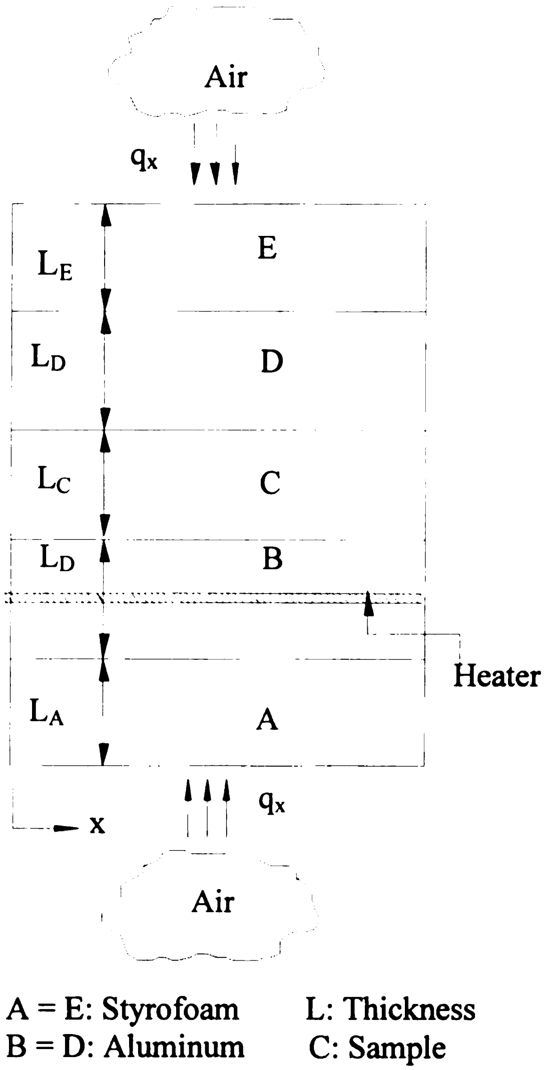


Figure 26 - Plane Wall when Temperature Controller System is Enabled

Figure 26 illustrates the composite wall for the box when the temperature controller is enabled. Figure 27 shows representation of electrical circuit for this composite wall.

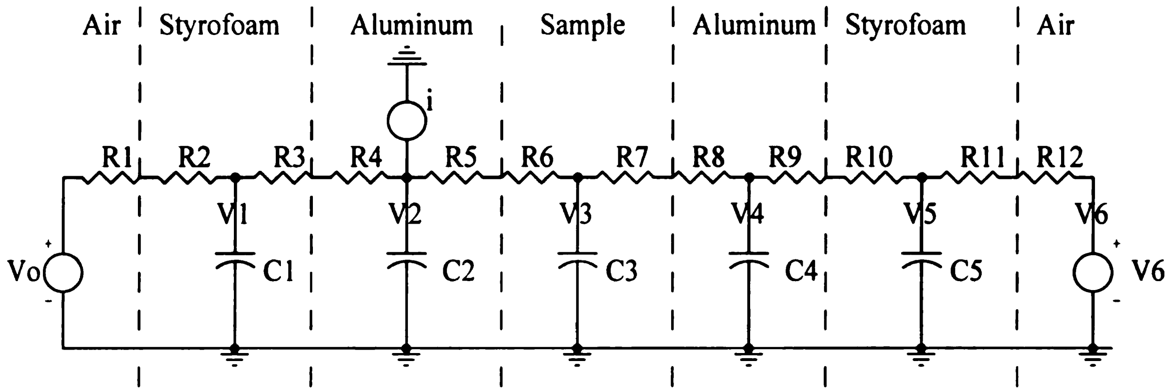


Figure 27 - Electrical Circuit Representation for the Composite Wall

From Figure 27, the heater acts as a current source instead of a voltage source since the heater generates heat through the box. In addition, the current source is put in the voltage node, V_2. This is because the plastic heater is placed underneath the aluminum. The node voltage equations for this circuit are described as:

$$\frac{V_o - V_1}{R_1 + R_2} = V_1' C_1 + \frac{V_1 - V_2}{R_3 + R_4} \tag{21}$$

$$Q(V_2) + \frac{V_1 - V_2}{R_3 + R_4} = V_2' C_2 + \frac{V_2 - V_3}{R_5 + R_6} \tag{22}$$

$$\frac{V_2 - V_3}{R_5 + R_6} = V_3' C_3 + \frac{V_3 - V_4}{R_7 + R_8} \tag{23}$$

$$\frac{V_3 - V_4}{R_7 + R_8} = V_4' C_4 + \frac{V_4 - V_5}{R_9 + R_{10}} \tag{24}$$

$$\frac{V_4 - V_5}{R_9 + R_{10}} = V_5' C_5 + \frac{V_5 - V_6}{R_{11} + R_{12}} \tag{25}$$

Where,

$V_o = V_6$ = Voltage source = Forcing function

$V_1 = V_5$ = Styrofoam temperature/voltage

$V_2 = V_4$ = Aluminum temperature/voltage

V_3 = Sample temperature/voltage

$C_1 = C_5$ = Styrofoam capacitance

$C_2 = C_4$ = Aluminum capacitance

C_3 = Sample capacitance

$R_1 = R_{12}$ = Air resistance

$R_2 = R_3 = R_{10} = R_{11}$ = Styrofoam resistance

$R_4 = R_5 = R_9 = R_8$ = Aluminum resistance

$R_6 = R_7$ = Water resistance

These equations and their logic statements for the automatic defrost condition are imbedded in the Mathematica software and are executed in order to obtain the temperature performance for each material. The comparison between the experimental results and the modeling results are shown in Figures 28a, 28b, and 28c. These figures referred to the plane wall composite in Figure 26.

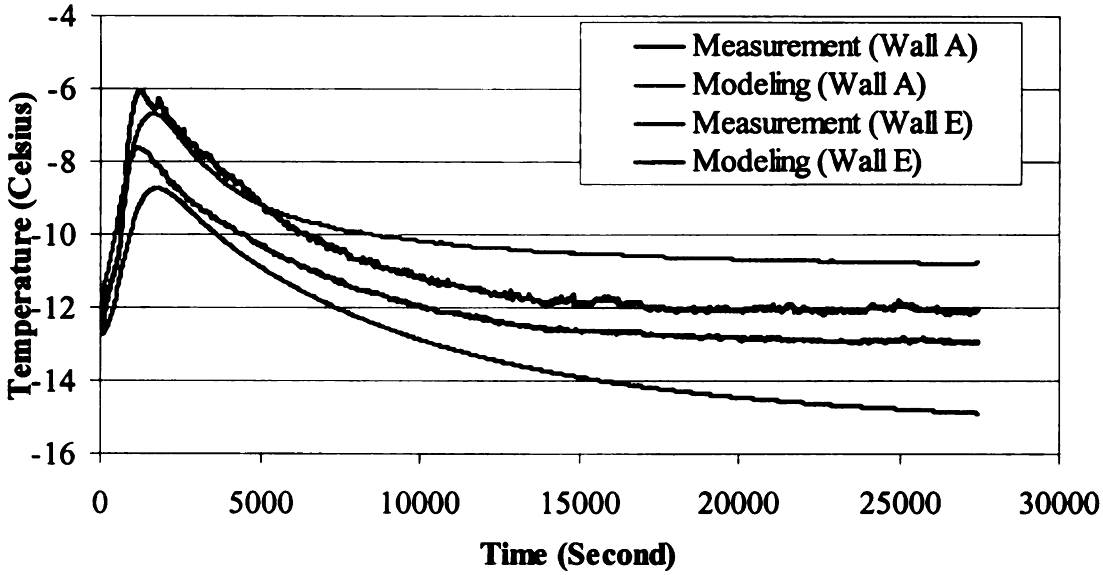


Figure 28a - Styrofoam Measurement vs. Modeling during Automatic Defrost (Heater is On)

Looking at Figure 28a, the maximum temperature difference for the styrofoam top and styrofoam bottom is approximately 1.5°C. This large temperature difference may be caused by resistance values used for each material. The calculated resistance values of the styrofoam bottom and top may be smaller or larger than the actual resistance of the material. Also, the temperature difference for the styrofoam bottom and top is larger as time increases.

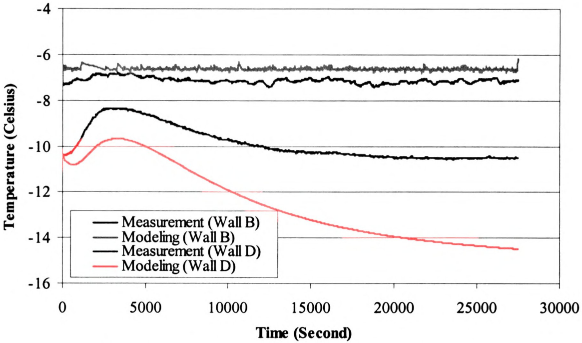


Figure 28b - Aluminum Measurement vs. Modeling during Automatic Defrost (Heater is On)

The modeling results and measurement results for aluminum bottom are represented in Figure 28b, showing that there is a small temperature difference about 0.3°C. Even though there is temperature difference for the aluminum bottom, this temperature difference is still acceptable since the temperature error is below 1°C. On the other hand, Figure 28b shows the temperature of the aluminum top between measurement results and modeling results are not similar and has a large error approximately 1.6°C. Furthermore, the longer the time, the larger the temperatue difference is. Also in Figure 28b, the error in the aluminum top may be caused by the capacitance value.

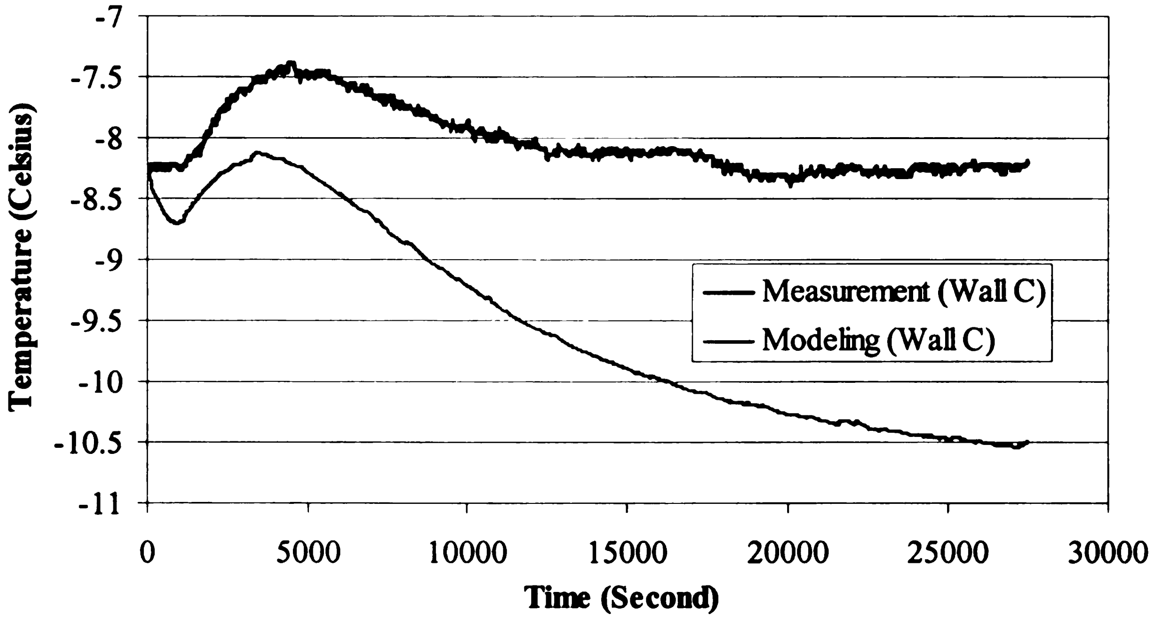


Figure 28c - Sample Measurement Vs. Modeling During Automatic Defrost (Heater is On)

For the sample temperature shown in Figure 28c, the graph between experimental results and modeling results are similar, but they still have a temperature difference approximately 1.5°C. Also, the temperature error is getting larger as the time becomes longer. This error may be caused by the resistance of the sample. Since the temperature difference for styrofoam bottom and top, aluminum top, and sample is above 1°C, this modeling is not acceptable.

The condition when opening the incubator door while the temperature controller enabled is not explained. Also, the condition for the opening incubator door and the top of the box is not explained. The reason these two conditions were not attempted is because the modeling results were not acceptable during the condition of automatic defrost condition while the temperature controller is enabled. These results are not acceptable due to the temperature difference between the modeling and the experimental results for styrofoam bottom and top, aluminum top, and sample are above 1°C.

3.3.3 *Sensitivity of Design Parameters*

The design parameters of the box consist of thermophysical properties of the materials, the heat transfer coefficients, the thickness of materials, and the area of the materials. Each of the parameters can play an important role in designing the box. Changing the values of the parameters will change the temperature performance of the box, thus affecting the temperature of the biological samples.

As for thermophysical properties, the values are different for each material. The thermophysical properties for a material are density (ρ), thermal conductivity (k), and specific heat capacity (Cp). These thermophysical properties cannot be changed to optimize the box. Since in this research, it has already been decided to use aluminum and styrofoam for the material of the box. Therefore, the thermophysical properties have a constant value and can be looked up in "Introduction to Heat Transfer (Appendix A) by Frank P. Incropera." Note that these thermophysical properties could be changed for more general optimization purpose.

A proportionality constant in Newton's Cooling Law equation is known as the heat transfer coefficient (h). This heat transfer coefficient (h) is influenced by the surface geometry and the nature of the fluid motion. Having a different surface geometry and different kind of the fluid motion nature will affect how heat diffuses in the object. From the mathematical modeling in Section 3.3.1, the value of the heat transfer coefficient (h) is not the same for each condition. For the condition when the auto defrost is enabled and when the door is opened, the heat transfer coefficient (h) is equal to 3 $W/m^2 \cdot K$. When the door and the lid of the box are opened, the heat transfer coefficient (h) is equal to 25 $W/m^2 \cdot K$. These show that the heat transfer coefficient affects the temperature performance of the box.

Other properties that have the ability to change the temperature performance of the box are adjusting the thickness and area of the materials. As shown in Figures 29 and 30, the thickness and area of the material from the composite wall can be associated with an equivalent resistor and capacitor electrical circuit.

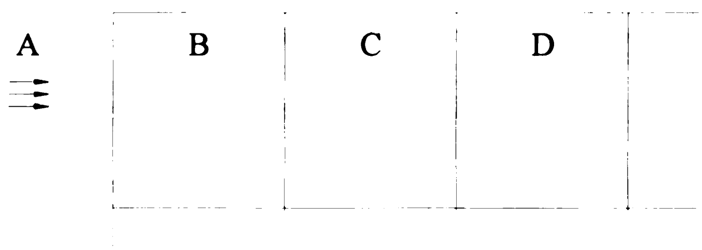


Figure 29 - Composite Wall

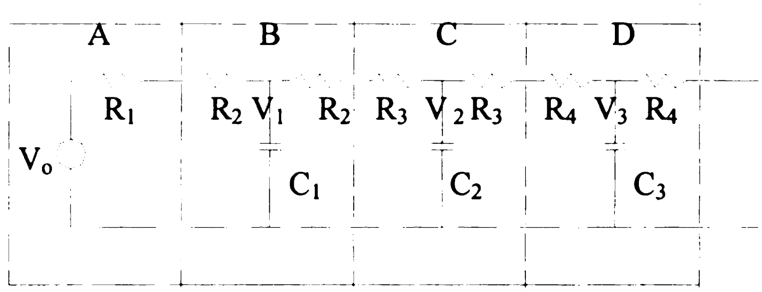


Figure 30 – Equivalent Electrical Circuit from the Composite Wall

From Figures 29 and 30 it can be seen that adjusting the thickness and area in a material would change the resistance and capacitance within the material. These resistance and capacitance values are used for the mathematical modeling. Within the mathematical modeling, adjusting the resistance and capacitance can help to improve the performance of the box. The paragraphs that follow show the relationship between the composite wall for the materials in the box and the electrical circuit showing the resistor and capacitor network for the materials in the box. Furthermore, a relationship between the electrical circuit and an equivalent thermal circuit is explained to show how heat is transferred to the sample.

For this analysis, the thickness and area of the cyro vials and petri dishes cannot be adjusted because they are obtained from the manufacturer. Furthermore, the aluminum is purchased from a manufacturer because the material is more expensive to manufacture individually. This means the thickness and area of the aluminum cannot be changed. As a result, the only change that can be made is the thickness and area of the sytorofoam or optimizing the design of the box. The changes of the thickness and area of the styrofoam would affect the styrofoam values of resistance and capacitance that are used in the mathematical modeling for this research. Figures 29 and 30 shows the composite wall for each material and how it can be shown as an equivalent electrical circuit for the mathematical modeling.

Compared to Figure 26 only half the circuit is shown for the styrofoam, aluminum, and sample. This is because the heat transferring through the other half of the circuit behaves the same due to symmetry of the box. In Figure 30, the voltage at each node represents the temperature in each material. This voltage is solved in Mathematica to determine the temperature in each material.

An easy way to understand the functionality in the electrical circuit is to explain it in terms of an equivalent thermal circuit shown in Figure 31.

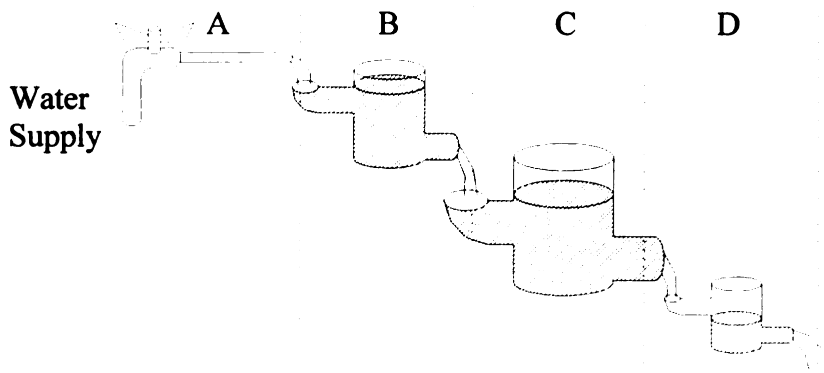


Figure 31 – Relationship between Electrical Circuit and Thermal Circuit (1)

The flow of water in Figure 31 represents the current flowing in the electrical circuit of Figure 30. The size of the pipes in the thermal circuit represents the resistance in the electrical circuit. Likewise, the size of the containers in the thermal circuit represents the capacitance in then electrical circuit. Adjusting the size of the thermal circuit affects the flow of water just like RC values affect the current in the electrical circuit. Figure 32 shows the result of adjusting the parameters of the styrofoam.

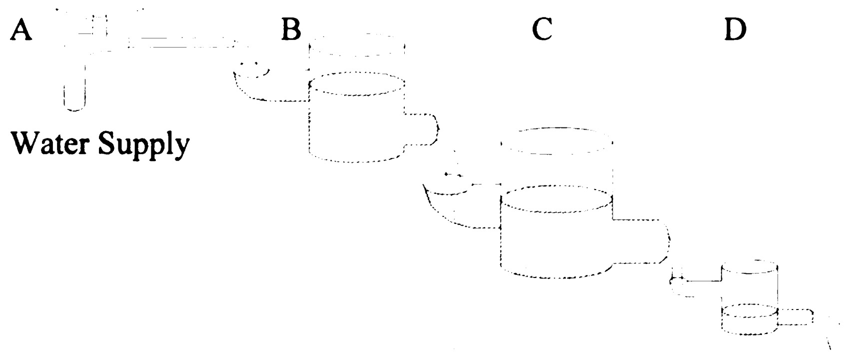


Figure 32 – Relationship between Electrical Circuit and Thermal Circuit (2)

Where,

A = Air

B = Styrofoam

C = Aluminum

D = Sample

R_1= Air Resistance = Pipe A Diameter

R_2= Styrofoam Resistance = Pipe B Diameter

R_3= Aluminum Resistance =Pipe C Diameter

R_4 = Sample Resistance = Pipe D Diameter

C_1= Styrofoam Capacitance = Water Tank B

C_2 = Aluminum Capacitance = Water Tank C

C_3 = Sample Capacitance = Water Tank D

V_o = Voltage Source = Water Supply

V_1 = Voltage Source = Water level B

V_2 = Voltage Source = Water level C

V_3 = Voltage Source = Water level D

Figure 32 shows the thickness of the styrofoam was made larger. Enlarging the thickness of styrofoam means the resistance and capacitance in the material is increased. If the resistance is increased, then the diameter of the pipes for the styrofoam in Figure 28 are decreased. This means there is more resistance in the flow of the water and it will take more time for the styrofoam container to be filled with water. Likewise, if the capacitance is increased then the volume of the container is increased so that it can store more water. As a result, Figure 32 shows the effect on the sample when increasing the thickness of the styrofoam. It shows that it takes more time for the water to reach the sample. This same analogy represents the heat transfer to the sample. By increasing the thickness of the styrofoam, the heat is diffused by the time it reaches the sample.

3.4 Design Optimization

As mentioned in Section 3.3.3, the design parameters that can affect the design of the box are the area and the thickness of each material. The area and the thickness of the aluminum box will be kept the same because it is custom made. As for the styrofoam box, the area and the thickness of the styrofoam are also kept the same. The reason is because of the limitation in the incubator space. There are twelve boxes that need to fit inside the incubator. Making the area and thickness of the styrofoam larger would mean the boxes may not fit inside the incubator. The position of the cyro vials inside the box can be changed to improve the temperature inside the box. According to heat transfer theory, the larger the area of the material, the more efficient the heat is transferred through the material. Due to using the z-direction for the heat transfer analogy, the cyro vials are inserted horizontally inside the box. By inserting the vials horizontally in the box, the vials will have a larger area for heat to transfer, meaning the heat from the plastic heater would diffuse faster through sample.

Adding a styrofoam lid to the samples is another way to improve the temperature performance inside the box. This is because cold air is flowing into the top and the bottom of the box (z-direction). By having a styrofoam cover on top of the samples, then the samples are insulated from the cold air moving into the top of the box. The cold air moving into the bottom of the box can be neglected since there is a plastic heater below the samples. Figure 33 shows the design of the box with the samples, styrofoam sample holder and styrofoam cover.

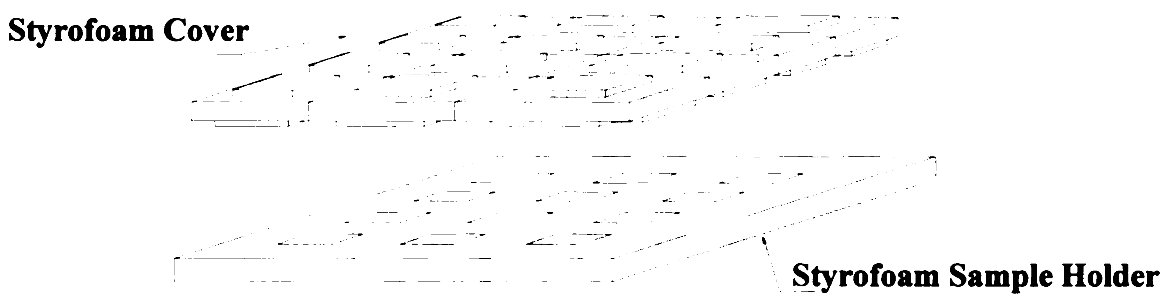
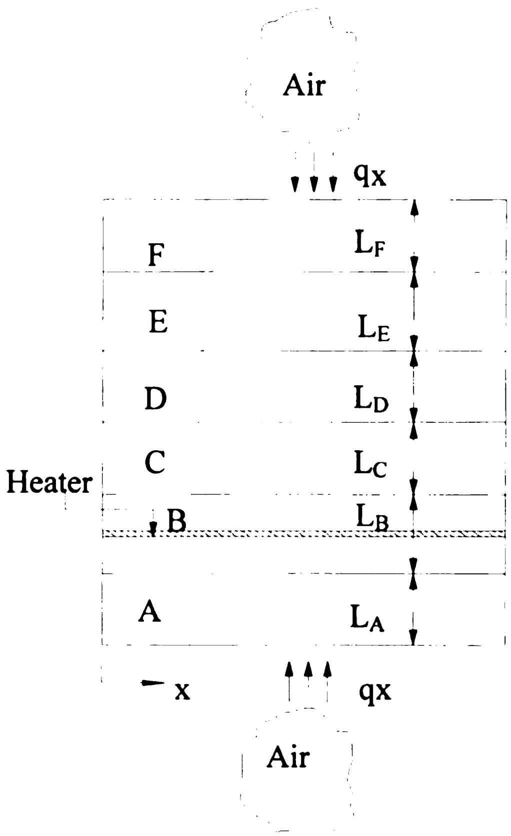


Figure 33 - Styrofoam Sample Holder and Cover

After the design of the styrofoam cover is finished, it is then modeled using the automatic defrost condition while the temperature controller is enabled. As can be seen in Figure 34, the sytrofoam cover is added to the composite wall.



A = G: Styrofoam
B = E: Aluminum
C : Sample
D : Styrofoam Cover
L : Thickness

Figure 34 - Composite Wall for the Condition when Automatic Defrost Enabled

The electrical circuit for Figure 34 is depicted as:

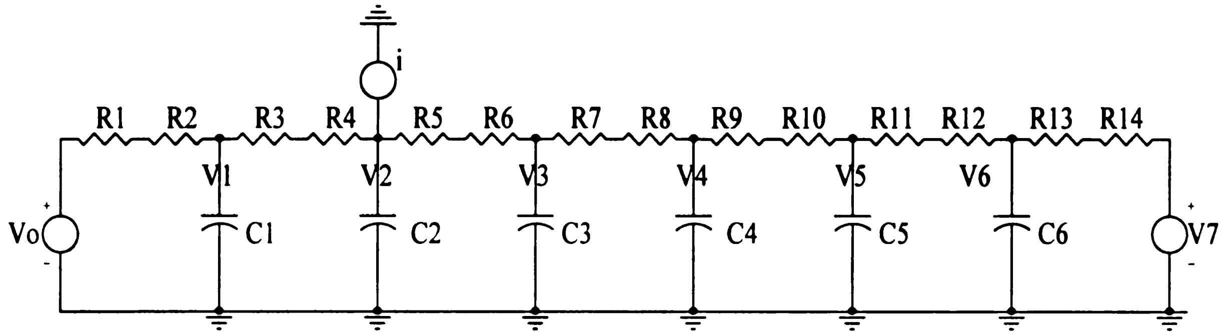


Figure 35 - Electrical Circuit for Condition when Automatic Defrost is Enabled

The equation for this electrical circuit is described as:

$$\frac{V_o - V_1}{R_1 + R_2} = V_1^{'} C_1 + \frac{V_1 - V_2}{R_3 + R_4} \tag{26}$$

$$Q(V_2) + \frac{V_1 - V_2}{R_3 + R_4} = V_2^{'} C_2 + \frac{V_2 - V_3}{R_5 + R_6} \tag{27}$$

$$\frac{V_2 - V_3}{R_5 + R_6} = V_3^{'} C_3 + \frac{V_3 - V_4}{R_7 + R_8} \tag{28}$$

$$\frac{V_3 - V_4}{R_7 + R_8} = V_4^{'} C_4 + \frac{V_4 - V_5}{R_9 + R_{10}} \tag{29}$$

$$\frac{V_4 - V_5}{R_9 + R_{10}} = V_5^{'} C_5 + \frac{V_5 - V_6}{R_{11} + R_{12}} \tag{30}$$

$$\frac{V_5 - V_6}{R_{11} + R_{12}} = V_6^{'} C_6 + \frac{V_6 - V_7}{R_{13} + R_{14}} \tag{31}$$

Where,

$V_o = V_7$ = Voltage source = Forcing function

$V_1 = V_6$ = Styrofoam temperature/voltage

$V_2 = V_5$ = Aluminum temperature/voltage

V_3 = Sample temperature/voltage

V_4 = Styrofoam cover temperature/voltage

$C_1 = C_6$ = Styrofoam capacitance

$C_2 = C_5$ = Aluminum capacitance

C_3 = Sample capacitance

C_4 = Styrofoam cover capacitance

$R_1 = R_{14}$ = Air resistance

$R_2 = R_3 = R_{12} = R_{13}$ = Styrofoam resistance

$R_4 = R_5 = R_{10} = R_{11}$ = Aluminum resistance

$R_6 = R_7$ = Water resistance

$R_8 = R_9$ = Styrofoam cover resistance

Figure 36 shows the comparison between the sample temperature using the modeling results from the old box and the new optimized box. As mentioned in the opening paragraph of Section 3.4, the old box did not use a lid for the styrofoam sample holder and the samples were inserted vertically inside the old box.

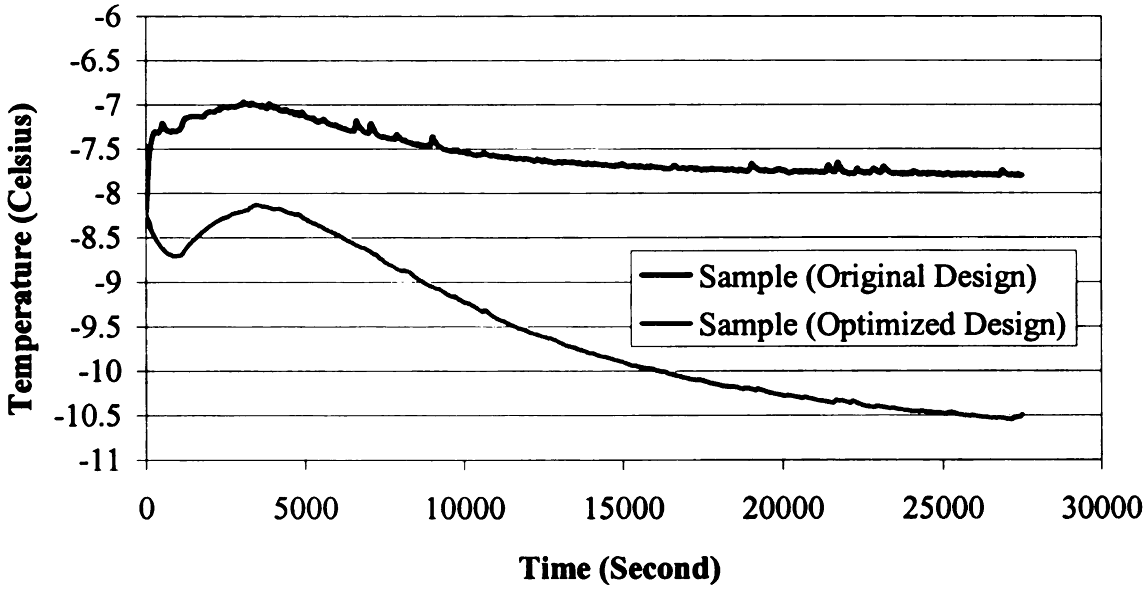


Figure 36 - Sample Modeling using Original Box vs. Modeling using Optimized Design during Automatic Defrost (Temperature Controller Enabled)

From this comparison, the new design provides an improved temperature regulation compared to the old design. But, the modeling is still not accurate for the automatic defrost condition. Since the modeling of the auto defrost condition is still not accurate, the optimization for the condition when the incubator door is opened is not considered. Also, the optimization when the incubator door and lid of the box is opened is not considered. Even though the mathematical modeling cannot be used as a tool to optimize the design of the box, this modeling is still useful for studying how heat is flowing inside the box. This study can be used as a foundation to achieve a better design for the box.

Chapter 4

RESULTS AND DISCUSSION

Twelve boxes were fabricated for the purpose of mantaining biological samples in a regulated low temperature environment. These boxes were tested during an automatic defrost condition, opening the incubator door, and opening the incubator door and lid of the box at the same time. The results for the twelve boxes are given to show that the boxes can fulfill the temperature requirements during these conditions. These temperature requirements are explained in Chapter 2. The results are given in the next paragraphs.

The first requirement defined by the Center for Microbial Ecology is that the sample shall be regulated with a set temperature between 0°C to -10°C. The Center for Microbial Ecology requested that the boxes 1-3 be tested with the petri dishes and boxes 4-12 be tested with the cyro vials. Tables 2 and 3 show the set temperature the boxes 1-12 are tested.

Table 2 - Set Temperature for Box 1, Box 2, and Box 3 (Petri Dishes)

# of the Box	Set Temperature (Celsius)
Box 1	-1.1
Box 2	-5
Box 3	-9.4

Table 3 - Set Temperature for Box 4 - Box 12 (Cyro Vials)

# of the Box	Set Temperature (Celsius)
Box 4	-1.1
Box 5	-5
Box 6	-9.4
Box 7	-3
Box 8	-7
Box 9	-9
Box 10	-4
Box 11	-6
Box 12	-8

The results of the set temperature can be compared with the sample temperature to verify the temperature regulation within the box. If the sample temperature is not within ±1°C, then a new calibration equation is generated. The comparison results between the sample temperature and set temperature are shown in Figures 37 – 48. The sample temperature was taken using thermocouples to measure the temperature in the left corner, middle, and right corner of the boxes. Figures 37-39 show the comparison results between the sample temperature and set temperature within the petri dishes for boxes 1-3 during an automatic defrost condition.

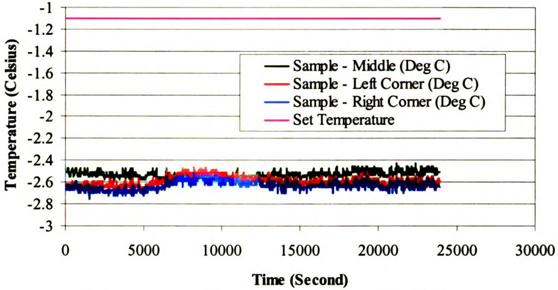


Figure 37 - Automatic Defrost Condition with Set Temperature inside the Box at -1.1 Deg C (Box 1)

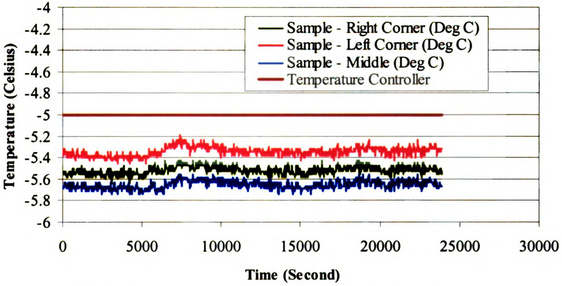


Figure 38 - Automatic Defrost Condition with Set Temperature inside the Box at -5 Deg C (Box 2)

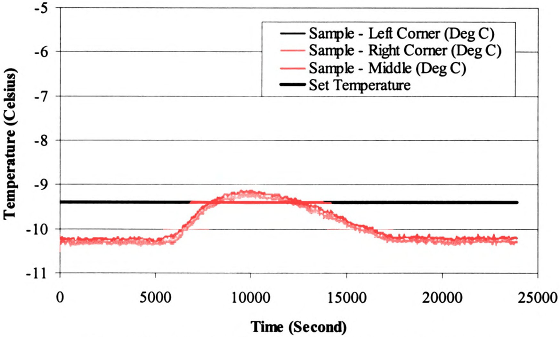


Figure 39 - Automatic Defrost Condition with Set Temperature inside the Box at -9.4 Deg C (Box 3)

Figures 40-48 illustrate the sample temperature performance in the cyro vials for boxes 4-12 during an auto defrost condition of the samples in the left corner, middle, and right corner of the box.

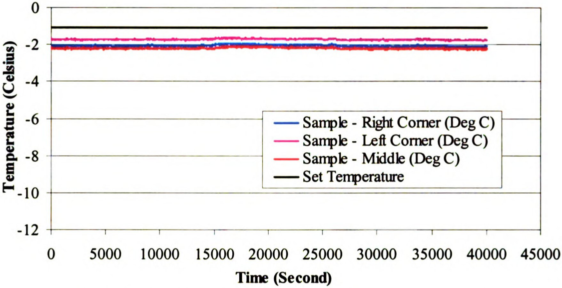


Figure 40 - Automatic Defrost Condition with Set Temperature inside the Box at -1.1 Deg C (Box 4)

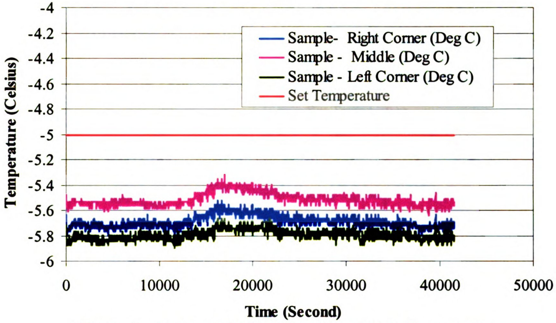


Figure 41 - Automatic Defrost Condition with Set Temperature inside the Box at -5 Deg C (Box 5)

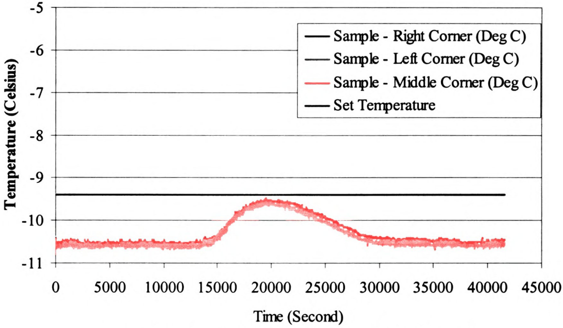


Figure 42 - Automatic Defrost Condition with Set Temperature inside the Box at -9.4 Deg C (Box 6)

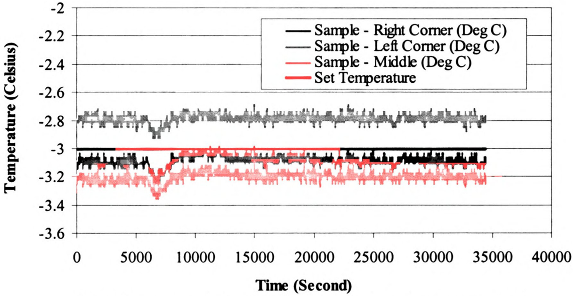


Figure 43 - Automatic Defrost Condition with Set Temperature inside the Box at -3 Deg C (Box 7)

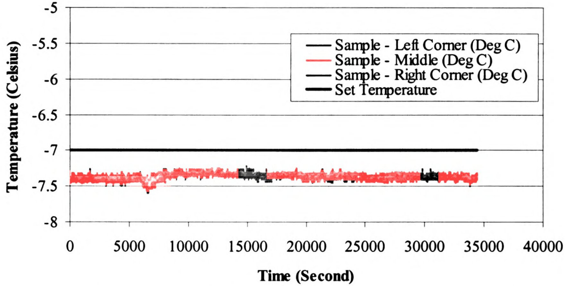


Figure 44 - Automatic Defrost Condition with Set Temperature inside the Box at -7 Deg C (Box 8)

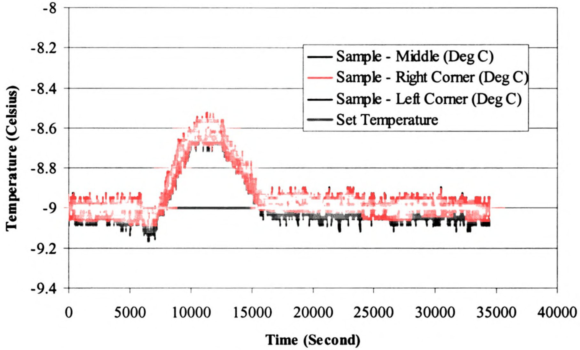


Figure 45 - Automatic Defrost Condition with Set Temperature inside the Box at -9 Deg C (Box 9)

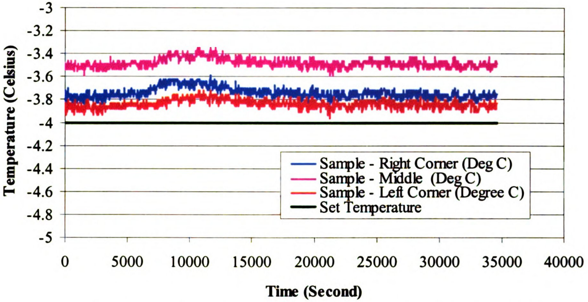


Figure 46 - Automatic Defrost Condition with Set Temperature inside the Box at -4 Deg C (Box 10)

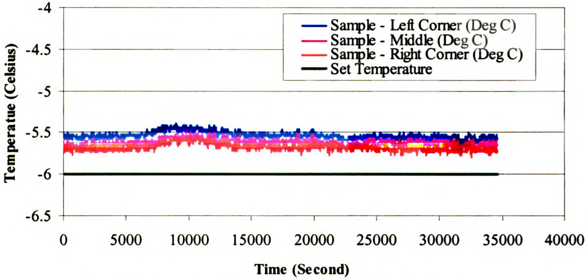


Figure 47 - Automatic Defrost Condition with Set Temperature inside the Box at -6 Deg C (Box 11)

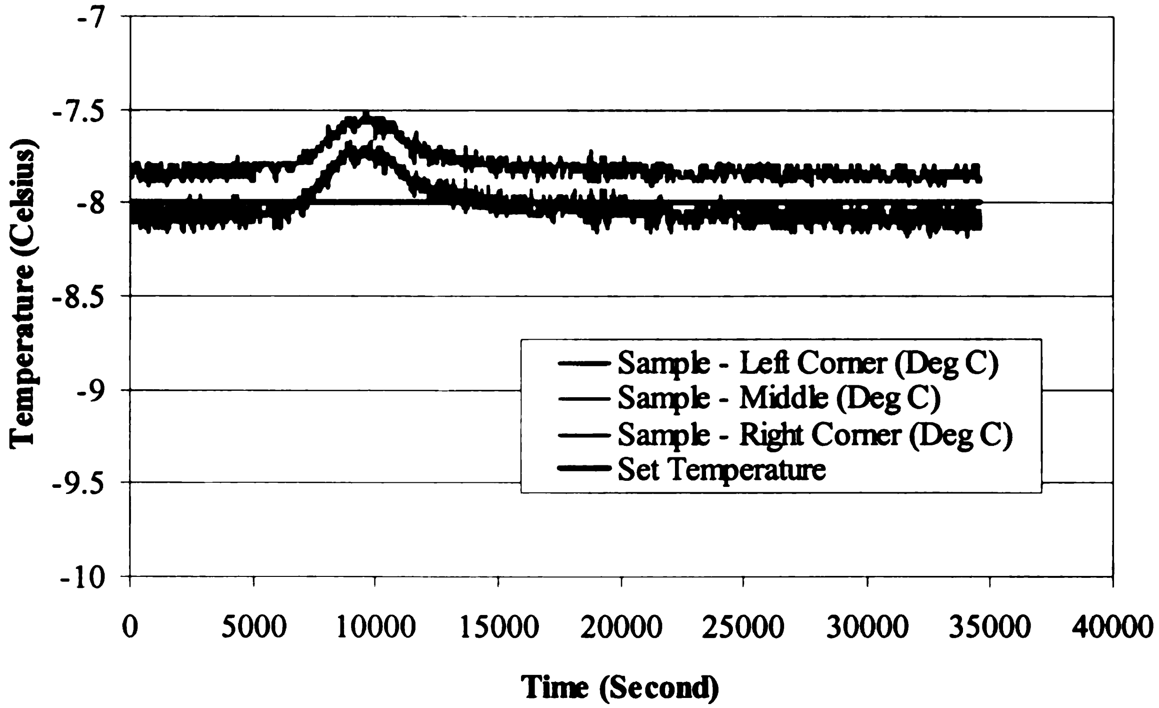


Figure 48 - Automatic Defrost Condition with Set Temperature inside the Box at -8 Deg C (Box 12)

Looking at Figures 37 – 48, the temperature difference between the sample temperature and the set temperature for some of the boxes is greater than 1°C. This temperature difference does not meet the requirements of ±1°C temperature difference explained in Chapter 2. As a result, a new calibration equation was generated to obtain a closer result between the sample temperature and set temperature. Tables 4 and 5 show which boxes did not meet the requirement for the ±1°C temperature difference between the sample and set temperature.

Table 4 – Temp Difference between Set and Sample Temperature (Petri Dishes)

# of Box	Set Temperature (Celsius)	Actual Sample Temperature (Celsius)					
		Left corner	Temp diff	middle	Temp Diff	right corner	Temp Diff
Box 1	-1.1	-2.63	1.53	-2.52	1.42	-2.66	1.56
Box 2	-5	-5.38	0.38	-5.67	0.67	-5.54	0.54
Box 3	-9.4	-10.24	0.84	-10.32	0.92	-10.31	0.91

Table 5 - Temp Difference between Sample and Set Temperature (Cyro Vials)

# of Box	Set Temp (Celsius)	Actual Sample Temperature (Celsius)					
		left corner	Temp Diff	middle	Temp Diff	right corner	Temp Diff
Box 4	-1.1	-1.74	0.64	-2.22	1.12	-2.08	0.98
Box 5	-5	-5.82	0.82	-5.54	0.54	-5.72	0.72
Box 6	-9.4	-10.56	1.16	-10.60	1.20	-10.49	1.09
Box 7	-3	-2.79	0.21	-3.22	0.22	-3.09	0.09
Box 8	-7	-7.37	0.37	-7.40	0.40	-7.42	0.42
Box 9	-9	-9.05	0.05	-8.98	0.02	-9.00	0.00
Box 10	-4	-3.87	0.13	-3.51	0.49	-3.78	0.22
Box 11	-6	-5.55	0.45	-5.64	0.36	-5.69	0.31
Box 12	-8	-7.84	0.16	-8.04	0.04	-8.09	0.09

Tables 4 and 5 show that the highest temperature difference is more than 1.56°C. This temperature difference occurs because the voltage sensor providing feedback to the temperature controller was placed on the bottom of aluminum box instead of inside the sample. As a result, a new calibration equation needs to be obtained in order for the sample temperature to be close to the set temperature within 1°C. In order to reduce this temperature difference, the sample temperature in Table 4 and Table 5 is plotted versus the reference voltage. From this plot, the new calibration equation is determined. This new calibration equation can be seen in Figure 49.

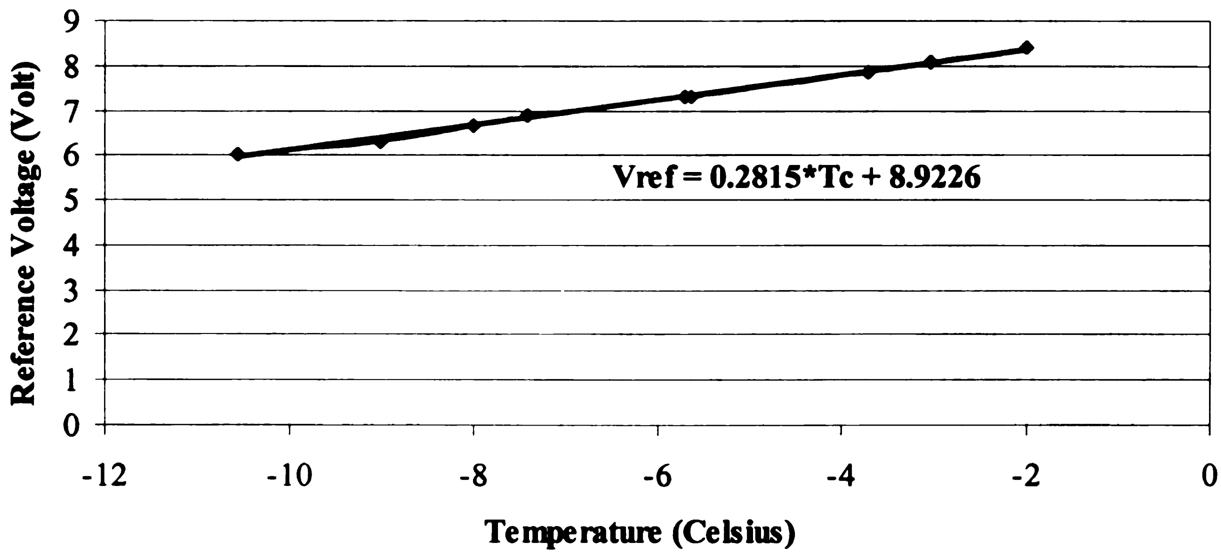


Figure 49 - New Voltage Reference Equation

Then this new calibration equation is used to reduce the temperature difference between the sample temperature and set temperature. The new calibration equation is tested on boxes 10-12 during the automatic defrost condition. Due to time constraints, the other nine boxes could not be tested. Figures 50 – 52 show the sample temperature and set temperature is below 1°C using the calibration equation.

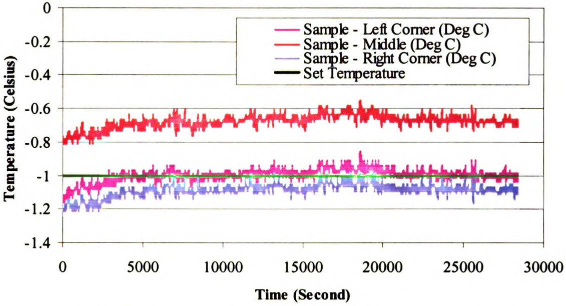


Figure 50- Automatic Defrost Condition with Set Temperature inside the Box at -1 Deg C (Box 10)

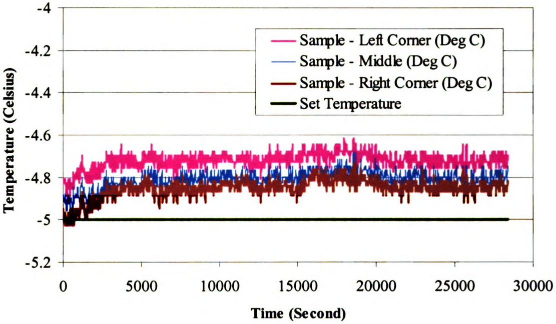


Figure 51 - Automatic Defrost Condition with Set Temperature inside the Box at -5 Deg C (Box 11)

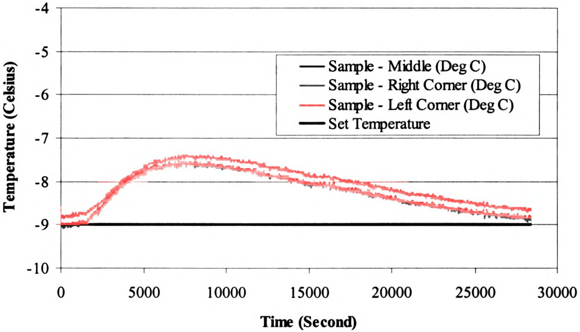


Figure 52 - Automatic Defrost Condition with Set Temperature inside the Box at -9 Deg C (Box 12)

Table 6 shows the temperature difference between the sample temperature and the set temperature for boxes 10-12 using the new calibration equation. Looking at Table 6, the new calibration equation is able to reduce the temperature difference between the sample temperature and the set temperature below 1°C.

Table 6 - Temp Difference between Sample and Set Temperature Using New Calibration Equation

# of Box	Set Temperature (Celsius)	Actual Sample Temperature (Celsius)					
		left corner	Temp diff	middle	Temp Diff	right corner	Temp Diff
Box 10	-1	-1.19	0.19	-0.79	0.21	-1.13	0.13
Box 11	-5	-4.84	0.16	-4.91	0.09	-4.99	0.01
Box 12	-9	-9	0	-9.04	0.04	-8.81	0.19

Next, the uniformity of sample temperature of each box is observed. Figures 37, 38, and 39 illustrate the sample temperature in the petri dishes for the right corner of the box, left corner of the box, and middle of the box. Also, Figures 40, 41, 42, 43, 44, 45, 46, 47, and 48 shows the uniformity for the sample temperature in cyro vials for the right corner of the box, left corner of the box, and middle of the box are close within each other. Table 7 shows the temperature difference between the samples at different positions of the box.

Table 7 - Temperature Error for the Sample at Different Locations in the Box

Petri Dishes						
	Actual Temperature			Temperature Difference		
# of Box	Left Corner	Middle	Right Corner	Left-Right	Left-Middle	Middle - Right
Box 1	-2.63	-2.52	-2.66	0.04	0.11	0.15
Box 2	-5.38	-5.67	-5.54	0.17	0.3	0.13
Box 3	-10.24	-10.32	-10.31	0.08	0.08	0.01
Cyro Vials						
	Actual Temperature			Temperature Difference		
# of Box	Left Corner	Middle	Right Corner	Left-Right	Left-Middle	Middle - Right
Box 4	-1.74	-2.22	-2.08	0.34	0.48	0.14
Box 5	-5.82	-5.54	-5.72	0.1	0.28	0.18
Box 6	-10.56	-10.6	-10.49	0.07	0.04	0.11
Box 7	-2.79	-3.22	-3.09	0.3	0.43	0.13
Box 8	-7.37	-7.4	-7.42	0.05	0.03	0.02
Box 9	-9.05	-8.98	-9	0.05	0.07	0.01
Box 10	-3.87	-3.51	-3.78	0.09	0.36	0.27
Box 11	-5.55	-5.64	-5.69	0.15	0.1	0.05
Box 12	-7.84	-8.04	-8.09	0.25	0.2	0.05

Looking at Table 7, the maximum temperature difference is less than 0.48°C, which is very small. As a result, it can be concluded that the temperature of sample is uniform throughout the box.

After observing the uniformity of the sample temperature in the box, the stability of the sample temperature when the incubator door is opened is observed. Figures 53-55 show the sample temperature in the petri dishes when the incubator door is opened.

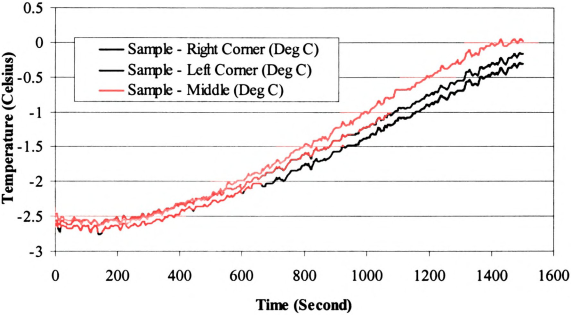


Figure 53 - Opening Incubator Door Condition with Set Temperature inside the Box at -1.1 Deg C (Box 1)

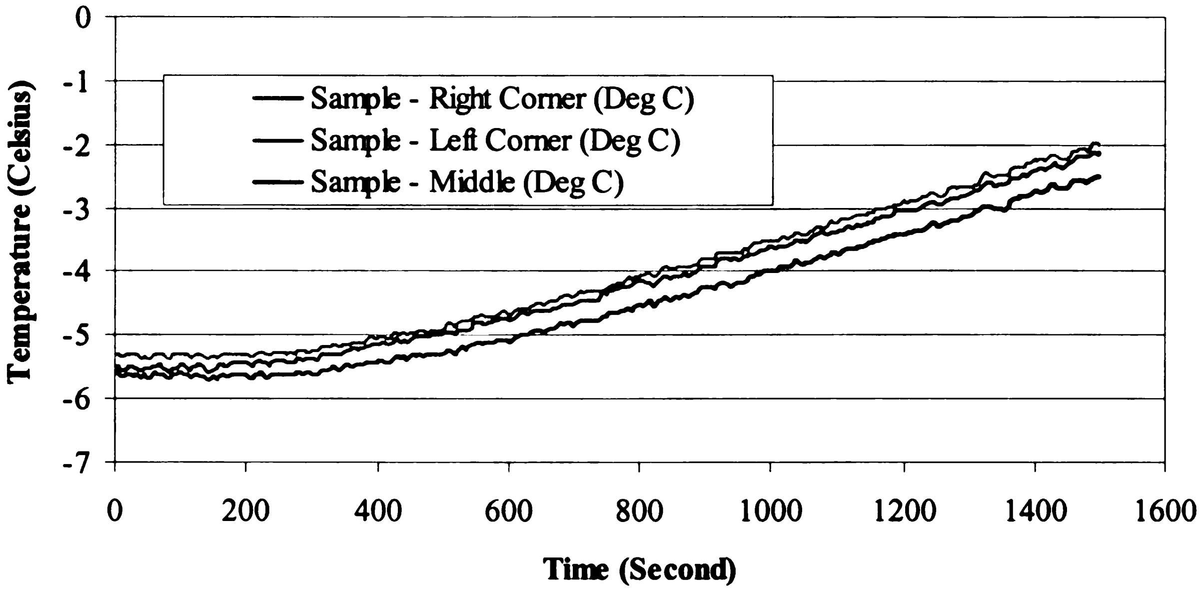


Figure 54 - Opening Incubator Door Condition with Set Temperature inside the Box at -5 Deg C (Box 2)

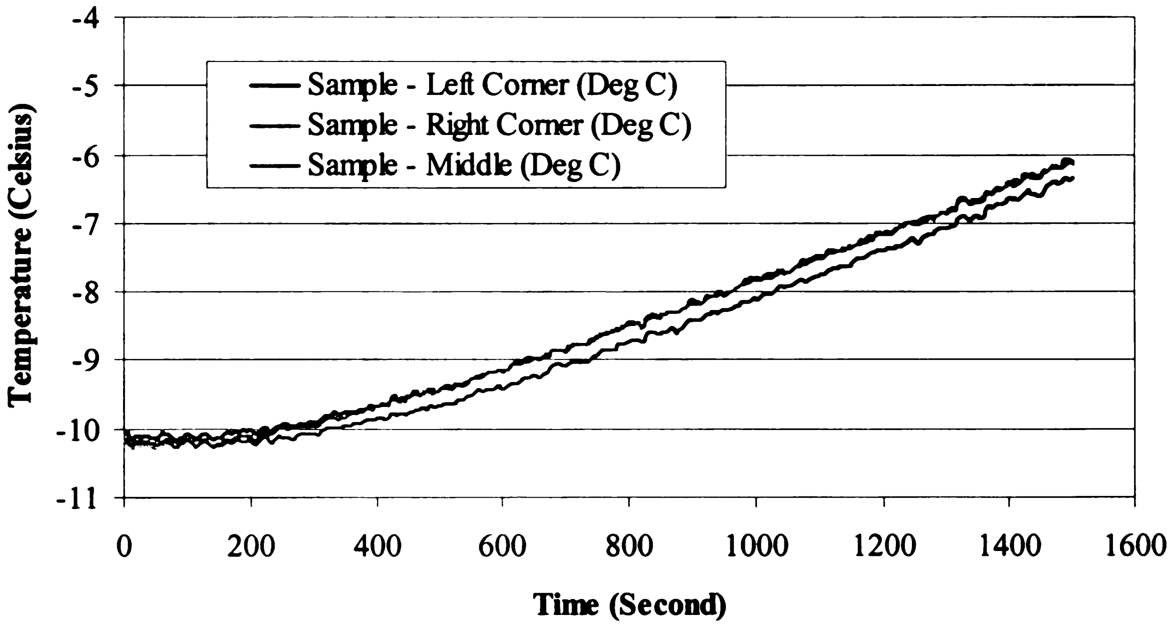


Figure 55 - Opening Incubator Door Condition with Set Temperature inside the Box at -9.4 Deg C (Box 3)

Figures 56-64 show the sample temperature in the cyro vials when incubator door is opened.

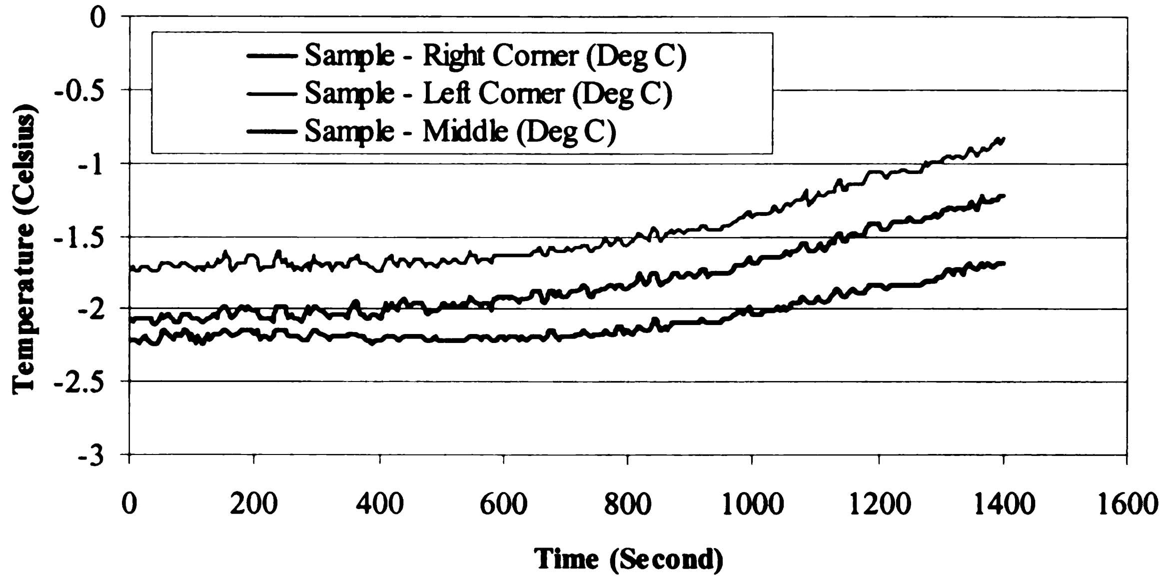


Figure 56 - Opening Incubator Door Condition with Set Temperature inside the Box at -1.1 Deg C (Box 4)

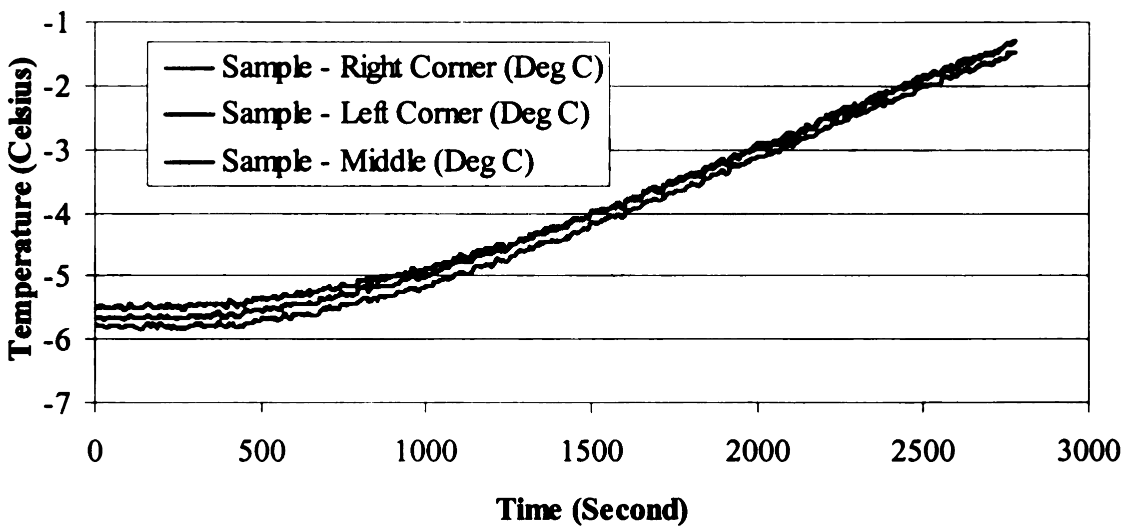


Figure 57 - Opening Incubator Door Condition with Set Temperature inside the Box at -5 Deg C (Box 5)

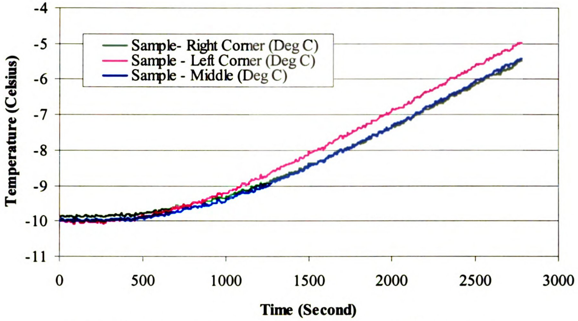


Figure 58 - Opening Incubator Door Condition with Set Temperature inside the Box at -9.4 Deg C (Box 6)

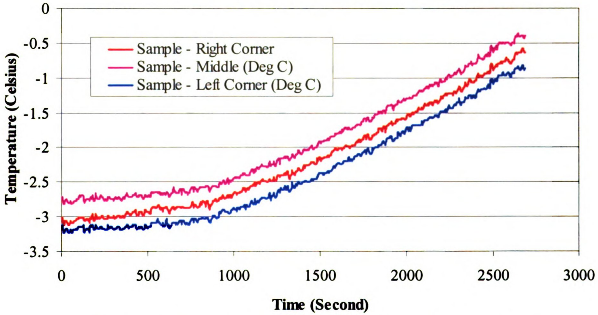


Figure 59 - Opening Incubator Door Condition with Set Temperature inside the Box at -3 Deg C (Box 7)

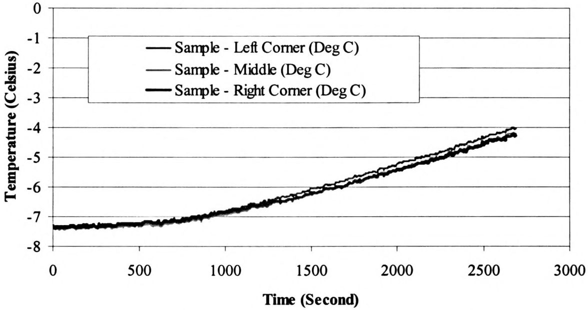


Figure 60 - Opening Incubator Door Condition with Set Temperature inside the Box at -7 Deg C (Box 8)

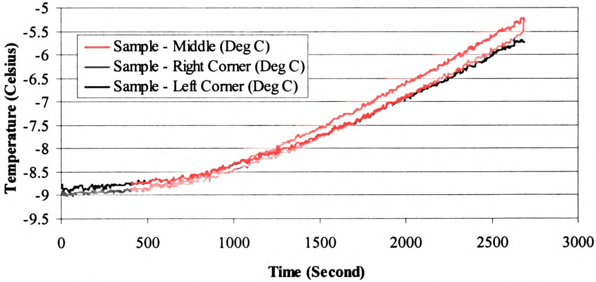


Figure 61 - Opening Incubator Door Condition with Set Temperature inside the Box at -9 Deg C (Box 9)

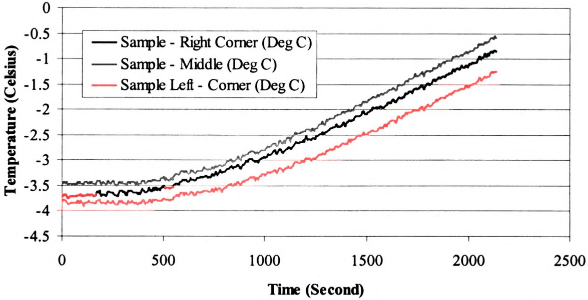


Figure 62 - Opening Incubator Door Condition with Set Temperature inside the Box at -4 Deg C (Box 10)

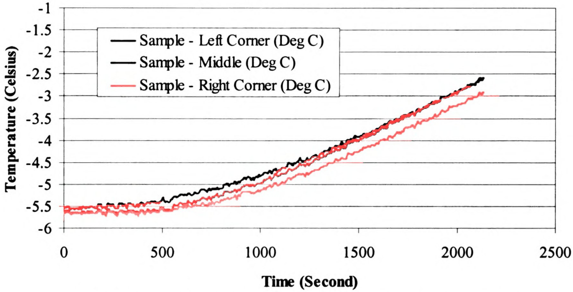


Figure 63 - Opening Incubator Door Condition with Set Temperature inside the Box at -6 Deg C (Box 11)

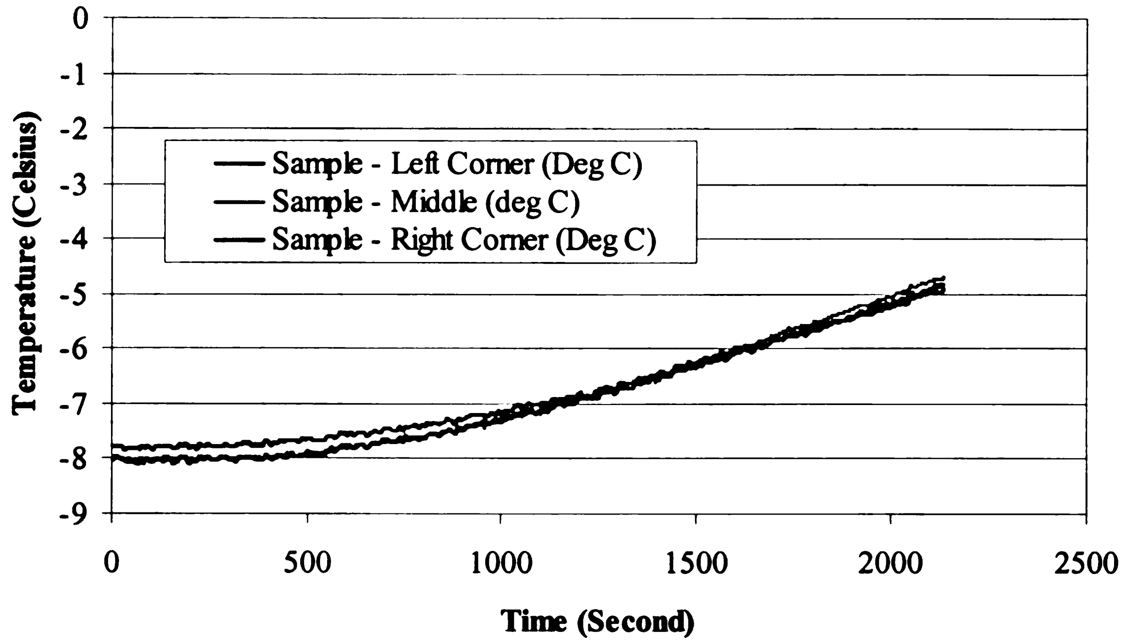


Figure 64 - Opening Incubator Door Condition with Set Temperature inside the Box at -8 Deg C (Box 12)

Based on the Figures 53 – 64, Table 8 shows the sample maintains temperature stability in the petri dishes and cyro vials for at least more than three and half minutes. Furthermore, it can be seen in Figure 8 that the amount of time the sample remains steady is different between the petri dishes and cryo vials. This is because the area of the petri dishes is larger than the area of the cryo vials meaning the warm air will diffuse at a faster rate in the petri dishes compared to the cryo vials. Likewise, using heat transfer theory, the heat transfer rate is proportional to the area of the media. This means that when the area of the media is increased, then the heat transfer rate will also be increased. As a result, the sample temperature in the petri dishes will become warmer faster compared to the sample temperature in the cyro vials.

Table 8 - The Amount of Time for the Boxes to Maintain Temperature Stability during Opening the Incubator Door

Petri Dishes	
# of Box	Steady Time (Minutes)
Box 1	3.5
Box 2	5.4
Box 3	3.5
Cyro Vials	
# of Box	Steady Time (Minutes)
Box 4	9
Box 5	7.6
Box 6	7.5
Box 7	9.3
Box 8	6.1
Box 9	7.3
Box 10	9.2
Box 11	6.9
Box 12	6.8

Table 8 shows that each box maintains the temperature about 3.5-9.0 minutes with a tolerance between 0.1°C -0.2°C. According to the requirements defined by Center of Microbial Ecology, it is important to have sample temperature to be steady at a minimum of 3 minutes for the petri dishes and at a minimum of 5 minutes for the cyro vials with approximately 1°C temperature tolerance. Table 8 shows the boxes fulfill the requirements defined by Center of Microbial Ecology.

Finally, the sample temperature results are examined during opening the incubator door and the lid of the box at the same time. Figures 65-67 show the sample temperature in the petri dishes when the incubator door and the lid of the box are opened at the same time.

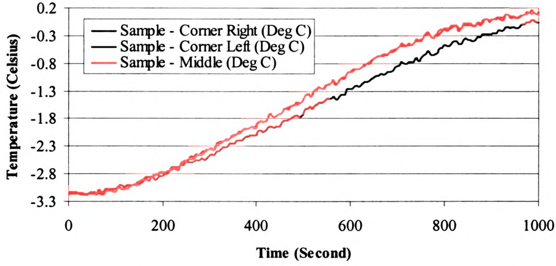


Figure 65. Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -1.1 Deg C (Box 1)

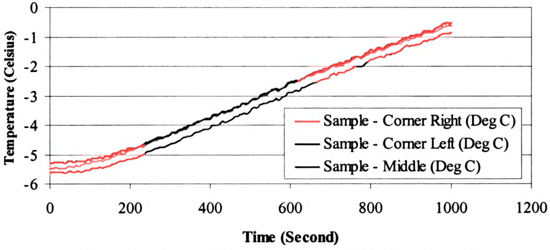


Figure 66 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -5 Deg C (Box 2)

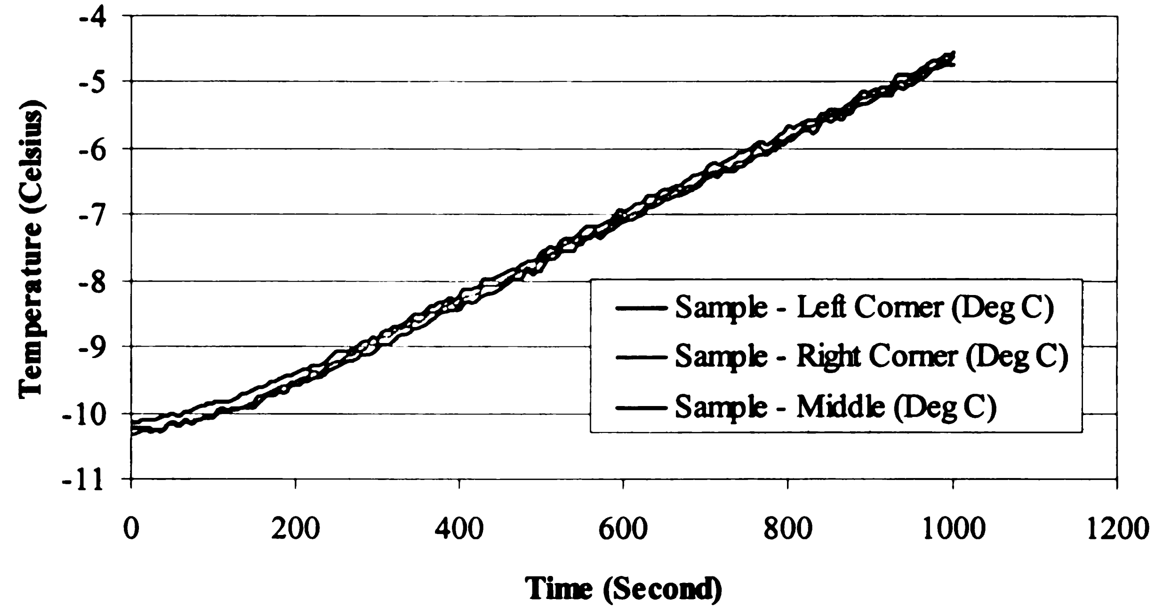


Figure 67 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -9.4 Deg C (Box 3)

Figures 68-76 show the sample temperature in the cyro vials during opening the incubator door and lid of the box at the same time.

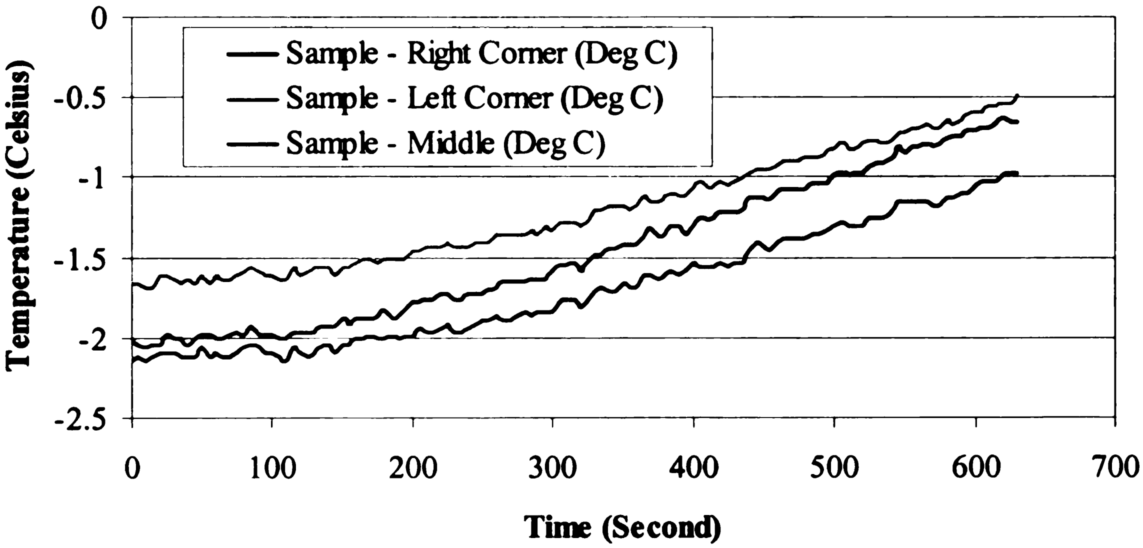


Figure 68 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -1.1 Deg C (Box 4)

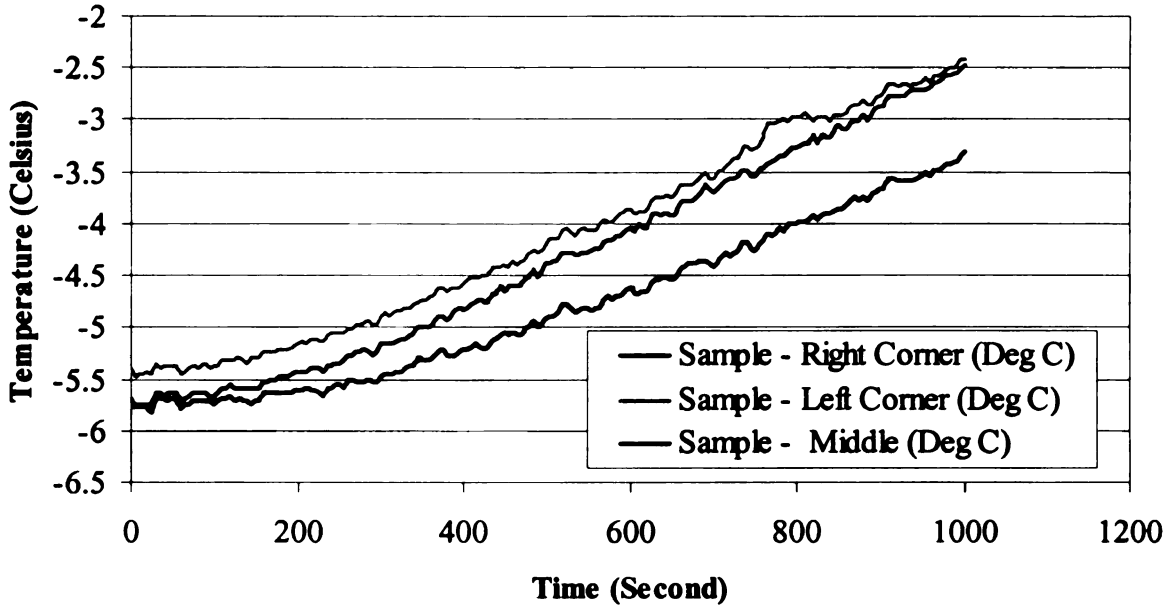


Figure 69 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -5 Deg C (Box 5)

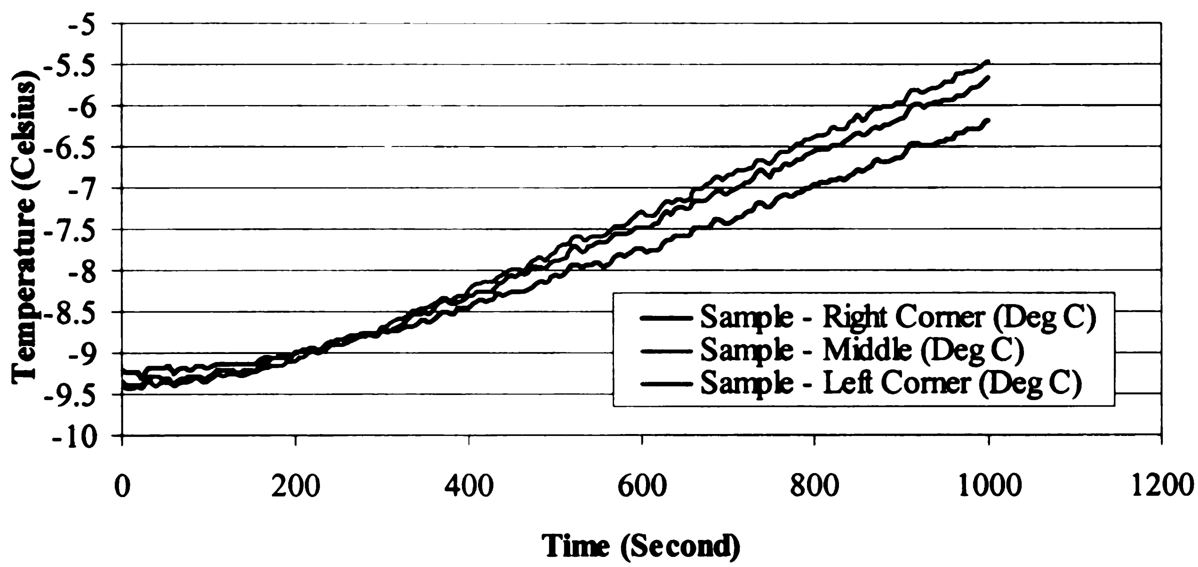


Figure 70 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -9.4 Deg C (Box 6)

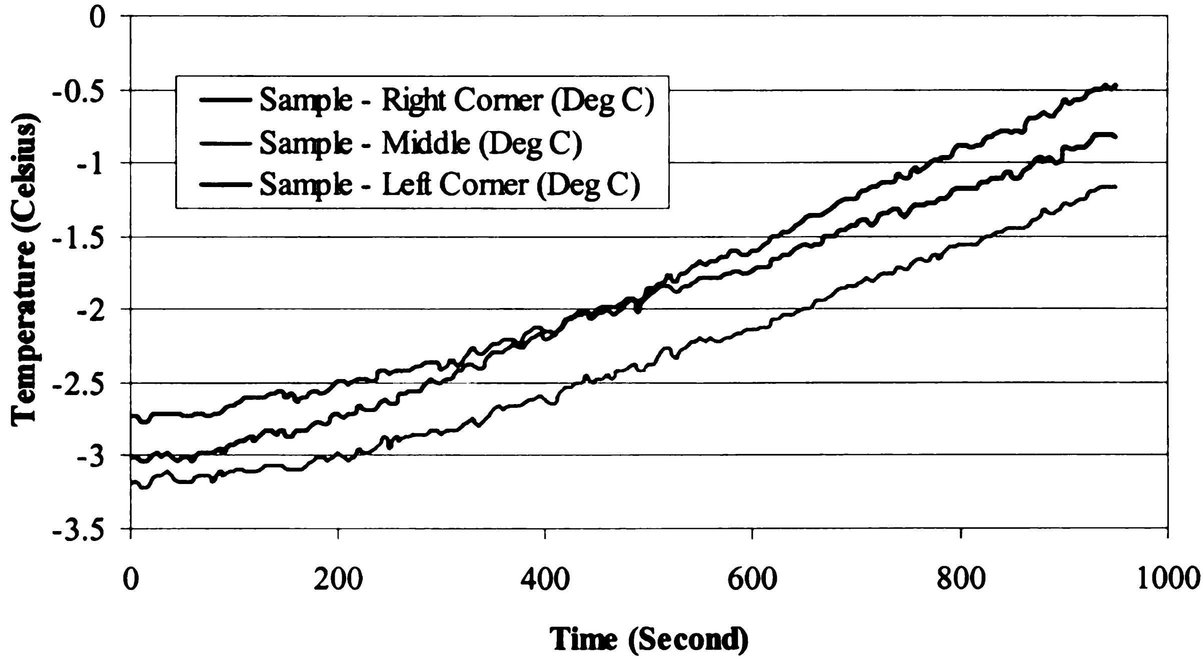


Figure 71 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -3 Deg C (Box 7)

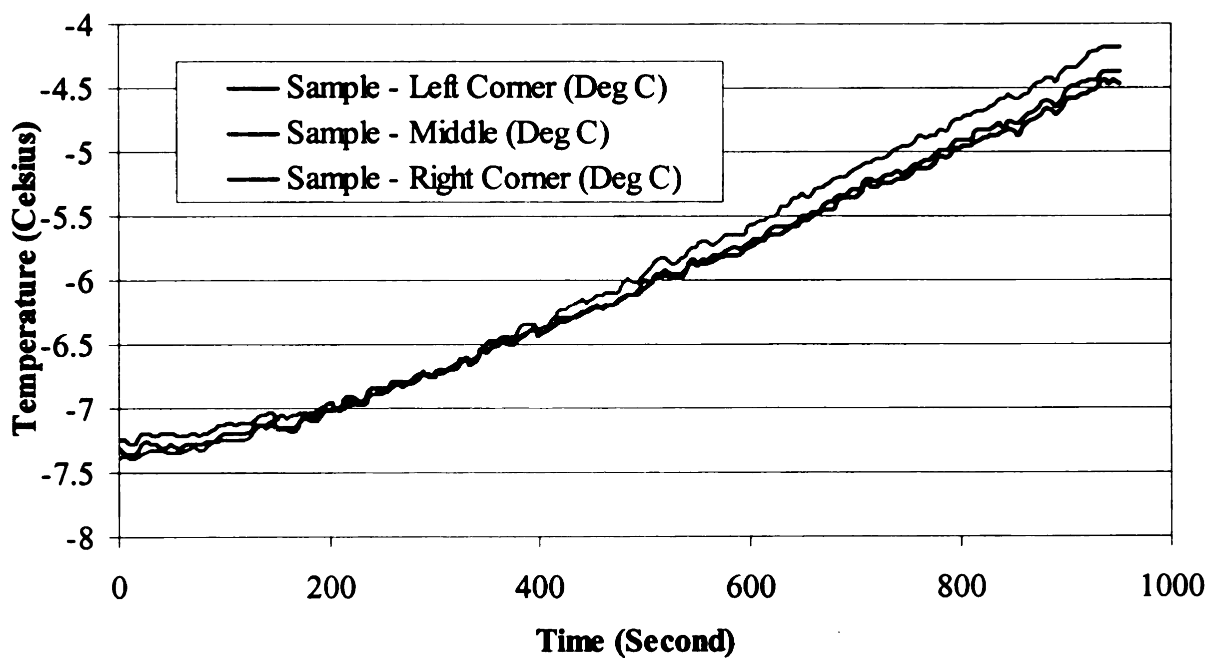


Figure 72 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -7 Deg C (Box 8)

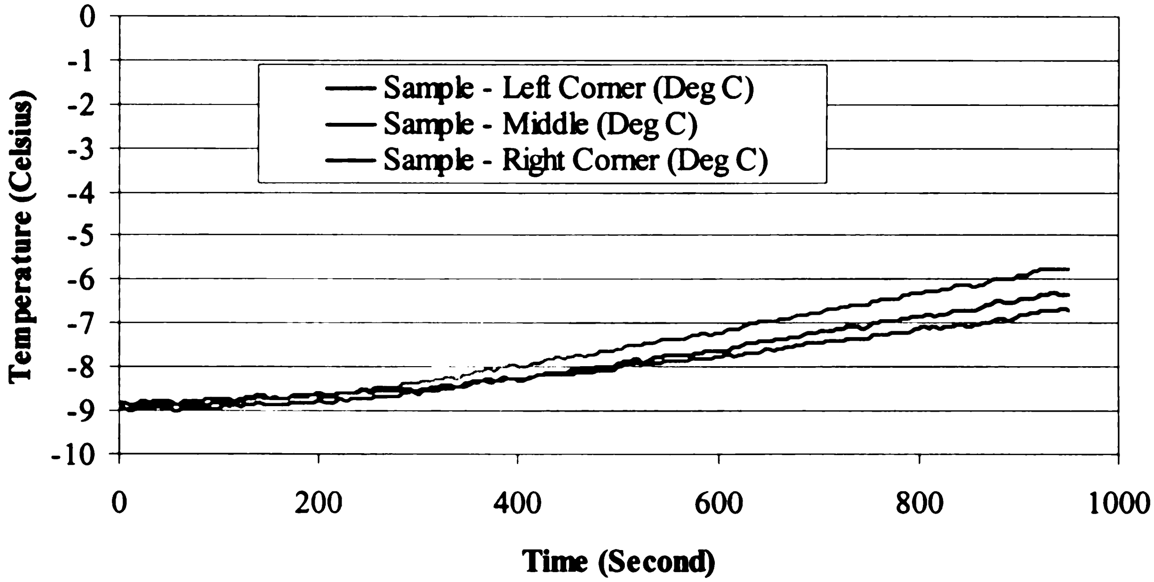


Figure 73 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -9 Deg C (Box 9)

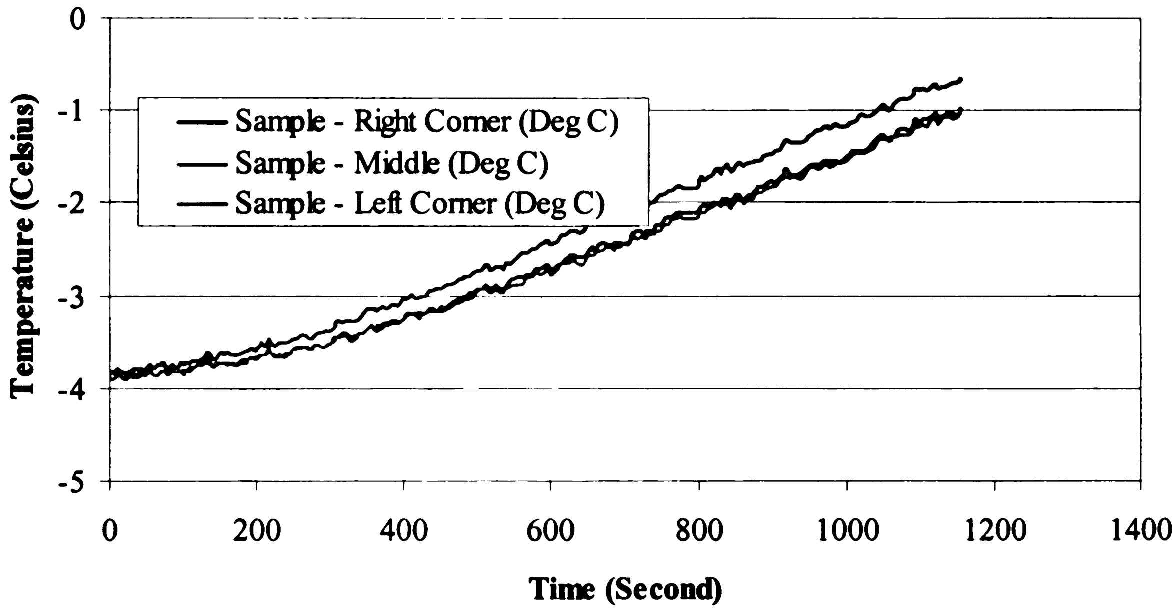


Figure 74 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -4 Deg C (Box 10)

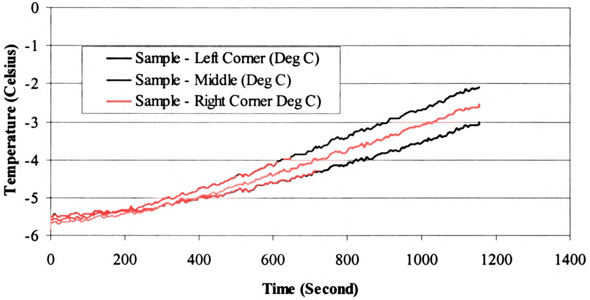


Figure 75 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -6 Deg C (Box 11)

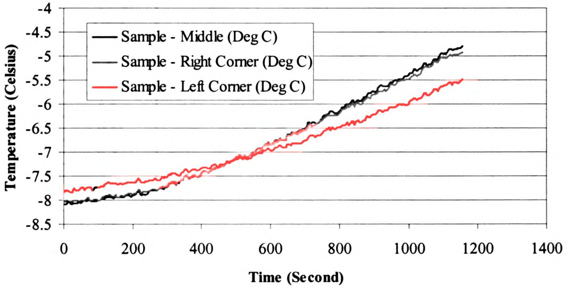


Figure 76 - Opening Incubator Door and Lid of the Box with Set Temperature inside the Box at -8 Deg C (Box 12)

Based on the Figures 65 – 76, Table 9 shows the sample maintains temperature stability in the petri dishes and cyro vials for at least more than 4.4 minutes. Similar as Table 8, the amount of time the sample remains steady is different between the petri dishes and cryo vials. The same argument used for Table 8 can be used for Table 9.

Table 9 - The Amount of Time for the Boxes to Maintain Temperature Stability during Opening the Incubator Door and Lid of the Box

Petri Dishes	
# of Box	**Steady Time (Minutes)**
Box 1	5.8
Box 2	4.9
Box 3	4.4
Cyro Vials	
# of Box	**Steady Time (Minutes)**
Box 4	9
Box 5	9.5
Box 6	6.1
Box 7	9.1
Box 8	6.3
Box 9	7.5
Box 10	9
Box 11	7.8
Box 12	8.4

It can be seen that each box is able to maintain the temperature approximately 4.4-9.1 minutes with a tolerance temperature about ±1°C. According to the design specification, the sample temperature needs to be steady at a minimum of 3 minutes for the petri dishes and at a minimum 5 minutes for the cyro vials and the maximum temperature tolerance that can be allowed is ±1°C. Table 15 shows the boxes fulfill the requirement by Center of Microbial Ecology. Note that images in this thesis are presented in color.

Finally, one box is tested for 10 days in the incubator to see if the sample can maintain its temperature for a long period of time. This testing also shows the reliability of the temperature controller that is regulating the temperature of sample. Figure 77 shows the sample temperature is able to maintain its temperature for 10 days. This means the performance of the controller system is reliable and can regulate the temperature of the box for a long period of time.

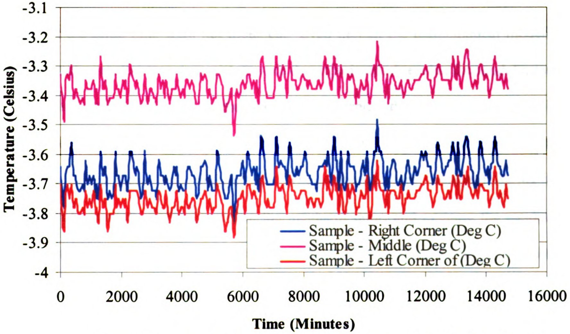


Figure 77 - Sample Temperature Performance after Testing for 10 Days

Chapter 5

CONCLUSION

The purpose of this research is to design, build, and test a thermally controlled environment (0°C to -10°C) that can maintain a regulated temperature for holding biological samples in a low temperature incubator. While in the process of designing the box, it was also important to attempt to improve the design of the box using mathematical modeling. This mathematical modeling was done when the controller system is disabled and when the controller system is enabled. The results showed that the mathematical modeling when the temperature controller system is enabled during the automatic defrost condition were not consistent with the experimental results. Because of this, the conditions for opening the incubator door, and opening the incubator door and the lid of the door at the same time were not modeled. Even though the mathematical modeling showed that the results are not consistent with the experimental results, the modeling was still used to optimize the design of the box. This is because the mathematical modeling helps to understand the flow of heat transfer within the box. Based on this understanding, the twelve boxes were built and tested. These results were tabulated during the conditions of automatic defrost, opening the incubator door, and opening the incubator door and the lid of the box at the same time. During these conditions, several investigations were conducted to see if the sample in the box fulfills the requirements defined by Center of Microbial Ecology. These investigations during the automatic defrost condition include the sample temperature stability, the longest time for the sample to maintain the stability, the comparison between the accuracy of the sample temperature and the set temperature, and the uniformity of the sample inside the box. As for testing the amount of time the sample is able to maintain the temperature stability, the results showed the sample was able to maintain its temperature stability for 10 days. The results for these investigations showed that there was temperature difference more than 1°C between the sample temperature compared to the set temperature. But, this temperature difference was corrected by defining a new calibration equation that is used to adjust the set temperature inside the box. Using this new calibration equation, the temperature difference between the sample temperatures and the set temperature was minimized. As for the temperature uniformity of the samples inside the box, the results showed that the sample temperature is uniform to within 0.48°C. This is because the sample temperature difference between the location of the left corner, middle corner, and right corner of the box was less than ±1°C, meeting the requirements defined by Center of Microbial Ecology. During the condition of opening door, and the condition of opening the door and lid of the box at the same time, the amount of time for sample temperature to be steady was investigated. The results showed that the samples were able to maintain the temperature stability between 3-7 minutes with approximately 0.2°C temperature tolerance during the condition of opening the door. Also, the sample was able to maintain the temperature stability between 4 -9 minutes with a maximum temperature tolerance of 1°C during the opening the door and lid of the box at the same time. As a result of this research, it can be concluded that the temperature controller system is able to regulate the sample temperature between a set temperature 0°C and -10°C. Moreover, the twelve boxes are able to function according to requirements defined by Center of Microbial Ecology.

Chapter 6

SUGGESTIONS FOR FUTURE RESEARCH

Even though this research has been completed, some suggestion can be made for future research. These suggestions can help to improve the design of the box for regulating the low temperature environment. One suggestion would be to improve the mathematical modeling that was evaluated in Chapter 3. One way to improve this mathematical modeling is to use three dimensions for modeling the flow of heat transfer in the box. Using three dimensional analysis, the results could be used to optimize the sample temperature performance during the conditions of automatic defrost, opening the incubator door, and opening the door incubator and lid of the box at the same time.

Another suggestion to improve the design of the box is to test the box in an incubator that does not have an automatic defrost condition. Using an incubator that does not have an automatic defrost condition will keep the temperature inside the incubator steady continuously except when opening the door incubator. But, this issue can be fixed by using an incubator with an air conditioning system that can flow cold air on to the sample when the incubator door is opened.

Besides improving the design of the box, it is suggested to conduct the research for building an ideal box that can regulate an internal temperature in a liquid nitrogen environment. Since liquid nitrogen can go down to -196°C, the possibility of building this box to function in liquid nitrogen environment may be useful for biologists to conduct research about the possibility of microorganism activity to survive in extreme cold temperature environments. This could help scientist to prove that microorganism activity can survive in extreme cold temperatures, meaning that there is possibility of a life on Mars or other planets.

APPENDICES

Instruction Manual for Operating the Temperature Controller

1. Place the box inside the incubator.
2. Connect the temperature sensor to the sensor terminals (S)
3. Connect the heater to the heater terminals (H).
4. Plug the temperature controller system into 115 VAC outlet and turn the unit on.
5. Calculate the desired reference voltage from the function:

   $V_{ref} = 0.2815*Tc + 8.9226$

6. Using a multimeter set the reference voltage (RV) to the desired voltage by adjusting the potentiometer (RA).
7. Monitor the temperature by checking the value of the temperature voltage (TV) to see if the value of the temperature voltage matches with the value of the reference voltage.

Procedure to Operate the Data Acquisition Software

1. Go to MS Dos Prompt.
2. Type CD DASTC and press enter.
3. Type CD STD and press enter.
4. Type tcdloger and press enter.
5. Press enter twice until data acquisition screen shows up.
6. Go to logging, click enabled.
7. Type file name and press enter.
8. Go to log scan and put the desired number of scan (1-10,000).
9. Go to log interval and put the desired number of interval in second or minutes.
10. Go to acquisition and click start.
11. Go to log and click start.

```
(*TEMPERATURE CONTROLLER DISABLED (X-DIRECTION)*)

(*Styrofoam Properties*)
La = 0.026 (*m*);
ka = 0.029 (*W/m.k*);
Aa = 0.022(*m^2*);
rhoa = 50 (*kg/m^3*);
Ca = 1000 (*J/kg.k*);
```

$$Ra = \frac{La}{ka\,Aa}$$

Csty = rhoa Ca Aa La

```
(*Styrofoam inner*)
Lc = 0.064 (*m*);
kc = 0.029 (*W/m.k*);
Ac =  0.0109(*m^2*);
rhoc = 50 (*kg/m^3*);
Cc = 1000 (*J/kg.k*);
```

$$Rc = \frac{Lc}{kc\,Ac}$$

Cstyi = rhoc Cc Ac Lc

```
(*aluminum properties*)
Lb = 0.0035 (* m*);
kb = 170 (*W/m.K*);
Ab = 0.011(*m^2*);
rhob = 2790 (*kg/m^3*);
Cb = 883(* J/kg.K*);
```

$$Rb = \frac{Lb}{kb\,Ab}$$

Cal = rhob Cb Ab Lb

```
(*Water properties*)
Lx = 0.005  (*m*);
kx = 2.03 (*W/m.k*);
Ax = 0.00049(*m^2*);
rhox = 912 (*kg/m^3*);
Cx = 1945 (*J/kg.K*);
```

$$Rx = \frac{Lx}{kx\,Ax}$$

Cwater = rhox Cx Lx Lx

```
(*unit area for slabs*)
A = 0.022(*m^2*);
h = 3 (*W/m^2 K*);

(*Convection and sytrofoam part*)
```

$$R1 = \frac{1}{h\,A} + 0.5\,Ra \text{ (*K/W*);}$$

C1 = Csty (*J/K*);

(*styrofoam and aluminum part*)

```
R2 = 0.5 Ra + 0.5 Rb (*K/W*);
C2 = Cal  (*J/K*);

(*aluminum and sample holder part*)
R3 = 0.5 Rb + 0.5 Rc(*K/W*);
C3 = Cstyi(*J/K*)

(*sample holder and water*)
R4 = 0.5 Rc + 0.5 Rx (*K/W*);
C4 = Cwater (*J/K*);

(*water and sample holder*)
R5 = 0.5 Rx + 0.5 Rc (*K/W*);
C5 = Cstyi (*J/K*);

(*sample holder and aluminum*)
R6 = 0.5 Rc + 0.5 Rb(*K/W*);
C6 = Cal(*J/K*)

(*aluminum and styrofoam*)
R7 = 0.5 Rb + 0.5 Ra (*K/W*);
C7 = Csty (*J/K*);

(*styrofoam and convection*)
R8 = 1/(h A) +  0.5 Ra (*K/W*);

maxT = 9980;
incT = 10;
Tout = (-11.82 + (13.63 / (1 + Exp[-(t - 575.87 + 0.000000000001 / 2) / 137.02])) *
    (1 - 1 / (1 + Exp[-(t - 575.87 + 0.000000000001 / 2) / 2579.22])))
solution = NDSolve[{C1 V1'[t] + (V1[t] - V2[t])/R2 == 1/R1 (Tout - V1[t]),
   (V1[t] - V2[t])/R2 == (V2[t] - V3[t])/R3 + C2 V2'[t],
   (V2[t] - V3[t])/R3 == C3 V3'[t] + (V3[t] - V4[t])/R4,
   (V3[t] - V4[t])/R4 == C4 V4'[t] + (V4[t] - V5[t])/R5, (V4[t] - V5[t])/R5 ==
    C5 V5'[t] + (V5[t] - V6[t])/R6, (V5[t] - V6[t])/R6 == C6 V6'[t] + (V6[t] - V7[t])/R7,


   (V6[t] - V7[t])/R7 == C7 V7'[t] + 1/R8 (V7[t] - (Tout)), V1[0] == -12.1,
   V2[0] == -12.02, V3[0] == -12.3, V4[0] == -12.2,
   V5[0] == -12.1, V6[0] == -12.3, V7[0] == -12.4},
  {V1, V2, V3, V4, V5, V6, V7}, {t, 0, maxT}]

g1 = Plot[Evaluate[V1[t] /. solution], {t, 0, maxT}]
g2 = Plot[Evaluate[V2[t] /. solution], {t, 0, maxT}]
g3 = Plot[Evaluate[V3[t] /. solution], {t, 0, maxT}]
```

```
g4 = Plot[Evaluate[V4[t] /. solution], {t, 0, maxT}]
g5 = Plot[Evaluate[V5[t] /. solution], {t, 0, maxT}]
g6 = Plot[Evaluate[V6[t] /. solution], {t, 0, maxT}]
g7 = Plot[Evaluate[V7[t] /. solution], {t, 0, maxT}]
g8 = Plot[Tout, {t, 0, maxT}]
Show[g1, g2, g3, g4, g5, g6, g7, g8]
```

```
(*TEMPERATURE CONTROLLER DISABLED (Y-DIRECTION)*)

(*Styrofoam Properties*)
La = 0.026 (*m*);
ka = 0.029 (*W/m.k*);
Aa = 0.0158(*m^2*);
rhoa = 50 (*kg/m^3*);
Ca = 1000 (*J/kg.k*);
Ra = La/(ka Aa)
Csty = rhoa Ca Aa La

(*Styrofoam inner*)
Lc = 0.10 (*m*);
kc = 0.029 (*W/m.k*);
Ac =  0.0069(*m^2*);
rhoc = 50 (*kg/m^3*);
Cc = 1000 (*J/kg.k*);
Rc = Lc/(kc Ac)
Cstyi = rhoc Cc Ac Lc

(*aluminum properties*)
Lb = 0.0035 (* m*);
kb = 170 (*W/m.K*);
Ab = 0.00725(*m^2*);
rhob = 2790 (*kg/m^3*);
Cb = 883(* J/kg.K*);
Rb = Lb/(kb Ab)
Cal = rhob Cb Ab Lb

(*Water properties*)
Lx = 0.01 (*m*);
kx = 2.03 (*W/m.k*);
Ax = 0.00049(*m^2*);
rhox = 912 (*kg/m^3*);
Cx = 1945 (*J/kg.K*);
Rx = Lx/(kx Ax)
Cwater = rhox Cx Lx Ax

(*unit area for slabs*)
A = 0.022 (*m^2*);
h = 10(*W/m^2 K*);

(*Convection and sytrofoam part*)
R1 = 1/(h A) +  0.5 Ra (*K/W*);
C1 = Csty (*J/K*);

(*styrofoam and aluminum part*)
```

```
R2 = 0.5 Ra + 0.5 Rb (*K/W*);
C2 = Cal (*J/K*);

(*aluminum and sample holder part*)
R3 = 0.5 Rb + 0.5 Rc(*K/W*);
C3 = Cstyi(*J/K*)

(*sample holder and water*)
R4 = 0.5 Rc + 0.5 Rx (*K/W*);
C4 = Cwater (*J/K*);

(*water and sample holder*)
R5 = 0.5 Rx + 0.5 Rc (*K/W*);
C5 = Cstyi (*J/K*);

(*sample holder and aluminum*)
R6 = 0.5 Rc + 0.5 Rb(*K/W*);
C6 = Cal(*J/K*)

(*aluminum and styrofoam*)
R7 = 0.5 Rb + 0.5 Ra (*K/W*);
C7 = Csty (*J/K*);

(*styrofoam and convection*)
R8 = 1/(h A) + 0.5 Ra (*K/W*);

maxT = 19990;
incT = 10;
Tout = (-14.27 + (15.45 / (1 + Exp[-(t - 903.09 + 0.000000000001 / 2) / 119.69])) *
    (1 - 1 / (1 + Exp[-(t - 903.09 - 0.000000000001 / 2) / 2900.73])))
solution = NDSolve[{C1 V1'[t] + (V1[t] - V2[t])/R2 == 1/R1 (Tout - V1[t]),
   (V1[t] - V2[t])/R2 == (V2[t] - V3[t])/R3 + C2 V2'[t],
   (V2[t] - V3[t])/R3 == C3 V3'[t] + (V3[t] - V4[t])/R4,
   (V3[t] - V4[t])/R4 == C4 V4'[t] + (V4[t] - V5[t])/R5, (V4[t] - V5[t])/R5 ==
   C5 V5'[t] + (V5[t] - V6[t])/R6, (V5[t] - V6[t])/R6 == C6 V6'[t] + (V6[t] - V7[t])/R7,


   (V6[t] - V7[t])/R7 == C7 V7'[t] + 1/R8 (V7[t] - (Tout)), V1[0] == -14.81,
  V2[0] == -14.87, V3[0] == -14.82, V4[0] == -14.96,
  V5[0] == -14.96, V6[0] == -14.96, V7[0] == -15.1},
 {V1, V2, V3, V4, V5, V6, V7}, {t, 0, maxT}]

g1 = Plot[Evaluate[V1[t] /. solution], {t, 0, maxT}]
g2 = Plot[Evaluate[V2[t] /. solution], {t, 0, maxT}]
g3 = Plot[Evaluate[V3[t] /. solution], {t, 0, maxT}]
```

```
g4 = Plot[Evaluate[V4[t] /. solution], {t, 0, maxT}]
g5 = Plot[Evaluate[V5[t] /. solution], {t, 0, maxT}]
g6 = Plot[Evaluate[V6[t] /. solution], {t, 0, maxT}]
g7 = Plot[Evaluate[V7[t] /. solution], {t, 0, maxT}]
g8 = Plot[Tout, {t, 0, maxT}]
Show[g1, g2, g3, g4, g5, g6, g7, g8]
```

```
(*TEMPERATURE CONTROLLER DISABLED (Z-DIRECTION)*)

(*Styrofoam Properties*)
La = 0.026 (*m*);
ka = 0.029 (*W/m.k*);
Aa = 0.055(*m^2*);
rhoa = 50 (*kg/m^3*);
Ca = 1000 (*J/kg.k*);
Ra = La/(ka Aa);
Csty = rhoa Ca La Aa;

(*aluminum properties*)
Lb = 0.0035 (* m*);
kb = 170 (*W/m.K*);
Ab = 0.0319(*m^2*);
rhob = 2790 (*kg/m^3*);
Cb = 883(* J/kg.K*);
Rb = Lb/(kb Ab);
Cal = rhob Cb Lb Ab;

(*unit area for slabs*)
A = 0.055 (*m^2*);
h = 3 (*W/m^2 K*);

(*water prop*)
Lx = 0.035 (*m*);
kx = 2.03 (*W/m.k*);
Ax = 0.0000785(*m^2*);
rhox = 912 (*kg/m^3*);
Cx = 1945 (*J/kg.K*);
Rx = Lx/(kx Ax);
Cwater = rhox Cx Lx Ax;

(*Convection and sytrofoam part*)
R1 = 1/(h A) +  0.5 Ra (*K/W*)
C1 = Csty (*J/K*)

(*styrofoam and aluminum part*)
R2 = 0.5 Ra + 0.5 Rb (*K/W*)
C2 = Cal (*J/K*)

(*aluminum & water part*)
R3 = 0.5 Rb + 0.5 Rx(*K/W*)
C3 = Cwater

(*water and aluminum part*)
R4 = 0.5 Rx + 0.5 Rb (*K/W*);
C4 = Cal (*J/K*);
```

```
(* aluminum and styrofoam part*)
R5 = 0.5 Rb + 0.5 Ra (*K/W*);
C5 = Csty (*J/K*);

(*Convection and sytrofoam part*)
R6 = 0.5 Ra + 1/(h A) (*K/W*);

maxT = 20000;
incT = 30;
Tout = (-12.69 + 0.011 t - 0.0000043 (t)^2 + 0.00000000059 (t)^3 -
     0.00000000000068 (t)^4 - 0.0000000000000053 (t)^5) /
   (1 - 0.00058 t + 0.00000025 (t)^2 - 0.000000000033 (t)^3 +
     0.00000000000073 (t)^4 + 0.000000000000000295 (t)^5)
solution = NDSolve[{C1 V1'[t] + (V1[t] - V2[t])/R2 == 1/R1 (Tout - V1[t]),
    (V1[t] - V2[t])/R2 == (V2[t] - V3[t])/R3 + C2 V2'[t], (V2[t] - V3[t])/R3 ==
     C3 V3'[t] + (V3[t] - V4[t])/R4, (V3[t] - V4[t])/R4 == C4 V4'[t] + (V4[t] - V5[t])/R5,
    (V4[t] - V5[t])/R5 == C5 V5'[t] + (V5[t] - Tout)/R6, V1[0] == -14.39,
    V2[0] == -14.45, V3[0] == -14.4522, V4[0] == -14.32, V5[0] == -14.92},
   {V1, V2, V3, V4, V5}, {t, 0, maxT}]

g1 = Plot[Evaluate[V1[t] /. solution],
    {t, 0, maxT}, PlotRange -> {Automatic, {5, -20}}];
g2 = Plot[Evaluate[V2[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {5, -20}}];
g3 = Plot[Evaluate[V3[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {6, -20}}];
g4 = Plot[Evaluate[V4[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {5, -20}}];
g5 = Plot[Evaluate[V5[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {5, -20}}];
g6 = Plot[{Tout}, {t, 0, maxT}];
Show[g1, g2, g3, g4, g5,
 PlotRange -> {Automatic, {5, -30}}, AspectRatio -> 1.0]
```

```
(*TEMPERATURE CONTROLLER DISABLED
  (OPENING THE INCUBATOR DOOR CONDITION)*)

(*Styrofoam Properties*)
La = 0.026 (*m*);
ka = 0.029 (*W/m.k*);
Aa = 0.055(*m^2*);
rhoa = 50 (*kg/m^3*);
Ca = 1000 (*J/kg.k*);
```

$$Ra = \frac{La}{ka\, Aa}$$

```
Csty = rhoa Ca La Aa

(*aluminum properties*)
Lb = 0.0035 (* m*);
kb = 170 (*W/m.K*);
Ab = 0.0319(*m^2*);
rhob = 2790 (*kg/m^3*);
Cb = 883(* J/kg.K*);
```

$$Rb = \frac{Lb}{kb\, Ab}$$

```
Cal = rhob Cb Lb Ab

(*unit area for slabs*)
A = 0.055 (*m^2*);
h = 3 (*W/m^2 K*);

(*water prop*)
Lx = 0.035 (*m*);
kx = 2.03 (*W/m.k*);
Ax = 0.0000785(*m^2*);
rhox = 912 (*kg/m^3*);
Cx = 1945 (*J/kg.K*);
```

$$Rx = \frac{Lx}{kx\, Ax}$$

```
Cwater = rhox Cx Lx Ax

(*Convection and sytrofoam part*)
```

$$R1 = \frac{1}{h\, A} + 0.5\, Ra \text{ (*K/W*);}$$

```
C1 = Csty (*J/K*);

(*styrofoam and aluminum part*)
R2 = 0.5 Ra + 0.5 Rb (*K/W*);
C2 = Cal (*J/K*);

(*aluminum & water part*)
R3 = 0.5 Rb + 0.5 Rx(*K/W*);
C3 = Cwater

(*water and aluminum part*)
R4 = 0.5 Rx + 0.5 Rb (*K/W*);
```

```
C4 = Cal (*J/K*);

(* aluminum and styrofoam part*)
R5 = 0.5 Rb + 0.5 Ra (*K/W*);
C5 = Csty (*J/K*);

(*Convection and sytrofoam part*)
R6 = 0.5 Ra + 1/(h A) (*K/W*);

maxT = 5000;
incT = 10;
Tout = (-15.65 + 15.73 (t)^0.5 - 0.022 * t) /
  (1 + 0.84 (t)^0.5 - 0.0035 t + 0.0000143 (t)^1.5)
solution = NDSolve[{C1 V1'[t] + (V1[t] - V2[t])/R2 == 1/R1 (Tout - V1[t]),
   (V1[t] - V2[t])/R2 == (V2[t] - V3[t])/R3 + C2 V2'[t], (V2[t] - V3[t])/R3 ==
    C3 V3'[t] + (V3[t] - V4[t])/R4, (V3[t] - V4[t])/R4 == C4 V4'[t] + (V4[t] - V5[t])/R5,
   (V4[t] - V5[t])/R5 == C5 V5'[t] + (V5[t] - Tout)/R6, V1[0] == -14.89,
   V2[0] == -14.79, V3[0] == -14.72, V4[0] == -14.79, V5[0] == -15.74},
  {V1, V2, V3, V4, V5}, {t, 0, maxT}]

g1 = Plot[Evaluate[V1[t] /. solution],
   {t, 0, maxT}, PlotRange -> {Automatic, {10, -20}}];
g2 = Plot[Evaluate[V2[t] /. solution], {t, 0, maxT},
   PlotRange -> {Automatic, {5, -20}}];
g3 = Plot[Evaluate[V3[t] /. solution], {t, 0, maxT},
   PlotRange -> {Automatic, {6, -20}}];
g4 = Plot[Evaluate[V4[t] /. solution], {t, 0, maxT},
   PlotRange -> {Automatic, {5, -20}}];
g5 = Plot[Evaluate[V5[t] /. solution], {t, 0, maxT},
   PlotRange -> {Automatic, {10, -20}}];
g6 = Plot[{Tout}, {t, 0, maxT}];
Show[g1, g2, g3, g4, g5, g6,
 PlotRange -> {Automatic, {5, -30}}, AspectRatio -> 1.0]
```

```
(*TEMPERATURE CONTRLLER DISABLED
  (OPENING THE INCUBATOR DOOR AND TOP OF THE BOX AT THE SAME TIME*)

(*Styrofoam Properties*)
La = 0.026 (*m*);
ka = 0.029 (*W/m.k*);
Aa = 0.055(*m^2*);
rhoa = 50 (*kg/m^3*);
Ca = 1000 (*J/kg.k*);
```

$$Ra = \frac{La}{ka\,Aa}$$

```
Csty = rhoa Ca La Aa

(*aluminum properties*)
Lb = 0.0035 (* m*);
kb = 170 (*W/m.K*);
Ab = 0.0319(*m^2*);
rhob = 2790 (*kg/m^3*);
Cb = 883(* J/kg.K*);
```

$$Rb = \frac{Lb}{kb\,Ab}$$

```
Cal = rhob Cb Lb Ab

(*unit area for slabs*)
A1 = 0.055 (*m^2*);
A2 = 0.0000785(*m^2*);
h = 20(*W/m^2 K*);

(*water prop*)
Lx = 0.035 (*m*);
kx = 2.03 (*W/m.k*);
Ax = 0.0000785(*m^2*);
rhox = 912 (*kg/m^3*);
Cx = 1945 (*J/kg.K*);
```

$$Rx = \frac{Lx}{kx\,Ax}$$

```
Cwater = rhox Cx Lx Ax

(*Convection and sytrofoam part*)
```

$$R1 = \frac{1}{h\,A1} + 0.5\,Ra \text{ (*K/W*);}$$

```
C1 = Csty (*J/K*);

(*styrofoam and aluminum part*)
R2 = 0.5 Ra + 0.5 Rb (*K/W*);
C2 = Cal (*J/K*);

(*aluminum & water part*)
R3 = 0.5 Rb + 0.5 Rx(*K/W*);
C3 = Cwater

(*water and convection part*)
```

```
R4 = 0.5 Rx + 1/(h A2) (*K/W*);

maxT = 5165;
incT = 5;
Tout = 16.08 - 0.006 (t) + 0.000043 (t)^1.5 + 0.32 (t)^0.5
solution = NDSolve[{C1 V1'[t] + (V1[t] - V2[t])/R2 == 1/R1 (Tout - V1[t]),
    (V1[t] - V2[t])/R2 == (V2[t] - V3[t])/R3 + C2 V2'[t],
    (V2[t] - V3[t])/R3 == C3 V3'[t] + (V3[t] - Tout)/R4, V1[0] == -0.58,
    V2[0] == -12.05, V3[0] == -12.84}, {V1, V2, V3}, {t, 0, maxT},
  MaxSteps -> 6000, PrecisionGoal -> 0.5, WorkingPrecision -> 1]

g1 = Plot[Evaluate[V1[t] /. solution],
    {t, 0, maxT}, PlotRange -> {Automatic, {-20, 20}}];
g2 = Plot[Evaluate[V2[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {-20, 20}}];
g3 = Plot[Evaluate[V3[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {-20, 20}}];
g4 = Plot[{Tout}, {t, 0, maxT}];
Show[g1, g2, g3, g4, PlotRange -> {Automatic, {-20, 20}}, AspectRatio -> 1.0]
```

```
(*TEMPERATURE CONTROLLER ENABLED (AUTOMATIC DEFROST CONDITION*)

(*Styrofoam Properties*)
La = 0.026 (*m*);
ka = 0.029 (*W/m.k*);
Aa = 0.055(*m^2*);
rhoa = 50 (*kg/m^3*);
Ca = 1000 (*J/kg.k*);
Ra = La/(ka Aa)
Csty = rhoa Ca La Aa

(*aluminum properties*)
Lb = 0.0035 (* m*);
kb = 170 (*W/m.K*);
Ab = 0.0319(*m^2*);
rhob = 2790 (*kg/m^3*);
Cb = 883(* J/kg.K*);
Rb = Lb/(kb Ab)
Cal = rhob Cb Lb Ab

(*unit area for slabs*)
A = 0.055 (*m^2*);
h = 25(*W/m^2 K*);

(*water prop*)
Lx = 0.035 (*m*);
kx = 2.03 (*W/m.k*);
Ax = 0.0000785(*m^2*);
rhox = 912 (*kg/m^3*);
Cx = 1945 (*J/kg.K*);
Rx = Lx/(kx Ax)
Cwater = rhox Cx Lx Ax

(*Convection and sytrofoam part*)
R1 = 1/(h A) +  0.5 Ra (*K/W*);
C1 = Csty (*J/K*);

(*styrofoam and aluminum part*)
R2 = 0.5 Ra + 0.5 Rb (*K/W*);
C2 = Cal (*J/K*);

(*aluminum & water part*)
R3 = 0.5 Rb + 0.5 Rx(*K/W*);
C3 = Cwater

(*water and  part*)
R4 = 0.5 Rx + 0.5 Rb (*K/W*);
C4 = Cal (*J/K*);
```

```
(* aluminum and styrofoam part*)
R5 = 0.5 Rb + 0.5 Ra (*K/W*);
C5 = Csty (*J/K*);

(*Convection and sytrofoam part*)
R6 = 0.5 Ra + 1/(h A) (*K/W*);

Q /: Q[y_ /; y < -6.7] = 10.87;
Q /: Q[y_ /; y >= -6.7] = 0;

maxT = 27480;
incT = 30;
Tout = (-16.48 + 0.86 (t)^0.5 - 0.013 (t)) / (1 - 0.046 (t)^0.5 + 0.00079 (t))
solution = NDSolve[{C1 V1'[t] + (V1[t] - V2[t])/R2 == 1/R1 (Tout - V1[t]),
    Q[V2[t]] + (V1[t] - V2[t])/R2 == (V2[t] - V3[t])/R3 + C2 V2'[t],
    (V2[t] - V3[t])/R3 == C3 V3'[t] + (V3[t] - V4[t])/R4,
    (V3[t] - V4[t])/R4 == C4 V4'[t] + (V4[t] - V5[t])/R5,
    (V4[t] - V5[t])/R5 == C5 V5'[t] + (V5[t] - Tout)/R6, V1[0] == -11.95,
    V2[0] == -7.26, V3[0] == -8.22, V4[0] == -10.42, V5[0] == -12.69},
   {V1, V2, V3, V4, V5}, {t, 0, maxT}, MaxSteps -> 5000,
   PrecisionGoal -> 1, WorkingPrecision -> 1]

g1 = Plot[Evaluate[V1[t] /. solution],
    {t, 0, maxT}, PlotRange -> {Automatic, {5, -20}}];
g2 = Plot[Evaluate[V2[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {5, -20}}];
g3 = Plot[Evaluate[V3[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {6, -20}}];
g4 = Plot[Evaluate[V4[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {5, -20}}];
g5 = Plot[Evaluate[V5[t] /. solution], {t, 0, maxT},
    PlotRange -> {Automatic, {5, -20}}];
g6 = Plot[Q[V2[t]] /. solution, {t, 0, maxT},
    PlotRange -> {Automatic, {0, 11}}];
g7 = Plot[{Tout}, {t, 0, maxT}];
Show[g1, g2, g3, g4, g5, g6, g7,
  PlotRange -> {Automatic, {5, -30}}, AspectRatio -> 1.0]
```

```
(*DESIGN OPTIMIZATION*)

(*Styrofoam Properties*)
La = 0.026(*m*);
ka = 0.029 (*W/m.k*);
Aa = 0.055(*m^2*);
rhoa = 50 (*kg/m^3*);
Ca = 1000 (*J/kg.k*);
```

$$Ra = \frac{La}{ka\,Aa}$$

```
Csty = rhoa Ca La Aa

(*aluminum properties*)
Lb = 0.0035 (* m*);
kb = 170 (*W/m.K*);
Ab = 0.0319(*m^2*);
rhob = 2790 (*kg/m^3*);
Cb = 883(* J/kg.K*);
```

$$Rb = \frac{Lb}{kb\,Ab}$$

```
Cal = rhob Cb Lb Ab

(*unit area for slabs*)
A = 0.055 (*m^2*);
h = 25(*W/m^2 K*);

(*water prop*)
Lx = 0.017 (*m*);
kx = 2.03 (*W/m.k*);
Ax = 0.000637(*m^2*);
rhox = 912 (*kg/m^3*);
Cx = 1945 (*J/kg.K*);
```

$$Rx = \frac{Lx}{kx\,Ax}$$

```
Cwater = rhox Cx Lx Ax

(*Styrofoam Properties*)
Ls = 0.025 (*m*);
ks = 0.029 (*W/m.k*);
As = 0.0319(*m^2*);
rhos = 50 (*kg/m^3*);
Cs = 1000 (*J/kg.k*);
```

$$Rs = \frac{Ls}{ks\,As}$$

```
Cst = rhos Cs Ls As

(*Convection and sytrofoam part*)
```

$$R1 = \frac{1}{h\,A} + 0.5\,Ra \text{ (*K/W*);}$$

```
C1 = Csty (*J/K*);

(*styrofoam and aluminum part*)
```

```
R2 = 0.5 Ra + 0.5 Rb (*K/W*);
C2 = Cal (*J/K*);

(*aluminum & water part*)
R3 = 0.5 Rb + 0.5 Rx(*K/W*);
C3 = Cwater

(*water and styrofoam cover part*)
R4 = 0.5 Rx + 0.5 Rs (*K/W*);
C4 = Cst (*J/K*);


(*Styrofoam cover and aluminum*)
R5 = 0.5 Rs + 0.5 Rb (*K/W*);
C5 = Cal (*J/K*);

(*aluminum and styrofoam part*)
R6 = 0.5 Rb + 0.5 Ra (*K/W*);
C6 = Csty (*J/K*);

(*Convection and sytrofoam part*)
R7 = 0.5 Ra + 1/(h A) (*K/W*);

Q /: Q[y_ /; y < -6.7] = 10.87;
Q /: Q[y_ /; y >= -6.7] = 0;

maxT = 27480;
incT = 30;
Tout = (-16.48 + 0.86 (t)^0.5 - 0.013 (t)) / (1 - 0.046 (t)^0.5 + 0.00079 (t))
solution = NDSolve[{C1 V1'[t] + (V1[t] - V2[t])/R2 == 1/R1 (Tout - V1[t]),
   Q[V2[t]] + (V1[t] - V2[t])/R2 == (V2[t] - V3[t])/R3 + C2 V2'[t],
   (V2[t] - V3[t])/R3 == C3 V3'[t] + (V3[t] - V4[t])/R4, (V3[t] - V4[t])/R4 ==
    C4 V4'[t] + (V4[t] - V5[t])/R5, (V4[t] - V5[t])/R5 == C5 V5'[t] + (V5[t] - Tout)/R6,
   (V5[t] - V6[t])/R6 == C6 V6'[t] + (V6[t] - Tout)/R7, V1[0] == -11.95, V2[0] == -7.26,
   V3[0] == -8.22, V4[0] == -9.22,  V5[0] == -10.42, V6[0] == -12.69},
  {V1, V2, V3, V4, V5,  V6}, {t, 0, maxT}, MaxSteps -> 5000,
  PrecisionGoal -> 1, WorkingPrecision -> 1]

g1 = Plot[Evaluate[V1[t] /. solution],
   {t, 0, maxT},  PlotRange -> {Automatic, {5, -20}}];
g2 = Plot[Evaluate[V2[t] /. solution], {t, 0, maxT},
   PlotRange -> {Automatic, {5, -20}}];
g3 = Plot[Evaluate[V3[t] /. solution], {t, 0, maxT},
   PlotRange -> {Automatic, {6, -20}}];
g4 = Plot[Evaluate[V4[t] /. solution], {t, 0, maxT},
   PlotRange -> {Automatic, {5, -20}}];
g5 = Plot[Evaluate[V5[t] /. solution], {t, 0, maxT},
```

```
   PlotRange → {Automatic, {5, -20}}];
g6 = Plot[Evaluate[V6[t] /. solution], {t, 0, maxT},
   PlotRange → {Automatic, {5, -20}}];
g7 = Plot[Q[V2[t]] /. solution, {t, 0, maxT},
   PlotRange → {Automatic, {0, 11}}];
g8 = Plot[{Tout}, {t, 0, maxT}];
Show[g1, g2, g3, g4, g5, g6, g7, g8,
 PlotRange → {Automatic, {5, -30}}, AspectRatio → 1.0]
```

BIBLIOGRAPHY

1. Gilichinsky, D. (1993). Viable Microorganisms in Permafrost: The Spectrum of Possible Applications to New Investigations in Science for Cold Regions.

2. Williams, D. (1989). Advances in Microbial Ecology. NY: Plenum Press.

3. McKay, P., Friedman, E., and Meyer, M. From Siberia to Mars.

4. Gilichinsky, D., McGrath, J., Rivkina, E., Tiedje, J., and Wagener, S. (1998). Biogeochemical Activity of Anaerobic Microorganisms from Buried Permafrost Sediments.

5. Kalinina, L., Aerobic Bacteria of Permafrost Siberia Coil.

6. Kalinina, L., McGrath, J., and Wagener, S. Cold Adaptation of Permafrost Microorganisms.

7. DeWitt, D. and Incropera, F. (1996). Introduction to Heat Transfer. NY: Wiley and Sons, Inc.

8. Holman, J. (1976). Heat Transfer. NY: McGraw-Hill, Inc.

9. Rizzoni, G. (1996). Principles and Applications of Electrical Engineering. Boston: Times Mirror Higher Education Group, Inc.

10. Nilsson, J. (1993). Electrical Circuits. NY: Addison-Wesley, Inc.