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Hypoxic Incubation Chamber

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HYPOXIC INCUBATION CHAMBER

A Master’s Project
presented to
the Faculty of California Polytechnic State University,
San Luis Obispo

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Biomedical Engineering

by
Makenzie Jones
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TITLE: Hypoxic Incubator Chamber

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EXEVCUTIVE SUMMARY

Hypoxic Incubator Chamber

Simone Helfrich & Makenzie Jones

This paper describes the design, manufacturing, and testing of a novel controllable hypoxic incubator with fully functional oxygen gas control and temperature control in a humid environment. On the current market, a majority of the few hypoxic incubators use pre-mixed gas that does not offer precise control over gas concentration. The objective for this project was to create a chamber that allows the user to set the O₂ concentration to varying set points of % O₂ while maintaining the chamber at a constant body temperature, CO₂ level, humidity, and sterility.

To start the project, multiple concepts were developed for the chamber design and the control system. These concepts were compared against developed engineering specs and were evaluated amongst the team and sponsor. From there a detailed CAD model was developed and utilized to design the structure and was used as a guide for manufacturing. The control system was prototyped on breadboards via Arduino. This breadboard testing served as the map to solder perf boards, which are utilized as the final structure for the control system.

Once all parts were sourced, machined, and assembled for the final chamber and the control system, these subassemblies were integrated together with a regulated gas system via various tubing. The integrated final design underwent a variety of testing to validate the incubator design and control system.

Testing was performed throughout the course of this project: material testing, gas leak testing, cell test, temperature control test, and gas control system optimization; however, the most important of these tests were those relating to the environmental control of the incubator. These tests confirmed whether the incubator design was functional as a practical incubator. Testing confirmed that O₂ and temperature control maintained in spec over a short and long period of time while maintaining a humid environment. CO₂ control optimization had more complications than the O₂ hypoxia system. During testing CO₂ concentration would typically overshoot the set point, likely due to a lack of precise control over the gas flow. CO₂ variability was reduced due to optimization in the code, but not fully mitigated. Future iterations of this chamber could improve upon the CO₂ control and streamline the user interface.

Keywords: [INCUBATOR, HYPOXIA, CELL CULTURE, CONTROL SYSTEM, GAS CONTROL, MECHANICAL DESIGN, PRODUCT DEVELOPMENT]
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Content</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>9</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>12</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>13</td>
</tr>
<tr>
<td>Project Objective</td>
<td>13</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>13</td>
</tr>
<tr>
<td>Biological Relevance</td>
<td>13</td>
</tr>
<tr>
<td>Engineering Relevance</td>
<td>15</td>
</tr>
<tr>
<td>Control Interface</td>
<td>17</td>
</tr>
<tr>
<td>Gas Control</td>
<td>19</td>
</tr>
<tr>
<td>Temperature Control</td>
<td>22</td>
</tr>
<tr>
<td>PROJECT BODY</td>
<td>23</td>
</tr>
<tr>
<td>Core Customer Charts</td>
<td>23</td>
</tr>
<tr>
<td>Success Criteria</td>
<td>23</td>
</tr>
<tr>
<td>Major Deliverables</td>
<td>23</td>
</tr>
<tr>
<td>Project Management</td>
<td>24</td>
</tr>
<tr>
<td>Specification Development</td>
<td>25</td>
</tr>
<tr>
<td>Engineering Specifications</td>
<td>25</td>
</tr>
<tr>
<td>Concept Generation and Evaluation</td>
<td>26</td>
</tr>
<tr>
<td>Initial Design Generation</td>
<td>26</td>
</tr>
<tr>
<td>Concept Sketches</td>
<td>27</td>
</tr>
<tr>
<td>Decision Matrix</td>
<td>29</td>
</tr>
<tr>
<td>Conceptual Models and Analysis</td>
<td>31</td>
</tr>
<tr>
<td>Cardboard Prototype</td>
<td>31</td>
</tr>
<tr>
<td>CAD Development</td>
<td>32</td>
</tr>
<tr>
<td>Control System Breadboard Prototype</td>
<td>41</td>
</tr>
<tr>
<td>Material Selection</td>
<td>45</td>
</tr>
<tr>
<td>Incubator Materials</td>
<td>45</td>
</tr>
<tr>
<td>Control System Materials</td>
<td>48</td>
</tr>
<tr>
<td>Prototype Manufacturing</td>
<td>50</td>
</tr>
<tr>
<td>Incubator Manufacturing</td>
<td>50</td>
</tr>
<tr>
<td>Control System Manufacturing</td>
<td>58</td>
</tr>
<tr>
<td>Electronics, Chamber, and Gas Integration</td>
<td>60</td>
</tr>
<tr>
<td>Test Protocol Development</td>
<td>63</td>
</tr>
<tr>
<td>Material Testing</td>
<td>63</td>
</tr>
<tr>
<td>Leak Testing</td>
<td>64</td>
</tr>
<tr>
<td>Control System Testing</td>
<td>65</td>
</tr>
<tr>
<td>Cell Incubation</td>
<td>67</td>
</tr>
</tbody>
</table>
Testing Data and Analysis ........................................................................................................68
  Material Testing ..................................................................................................................68
  Leak Testing .......................................................................................................................69
  Control System Testing ......................................................................................................74
  Cell Incubation ....................................................................................................................80
Discussion and Future Directions ..........................................................................................83
  Engineering Specifications and Success Criteria .................................................................83
  Limitations ...........................................................................................................................84
  Future Directions ................................................................................................................85
REFERENCES/WORKS CITED/BIBLIOGRAPHY ..................................................................87
APPENDICES .........................................................................................................................90
A.  Core Customer Charts .......................................................................................................90
  A.1 SUCCESS CRITERIA .................................................................................................90
  A.2 MAJOR DELIVERABLE CHARTS .............................................................................90
B.  Project Management Tools ..............................................................................................92
  B.1 FLEXIBILITY MATRIX ............................................................................................92
  B.2 RISK MANAGEMENT ...............................................................................................93
  B.3 BUDGET ....................................................................................................................93
  B.4 GANTT CHART ..........................................................................................................98
  B.5 PENTA CHART ..........................................................................................................102
C.  House of Quality .............................................................................................................103
D.  Prototype Survey .............................................................................................................103
E.  Bill of Materials ...............................................................................................................104
F.  Detailed Drawings: Incubator chamber ..........................................................................107
  F.1 WATER JET DXF FILES .........................................................................................107
  F.2 POLYCARBONATE MECHANICAL DRAWINGS ....................................................107
  F.3 ALUMINUM MECHANICAL DRAWINGS ...............................................................111
  F.4 ASSEMBLY DRAWINGS .........................................................................................25
G.  Control System Progression ............................................................................................26
H.  Data Sheets: Off-the-shelf Components .........................................................................27
I.  Detailed Manufacturing Protocols ....................................................................................30
  I.1 CHAMBER MANUFACTURING .................................................................................31
  I.2 PERF BOARD MANUFACTURING ..........................................................................67
  I.3 INTEGRATION PROTOCOL ......................................................................................74
J.  Additional Manufacturing Images .....................................................................................75
  J.1 INNER CHAMBER .....................................................................................................90
  J.2 SENSOR BOX .............................................................................................................90
  J.3 INNER DOOR ............................................................................................................90
  J.4 OUTER DOOR ...........................................................................................................91

7
LIST OF FIGURES

Figure 1. Shows increased HAECs, which will form blood vessels, from hypoxic environment. A shows HAECs suspended in serum-free endothelial basal medium (eBM*), conditioned media prepared under normoxic conditions (CdMNor), and conditioned media prepared under hypoxic conditions (CdMHyp) seeded on GFR-Matrigel in 96-well plates at 4x 10⁴ cells per well and cultured in hypoxic conditions for 48 hours. B is the same as A but seeded in standard Matrigel (Hung et al., 2007). .............................................. 14

Figure 2. Cell viability over time as a percentage of initial value (Baldea et al. 2018). ......................................................... 14

Figure 3. Schematic of Incubation System (Desai et al., 2011). ................................................................. 16

Figure 4. Silicone gas chamber including gas concentrations, flow regulation, and flow rate (Kreutzer et al., 2017). We will not be using an insert solution for our design, but this proposed chamber demonstrates a possible way to regulate gas flow. .................................................. 17

Figure 5. Incubator system layout and necessary components (Feki et al., 2017). ...................................................... 18

Figure 6. Circuit diagram of the Arduino phase-controlled system (Feki et al., 2017). ...................................................... 18

Figure 7. Gas concentrations in a CO2 incubator at 37°C and 0.5 km altitude. “Input air” is room air and “Incubator” is incubator air (Wenger et al., 2015). .................................................. 19

Figure 8. Setup of sensor circuit (Mathupala et al., 2016). ............................................................. 20

Figure 9. NDIR sensor diagram (Shen, 2014). ........................................................................................................ 21

Figure 10. DS18B20 Data Flow Chart (Iggy, 2022). ............................................................................................. 22

Figure 11. Major Deliverables Spider Chart. ........................................................................................................ 24

Figure 12. Design concept one.............................................................. 27

Figure 13. Design concept two........................................................................................................................................ 28

Figure 14. Design concept three................................................................................................................................ 29

Figure 15. Cardboard prototype: Outer incubator structure......................................................................................... 32

Figure 16. Cardboard prototype: incubator structure with model shelf and fan.............................................................. 32

Figure 17. Exploded view of the incubator structure................................................................................................. 33

Figure 18. Full incubator structure: door open view. ................................................................................................. 34

Figure 19. Door assembly CAD images featuring final design & hardware ................................................................. 35

Figure 20. Original sensor box design....................................................................................................................... 35

Figure 21. Second Iteration of sensor box design with incubator assembly................................................................. 36

Figure 22. Final design of sensor box assembly (front face hidden). .............................................................................. 37

Figure 23. Original fan assembly and inlet orientation................................................................................................ 37

Figure 24. Final design of fan assembly allowing for gas mixing.................................................................................... 38

Figure 25. Final design of fan assembly showing relationship to sensor box and gas inlets.......................................... 38

Figure 26. Display stand QTY 2............................................................................................................................... 39

Figure 27. Display stand assembly front and back view. .............................................................................................. 39

Figure 28. Solenoid stand QTY 3............................................................................................................................ 40

Figure 29. Hinge spacer QTY 2.............................................................................................................................. 40

Figure 30. Magnet holder QTY 2............................................................................................................................ 40

Figure 31. Magnet holder assembly including heat insert, standoff, and magnet. .............................................................. 41

Figure 32. Circuit setup for solenoids and heating pad control. ..................................................................................... 41

Figure 33. Pin layout of O₂ sensor............................................................................................................................. 43

Figure 34. CO₂ sensor pin layout............................................................................................................................... 44

Figure 35. Temperature sensor circuit with two sensors................................................................................................. 44

Figure 36. Inner aluminum chamber without OTS attachments. .................................................................................... 51

Figure 37. Sensor box including pressure relief valve................................................................................................. 52

Figure 38. Polycarbonate chamber epoxied & dried........................................................................................................ 53

Figure 39. Inner door front panel............................................................................................................................... 54

Figure 40. Outer door final assembly........................................................................................................................ 55

Figure 41. Final assembled removable shelf fully leveled. ............................................................................................ 55

Figure 42. 3D Printed per board holder.................................................................................................................... 56

Figure 43. 3D Printed display stands.......................................................................................................................... 57

Figure 44. 3D printed solenoid holders. ...................................................................................................................... 57

Figure 45. Back view of completed incubator with wire covering.................................................................................... 57

Figure 46. Front view, closed door, of completed incubator............................................................................................. 58

Figure 47. Close-up of gas inlets .................................................................................................................................. 58
Figure 48. Basic perf board setup..........................................................59
Figure 49. All four basic relay circuits set up..............................................60
Figure 50. Perf board final setup.................................................................60
Figure 51. Current control system setup.....................................................61
Figure 52. Control system with solenoids.....................................................62
Figure 53. Gas system setup. O₂ is the left tank, N₂ is the middle tank, and CO₂ is the right tank. .................................62
Figure 54. Solenoid setup...............................................................................63
Figure 55. Material testing setup.................................................................64
Figure 56. Materials before liquid immersion..............................................69
Figure 57. Materials after testing.................................................................69
Figure 58. Front view of initial door seal displaying bending at latch point. .............................................................70
Figure 59. Side view of initial door seal displaying side gapping....................70
Figure 60. Front view of inner door assembly displaying support material and seal. .......................................................71
Figure 61. Long term leak/seal test: percent O₂ over four-hour time period. O₂ concentration is in percent and the sample interval is 1 measured value per 1 second. .................................................................72
Figure 62. A. Change in temperature (°C) over 3 hours with no additional heat applied. Temperature is in °C and the sample interval is 1 measured value per 3 seconds. B. Rate of heat transfer (W) across the incubator wall over time. Q is in Watts and the sample interval is 1 measured value per 3 seconds. ............73
Figure 63. Linear fits for sections of O₂ data (left) and CO₂ data (right). O₂ is in percent and samples are taken every second. CO₂ is in percent and samples are taken every 3 seconds. ........................................................................................................75
Figure 64. Pareto charts from Minitab showing N₂ (B), O₂ (C), and their interaction term (BC) as significant factors in time out of spec for both conditions. .........................................................................................76
Figure 65. Pareto chart showing the significant factors N₂ (B), and O₂ (C) for time in spec for door open. ..........................77
Figure 66. Contour plot for time out of spec for CO₂ concentration, stationary. For both N₂ and O₂, -1 refers to a 1ms solenoid on time and 1 refers to a 3ms solenoid on time. .........................................................77
Figure 67. Contour plot for time out of spec for CO₂ concentration, open door. For both N₂ and O₂, -1 refers to a 1ms solenoid on time and 1 refers to a 3ms solenoid on time. .........................................................78
Figure 68. Contour plot for time in of spec for CO₂ concentration, open door. For both N₂ and O₂, -1 refers to a 1ms solenoid on time and 1 refers to a 3ms solenoid on time. .........................................................78
Figure 69. 1-hour testing data for CO₂ concentration (left) and O₂ concentration (right). CO₂ concentrations is in percent and samples are taken every 3 seconds. O₂ concentration is in percent and samples are taken every second.................................................................80
Figure 70. Overnight testing data for CO2 concentration (left) and O2 concentration (right). CO₂ concentrations is in percent and samples are taken every 3 seconds. O₂ concentration is in percent and samples are taken every second......................................................................................80
Figure 71. CO₂ concentration on day 1 (left) and day 2 (right) of cell testing. CO₂ concentrations is in percent and samples are taken every 3 seconds. .................................................................81
Figure 72. O₂ concentration on day 1 (left) and day 2 (right) of cell testing. O₂ concentration is in percent and samples are taken every second. .........................................................................................81
Figure 73. Temperature on day one (left) and day two (right) of cell testing. Temperature is in °C and samples are taken every 3 seconds. .........................................................................................82
Figure 74. Well plate from standard incubator after two days. ..........................82
Figure 75. Well plate from experimental incubator after two days. .................83
Figure 76. Evidence of humidification............................................................83
Figure 77. Network diagram........................................................................102
Figure 78. Penta chart..................................................................................102
Figure 79. House of quality..........................................................................103
Figure 80. DXF files used to water jet cut stock: polycarbonate left, aluminum right......................................................107
Figure 81. Breadboard relay circuit..............................................................114
Figure 82. Basic temperature sensor circuit.................................................114
Figure 83. Temperature relay test setup......................................................115
Figure 84. Perf board map..........................................................................115
Figure 85. Backside of perf board...............................................................116
Figure 86. Cutouts of the aluminum inner chamber........................................120
Figure 87. Tube adaptor with hex nut on gas inlet cutout..............................121
Figure 88. Fan assembly on top plate of aluminum chamber.......................121
Figure 89. Alignment of sensor box on top plate of aluminum chamber. .................................................................121
Figure 90. Drying epoxy for aluminum chamber assembly: 90˚ made using carpenter square & held in place with paint cans. .............................................................................................................................122
Figure 91. Applying and drying epoxy for sensor box. ...............................................................................................122
Figure 92. Sensor box with press fit components: pressure relief valve includes O-ring. .................................123
Figure 93. Latches fastened on inner door assembly displaying O-rings & spacers. .................................................123
Figure 94. Inner door assembly/front panel unassembled. .........................................................................................123
Figure 95. Polycarbonate half boxes for clearance with outer door hinges. ..............................................................124
Figure 96. Polycarbonate inner door support material. ..........................................................124
Figure 97. Plastic heat set insert in outer door used for threading standoffs. ........................................................125
Figure 98. Magnet attachment installed on outer door. .............................................................................................125
Figure 99. Holes drilled out for outer chamber pre epoxying. ..................................................................................126
Figure 100. Epoxied outer chamber with feet and nylon spacer. .............................................................................126
Figure 101. Shelf top plate: each hole measured, drilled, and deburred. ...............................................................127
Figure 102. Each leg was hand sawed, filed, & leveled. .........................................................................................127
Figure 103. Four legs were epoxied first, the second bottom plate was leveled and epoxied second. ....128
Figure 104. Measuring and cutting insulation material. .........................................................................................128
Figure 105. Fitting inner insulation material. ......................................................................................................129
Figure 106. Inner chamber with heating pad, fan, temperature sensors, and removable shelf. .........................129
Figure 107. O2 Regulator. .................................................................................................................................130
Figure 108. N2 Regulator. .................................................................................................................................130
Figure 109. CO2 Regulator. ...............................................................................................................................131
Figure 110. In-line HEPA filter. ..........................................................................................................................131
Figure 111. Sensors in place in sensor box. ..........................................................................................................132
Figure 112. Display in stand. ...............................................................................................................................132
Figure 113. Functioning display. ........................................................................................................................133
Figure 114. 12 V power supply. ........................................................................................................................133
LIST OF TABLES

Table I. Engineering specifications.................................................................25
Table II. Features for three overall concept designs.......................................26
Table III. Incubator material selections for three concept designs..................27
Table IV. Hierarchy of needs for an alpha build...........................................30
Table V. Concept evaluation chart.................................................................31
Table VI. Table of factors and levels for factorial analysis............................67
Table VII. Sensor testing results......................................................................74
Table VIII. Control system response testing results........................................75
Table IX. Overall success criteria.................................................................90
Table X. Major sub-deliverable 1: Literature review.......................................90
Table XI. Major Sub-deliverable 2: Incubation chamber structure...................90
Table XII. Major sub-deliverable 3: Gas system...........................................91
Table XIII. Major sub-deliverable 4: Temperature and humidity system..........91
Table XIV. Major Sub-deliverable 5: System integration and assembly testing...91
Table XV. Major sub-deliverable 6: User interface........................................92
Table XVI. Major sub-deliverable 7: User documentation.............................92
Table XVII. Flexibility matrix........................................................................92
Table XVIII. Risk assessment.........................................................................93
Table XIX. Initial budget................................................................................93
Table XX. Final budget..................................................................................94
Table XXI. Full project Gantt chart...............................................................99
Table XXII. Bill of materials used in incubator structure...............................104
Table XXIII. Refined data for CO₂ - stationary...........................................141
Table XXIV. Refined data for CO₂ - door open............................................142
Table XXV. Refined data for O₂ - stationary................................................143
Table XXVI. Refined data for O₂ - door open...............................................144
Table XXVII. Factorial setup – CO₂ and O₂ data..........................................145
Table XXVIII. CO₂ data - stationary.........................................................145
Table XXIX. CO₂ data – door open..............................................................145
Table XXX. O₂ data – stationary.................................................................146
Table XXXI. O₂ data – door open...............................................................146
Chapter 1

INTRODUCTION

Project Objective

Design and create a prototype of a cleanable and sterilizable hypoxic incubation chamber with functional, controllable gas and heat systems and humidity that can successfully be used to incubate cells in hypoxic conditions for $2000 by August 31, 2022.

LITERATURE REVIEW

Biological Relevance

Hypoxia is defined as insufficient oxygen concentrations in tissues and cells and is relevant in many diseases and physiologic processes (Pavlacky & Polak, 2020). Hypoxia contributes to the development of pathological conditions including systemic inflammatory response, tumorigenesis, and cardiovascular disease (Brennan et al., 2014). Traditionally to model hypoxia, a concentration of 1% O₂ has been used. However, this choice of concentration is flawed as it does not match physiological situations in vivo. There is no precise answer to what hypoxia values are in vivo because it depends on tissue type (Wenger et al., 2015). Therefore, it is important to develop a hypoxic incubation chamber that can deliver a range of concentrations of O₂ rather than using pre-mixed gases.

Hypoxia in biological tissue will lead to the release of vascular endothelial growth factor (VEGF) among other growth factors, which induces the process of angiogenesis (Berger et al., 2003). The connection between hypoxia and angiogenesis has been verified through research, specifically its effect on endothelial cells. When cultured in human multipotent stromal cell conditioned media in a hypoxic environment, it was found that formation of human aortic endothelial cells (HEACs) was promoted, as seen in Figure 1 (Hung et al., 2007). This was relevant to the intended lab use of the incubator for encouraging blood vessel growth on a tumor model.
Figure 1. Shows increased HAECs, which will form blood vessels, from hypoxic environment. A shows HAECs suspended in serum-free endothelial basal medium (eBM*), conditioned media prepared under normoxic conditions (CdM*Nor), and conditioned media prepared under hypoxic conditions (CdM*Hyp) seeded on GFR-Matrigel in 96-well plates at 4x 10^4 cells per well and cultured in hypoxic conditions for 48 hours.

B is the same as A but seeded in standard Matrigel (Hung et al., 2007).

It is important to note that incubation in hypoxic conditions can also negatively affect cells. In a time-point study done on human umbilical vein endothelial cells, it was found that that prolonged exposure to hypoxia, especially 0% O_2 reduced cell viability. Additionally, severe hypoxia and longer exposures to mild hypoxia induced high oxidative stress related damage and eventually led to apoptosis (Baldea et al., 2018). The effect on cell viability can be seen in Figure 2.

Figure 2. Cell viability over time as a percentage of initial value (Baldea et al. 2018).
Various companies, individuals, and labs have tested cells in a controlled setting in hypoxic environment. In order to perform experiments such as encouraging blood vessel growth on a tumor model or understanding cell viability, a controlled hypoxic environment is necessary. On the current market there are a few hypoxic incubator models, but many are limited in user oxygen control.

**Engineering Relevance**

Before designing the novel hypoxic incubator, an understanding of current incubator designs is needed. There are many incubators currently available, but few with oxygen controls. OpenTCC, is a current open-source low-cost temperature-control chamber. The chamber controls temperatures between 20-55˚C, is cheap (less than $400), and is made from a light aluminum, eight heating pads, two cooling modules, and electronics that are open-source only (Sánchez et al., 2020). This incubator uses an Arduino microcontroller and a Python interface. The chamber includes extruded polystyrene sheets that help insulate the chamber for long periods of time. Both using polystyrene to insulate the chamber and an aluminum outer shell for structural support could be viable options as materials for the Hypoxic Incubator design. Looking into this design gives us a look into an inexpensive design strategy. Another affordable design was a fabricated on-stage cell incubator. Ragazzini et al. developed a less than 300-dollar microscope cell incubator, which also uses Arduino control. Instead of a python interface this system uses LabView, connecting via a USB port to monitor and display all the parameters desired, which in this instance is CO₂ concentration, temperature, and humidity (Ragazzini et al., 2019). Using a LabView interface was considered as a solution for our incubator design. A separate group also used LabView to make an on-stage incubator focusing on two separate chambers: one for humidity and specifically for CO₂. This design solution is shown in the Figure 3 below.
Figure 3. Schematic of Incubation System (Desai et al., 2011).

A more complex, yet more expensive current solution is the Incuver incubator. The Incuver incubator allows for control of oxygen and carbon dioxide concentration, is compact and easily transportable, and has automation to simulate conditions including live cell imaging and remote monitoring. This incubator contains all the technology that will be incorporated in the hypoxic incubator. Although, the Incuver has an estimated price of 6,000 euros (Sánchez et al., 2020).

One incubator design that differed from others was a cell culture chamber with a gas supply for prolonged recording of human neuronal microelectrode array. The chamber has a silicone structure which was gas permeable, allowing gas transfer to and from the chamber (Kreutzer et al., 2017). This chamber was inserted into an incubator and acted more as an insert, similar to the prior senior project design for the gas-controlled chamber. The membrane thickness itself between the gas supply and culture chamber affected the distribution of CO₂. Figure 4 below shows the chamber system flow chart.
Figure 4. Silicone gas chamber including gas concentrations, flow regulation, and flow rate (Kreutzer et al., 2017). We will not be using an insert solution for our design, but this proposed chamber demonstrates a possible way to regulate gas flow.

Control Interface

An integral part of our incubator design is using control systems to have automated gas-controlled and temperature-controlled environment. Background on basic control systems as well as understanding more complex temperature and gas feedback systems will be used utilized in the final design of the hypoxic incubator. Control systems contain both electrical and coding elements and are often modeled and simulated using mathematical models. Feki et al. used a generalized predictive control (GPC) temperature control system and created a Matlab/Simulink model to demonstrate and optimize the ideal air temperature for a newborn incubator system (Feki et al., 2017). In this study, they also compare their predictive control system’s effectiveness to a proportional–integral–derivative controller (PID). PID controls use a “on/off” mechanism vs. an adaptive control. Their control of heating element was applied under the constraints of the air temperature and the power of the actuator. They also incorporated skin temperature and core temperature at parameters in the MATLAB-Simulink model. Using a mathematical model, all the necessary parameters were defined, and equations were used to create looping control heating system. They then used an Arduino board to design the heat control circuit, which uses an analogue input to acquire the desired temperature (Feki et al., 2017). A similar Arduino board circuit can be utilized for the hypoxic incubator design with a similar incubator set up, shown in Figures 5 & 6 below.
The Arduino board was able to interface with the MATLAB-Simulink model to create a controllable temperature environment. A separate group used a temperature and humidity decoupled proportional integral derivative (PID) controller for a bioprinter atmospheric enclosure system (Matamoros et al., 2020). This bioprinter is dependent on CO$_2$, temperature, and humidity, three components of equal relevance of the Hypoxic Incubator. This bioprinter control had three main processes all controlled by Arduino UNO boards, a Raspberry Pi, and a Python Script. Their report included relevant temperature, humidity, and CO$_2$ sensors, as well as useful information on Laplace transform equations and PID control. Similar to the newborn incubator, experimental transfer functions were obtained from test data using the MATLAB/Simulink mathematical model (Matamoros et al., 2020). For the design of the control
systems for both temperature and gas levels for the Hypoxic Incubator a similar approach can be utilized either using a PID or GPC control system, and it can also be modeled on MATLAB/Simulink.

**Gas Control**

Before controlling the gases, it is important to understand normoxic and hypoxic concentrations of gases in human tissues. Normoxic conditions for dry air are 20.9% concentration of O$_2$, which when used as input for an incubator becomes 18.6% concentration (Wenger et al., 2015). This is due to the incubator input of 5% CO$_2$ and water vapor. Figure 7 below illustrates this concept.

![Figure 7](image)

**Figure 7.** Gas concentrations in a CO2 incubator at 37°C and 0.5 km altitude. “Input air” is room air and “Incubator” is incubator air (Wenger et al., 2015).

However, elevation will affect gas concentrations as well. When inputting gas into the hypoxic chamber, similar considerations will have to be kept in mind.

Oxygen control is the focus of this hypoxic incubator. In microfluidics, oxygen can be controlled by devices that rely on diffusion from liquid or gas, utilizing on-or-off-chip mixers, leveraging cellular oxygen uptake to deplete the oxygen, relying on chemical reactions in channels to generate oxygen gradients in a device, or electrolytic reactions to produce oxygen directly on chip (Brennan et al., 2014). A similar method was considered for employing oxygen control for the hypoxic incubator.
Ideally, gas can be controlled with a microcontroller and solenoid valves. Others have had success building an Arduino controlled sensor module for continuous oxygen measurement for a hypoxic chamber, the configuration of which can be seen in Figure 8.

![Figure 8. Setup of sensor circuit (Mathupala et al., 2016).](image)

They were able to run multiple experiments with sensor setup to test and validate its use as a continuous oxygen sensor. One important aspect they were able to achieve was setting up the circuit so that O₂ values could be adjusted mid-experiment (Mathupala et al., 2016). This is relevant because they needed more than one gas purge throughout their experiments to maintain hypoxia. This was used as a model when designing the oxygen/gas control system for the hypoxic incubator. Another, more complicated model for oxygen control can be seen in Figure 10. This setup utilized a PIC microcontroller.

While this may be too complicated for practical use in this project, it would still be useful in developing ideas for oxygen sensing and integrating a solenoid valve with a sensing circuit. The response time of the sensor was 10-13 seconds, and the response time of the control system was 5.5 seconds (Burunkaya & Guler, 2006).

One way of controlling gas would be to simply input the desired concentrations of gas, as done by a group of researchers developing a new experimental hypoxia chamber. However, this depends on the design of the incubator. The new hypoxia chamber that was developed was modeled after a Ziploc bag, which allowed for easy control of gas concentrations since air could be vacuumed out of the bag and then filled with desired concentration of each gas (Wang et al., 2014). As the chamber developed for this project was
more structural than this, vacuuming out the air would prove to be a challenge. A similar method could be employed by flushing the incubator with inert gas for a set amount of time so the concentration would initially be 100% inert gas. The chamber concentrations could be altered from there. This is similar to the tactic used by hypoxic incubators that utilize pre-mixed gases; however, the chambers are flushed with the mixed gas, not an inert gas (Shay & Wright, 2006).

For these control systems to function, it is important to understand how the sensors used within the system work. The carbon dioxide sensor used in this project is an NDIR sensor, which stands for nondispersive infrared. This is a commonly used sensor for measuring CO₂ concentrations. It’s transduction mechanism utilizes the infrared light (IR) by directing through a tube filled with a sample of air which contains an optical filter and an IR light detector. As the IR light is passing through the tube, certain frequencies are absorbed by CO₂ while others continue down the tube to the detector, as seen in Figure 9. To obtain the measurement for CO₂, the difference between the light emitted and light absorbed by the detector is measured. This difference is directly proportional to the number of CO₂ molecules in the air sample. The measurement is outputted as an analog micro-voltage, which is then converted to a digital signal and further converted to a serial output which is compatible with many microcontrollers (How does an NDIR..., 2022).

![NDIR CO2 Sensor](https://example.com/ndir_sensor_diagram.png)

**Figure 9.** NDIR sensor diagram (Shen, 2014).

The LuminOx oxygen sensor, used in this project, uses photodiode technology. The sensor has a PTFE filter, outer casing, a carrier containing a UV filter and a Dichroic Mirror/Ruthenium Complex, a PCB with a pressure and temperature sensor and a photodiode, and an outer casting (SST, 2019). The dichroic mirror acts as an interference filter only allowing light of a specific wavelength to reach the photodiode. A timing circuit receives and measures the rate of decay of the excited fluorophore from the electrical current

21
produced by the photodiode. The rate of decay is inversely related to the oxygen level at a certain temperature and pressure. The sensor has a wideband pressure sensor that allows for high altitude and vacuum settings. Using the rate of decay, the sensor calculates and outputs the partial pressure of oxygen (SST, 2019 & The Science Behind…, 2022).

**Temperature Control**

This project utilizes DS18B20 temperature sensors for the control system. These are 1-wire digital temperature sensors with a resolution that ranges for 9 to 12-bits (Agarwal, 2019). The sensors are wired parasitically for this project, which allows multiple sensors to run simultaneously (Das, 2022). A data flow diagram of the sensor may be seen in Figure 10, below. The low temperature coefficient oscillator is kept at a constant frequency and the signal is sent to the first subtraction counter. The high temperature coefficient oscillator changes its frequency in response to a change in temperature. This signal is sent to the second subtraction counter. The subtraction counter and ramp accumulator are compared and open a counting gate. When this gate is open, the sensor counts the clock pulses generated by the low temperature coefficient oscillator which completes the temperature measurement (Iggy, 2022).

![Figure 10. DS18B20 Data Flow Chart (Iggy, 2022).](image-url)
PROJECT BODY

Considering past incubator designs, possible sensors, control systems, and the overall biological relevance for hypoxia, the hypoxic incubator project requirements and goals were established. In the following section, the project development from goals to the end product was tracked. The report body will move through and discuss the project management, design process, conceptual model development, manufacturing, testing, and overall project outcomes.

Core Customer Charts

Success Criteria

The goal of this project was to design, manufacture, and test a prototype of a sterilizable and easy to clean hypoxic incubation chamber with functional, controllable gas and heat systems and passive humidity control that can successfully incubate cells in hypoxic conditions. The overall success criteria and scope of the project was outlined and broken down, shown in Appendix A.1. Included within the scope of this project was a functional, controllable gas systems to set and control oxygen and carbon dioxide gas levels, a functional and controllable heating system to maintain an in vivo environment, and to maintain uniform gas and heat distribution in the chamber environment. The physical chamber also needed to be built within a set budget and be airtight to minimize gas loss and maintain the set gas concentrations, temperature, and humidity. Overall, project success was determined by the ability to create a hypoxic environment in which cells may be cultured. Success for this project did not include a device that was market ready or that contained a streamlined user interface. Validating that the chamber can create a hypoxic environment was necessary but testing that angiogenesis can occur in the hypoxic chamber was not within the scope of this project.

Major Deliverables

The major project deliverables, to achieve a final functional prototype, were broken into seven distinct sub-deliverables. Figure 11 details the major deliverable for the project and the sub-deliverables within the ultimate project goal. The success criteria for each subsystem can be viewed in greater detail in Appendix A.2.
Each sub-deliverable was assigned to project lead to maintain the deliverable timeline and make any final decisions regarding the goals of the subsystem. Simone was assigned to lead the incubator chamber structure, temperature and humidity system, and the user interface and documentation. Makenzie was assigned to lead the literature review, the gas control system, and the system integration, assembly, and testing. Overall, each subsection was worked on by both team members and responsibilities were split accordingly as project tasks arose. In order to deliver the overall goal of a working prototype with functional subsystems, project management tools were utilized from the start of the project.

**Project Management**

To manage the project spending, a detailed budget was updated throughout the project. An initial budget was crafted to estimate costs before purchasing through extensive material research. Throughout the project, the initial estimate was modified based on items purchased into a final budget, which may be seen in Appendix B.3. There was around an $800 discrepancy between the estimated total and actual total of our budget. This was partially due to an underestimation of shipping costs for certain large materials, along with the need for additional components used in the incubator structure and control system that were not initially considered as the design was manufactured and tested. Throughout project planning, a detailed Gantt chart was maintained and updated each quarter to track our progress and maintain a steady work pace. Our project plan has been outlined in the Gantt chart and through the network diagram, shown in
Appendix B.4. Our final project management tool was the Penta chart, which fully outlines the overall purpose and goals of the project. This chart was used for communicating the project to committee members and more general audiences, found in Appendix B.5.

**Specification Development**

Engineering Specifications

Engineering specifications were developed based on major deliverables and the customer requirements. The customer requirements included in the functional gas control system were maintaining and reaching a set hypoxic oxygen concentration, maintaining 5% CO$_2$ levels, and having a sealed incubator environment. The customer also needed the system to maintain a humid environment at a set temperature, as well as have the chamber easy to clean and sterilize. Components were required to last 2 years or longer and need minimal charging or battery replacement if any. These requirements were refined and established into engineering specifications with measurable quantifiable target values and set tolerances, detailed in Table I below.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Target</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>gas sealed incubator environment</td>
<td>&lt; 4% of O$_2$ setpoint over 4 hours with no control system</td>
<td>Max</td>
</tr>
<tr>
<td>fit T-75 flasks</td>
<td>2 flasks</td>
<td>Min</td>
</tr>
<tr>
<td>maintain O$_2$ concentration</td>
<td>&lt; 5% O$_2$ for 48hrs</td>
<td>±0.1% O$_2$</td>
</tr>
<tr>
<td>reach O$_2$ concentration quickly</td>
<td>&lt; 3 hours</td>
<td>+/− 30 minutes</td>
</tr>
<tr>
<td>high temperature resistance</td>
<td>Withstand up to 121°C</td>
<td>± 10˚C</td>
</tr>
<tr>
<td>pressure resistant</td>
<td>Withstand up to 16 psig</td>
<td>± 1 psig</td>
</tr>
<tr>
<td>cleanable &amp; sterilizable</td>
<td>5 cycles/day with no sign of material alteration</td>
<td>± 1 cycle</td>
</tr>
<tr>
<td>maintain a humid environment</td>
<td>Visible condensation within 30 min at setpoint temperature</td>
<td>Within ± 15 min</td>
</tr>
<tr>
<td>maintain incubator CO$_2$ concentration</td>
<td>At 5% CO$_2$ for 48hrs</td>
<td>±0.5% CO$_2$</td>
</tr>
</tbody>
</table>
**maintain stable incubator temperature**

<table>
<thead>
<tr>
<th>Component lifetime</th>
<th>2 years</th>
<th>+/- 2 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component battery life</td>
<td>48 hours w/o charge or change</td>
<td>+/- 6 hours</td>
</tr>
</tbody>
</table>

Conditions, dimensions, and other categorized qualifications were defined based on the specifications developed. The incubator structural dimensions must allow for the number of T-75 flasks necessary; the control system must maintain and measure O₂, CO₂, and temperature, and the structure must be a sterilizable, cleanable, reusable environment. To assess the importance of each criterion and compare our design to the industry standards the House of Quality was utilized, which can be referenced in greater detail in Appendix C.

**Concept Generation and Evaluation**

**Initial Design Generation**

To begin the design process, three initial designs were developed for the general incubator design via breaking down major components of the structure. For concept generation two separate design decision matrices were developed on Excel. First, generic feature categories were identified for the overall incubator design: sensor location, incubator shape, door concept, electronics coding interface, heating component, and incubator material (outer, insulation, & inner). Then for each of these categories, three separate possible options were identified. These developed options were all listed out under its respected feature category. All the developed options were then sorted and assigned into three separate concepts. The features for each concept are described in Table II & III below.

*Table II. Features for three overall concept designs.*

<table>
<thead>
<tr>
<th>Features</th>
<th>Sensor Location</th>
<th>Incubator Shape</th>
<th>Door Concept</th>
<th>Coding Interface</th>
<th>Heating Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concept 1</td>
<td>In the chamber</td>
<td>Rectangle</td>
<td>Double door</td>
<td>Arduino</td>
<td>Heating element</td>
</tr>
<tr>
<td>Concept 2</td>
<td>In an incubator enclosed chamber</td>
<td>Irregular shape</td>
<td>Single door</td>
<td>Lab View</td>
<td>Heating pad</td>
</tr>
<tr>
<td>Concept 3</td>
<td>In an external gas sampling chamber</td>
<td>Square</td>
<td>Circle door</td>
<td>Python</td>
<td>Pre-heated air inlet</td>
</tr>
</tbody>
</table>
Table III. Incubator material selections for three concept designs.

<table>
<thead>
<tr>
<th>Incubator Material</th>
<th>Inner Surface</th>
<th>Insulation</th>
<th>Outer Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concept 1</td>
<td>Aluminum</td>
<td>Sheet Foam</td>
<td>Stainless Steal</td>
</tr>
<tr>
<td>Concept 2</td>
<td>Copper</td>
<td>Spray Foam</td>
<td>Plastic/Resin</td>
</tr>
<tr>
<td>Concept 3</td>
<td>Copper</td>
<td>Foam w/ water seal</td>
<td>Acrylic</td>
</tr>
</tbody>
</table>

Concept Sketches

Three separate sketches were then made to incorporate the pre-selected features. These sketches are included in Figure 12-14, shown below. Concept one, shown in Figure 12, uses an aluminum sheet inner material, with a foam sheet insulation insert, and an outer stainless-steel panel. Inside the wall plate there would be a heating element inserted to provide the heating component of the incubator. The overall shape of this design is rectangular, and it would use a double door design within chamber sensors. On the back panel there would be pressure release values and O₂, CO₂, and temperature sensors. The double door design contains a thin inner door made of a glass or acrylic material that is used to create the airtight seal against the door opening and a second thicker door that will help insulate the chamber. The interface used for the temperature and gas control circuits would be Arduino.

Figure 12. Design concept one.
Concept two, shown in Figure 13, uses a copper sheet for the inner material, with a spray on foam insulation insert, and an outer plastic panel material. Inside the wall plate there would be a heating pad inserted to provide the heating component of the incubator. The overall shape of this design is irregular, with a back panel extrusion providing a separated and protected space of the gas level sensors. This incubator uses a single door design, which uses a pressure/spring system to maintain the airtight environment. Like concept one the door would be made of a glass, plexiglass, or acrylic material. On the back panel there would be pressure release values and O₂, CO₂, and temperature sensors. The interface used for the temperature and gas control circuits would be LabView.

![Design 2: Control interface.](image)

**Figure 13.** Design concept two.

The final concept, concept three shown in Figure 14, uses a copper sheet for the inner material, a sheet of foam that has a water seal attached for an inner insulation layer, and an outer acrylic panel material. To provide the heating component of the incubator, there would be a hot air inlet in the chamber, which would be produced by a separated heating element. The overall shape of this design is square, with an additional small, separated chamber where the gas level sensors will reside. This incubator uses a single circular door design, which uses a system similar to a washing machine to maintain the airtight environment. On the back panel there would be pressure release values, and the three inlets for O₂, CO₂, and N₂ gas. The interface used for the temperature and gas control circuits would be Python.
Decision Matrix

To evaluate these three concepts, a hierarchy of needs for an alpha build was developed and weighted using previously made customer requirements and engineering specifications, shown in Table IV. The “needs” were broken into four categories of Usability, Cost, Time, and Reliability. The weighting consisted of scoring each “need” with a value of 1, 3, or 9, where 9 was most important. From this weighting we determined that Usability and Reliability categories are most important to our project.
Table IV. Hierarchy of needs for an alpha build.

<table>
<thead>
<tr>
<th>Specification Needs</th>
<th>Weighting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy to Open door</td>
<td>9</td>
</tr>
<tr>
<td>Intuitive Controls</td>
<td>9</td>
</tr>
<tr>
<td>Easy to Clean (wipe down)</td>
<td>3</td>
</tr>
<tr>
<td>Easy to Sterilize</td>
<td>9</td>
</tr>
<tr>
<td>Easy to read display</td>
<td>3</td>
</tr>
<tr>
<td>Easy to culture in (fits flasks well)</td>
<td>9</td>
</tr>
<tr>
<td>Intuitive Interface: setting parameters</td>
<td>9</td>
</tr>
<tr>
<td>Long lasting electronics</td>
<td>9</td>
</tr>
<tr>
<td>Durable sensors</td>
<td>9</td>
</tr>
<tr>
<td>Seal that survives (&gt;1,000,000) cycles</td>
<td>9</td>
</tr>
<tr>
<td>Corrosion resistant material</td>
<td>3</td>
</tr>
<tr>
<td>Cost Effective Materials</td>
<td>9</td>
</tr>
<tr>
<td>Open-Source Circuit Board</td>
<td>3</td>
</tr>
<tr>
<td>Easy to Assemble</td>
<td>3</td>
</tr>
<tr>
<td>Inexpensive Display</td>
<td>1</td>
</tr>
<tr>
<td>Fewer Custom Parts</td>
<td>3</td>
</tr>
<tr>
<td>In-house manufacturing</td>
<td>3</td>
</tr>
<tr>
<td>Easy sourcing From the U.S.</td>
<td>9</td>
</tr>
<tr>
<td>Quick Manufacturing Time</td>
<td>3</td>
</tr>
<tr>
<td>Ease of Testing</td>
<td>3</td>
</tr>
</tbody>
</table>

Each concept was then ranked either as worse, same, or better than a baseline design. For this chart, the Stem Cell Technologies incubator was used, a currently on the market hypoxic incubator that uses pre-mixed gas. Using this concept evaluation system, the top concept was determined (Table V). From these evaluations, Concept one ranked the highest with a total score of 44 and Concept three ranked the lowest with a total score of -7. Using the general form of Concept one and the defined specifications, a CAD model was then developed.
Table V. Concept evaluation chart.

<table>
<thead>
<tr>
<th>Heirarchy of Needs</th>
<th>STEM Cell Technologies</th>
<th>Design 1</th>
<th>Design 2</th>
<th>Design 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Alpha Build)</td>
<td>(Baseline concept)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Usability</th>
<th>Lab Use</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy to Open door</td>
<td>9 same</td>
<td>better</td>
<td>better</td>
<td>better</td>
</tr>
<tr>
<td>Intuitive Controls</td>
<td>9 same</td>
<td>better</td>
<td>same</td>
<td>worse</td>
</tr>
<tr>
<td>Easy to Clean</td>
<td>3 same</td>
<td>better</td>
<td>better</td>
<td>worse</td>
</tr>
<tr>
<td>Easy to Sterilize</td>
<td>9 same</td>
<td>better</td>
<td>better</td>
<td>worse</td>
</tr>
<tr>
<td>Easy to read display</td>
<td>3 same</td>
<td>same</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td>Easy to Culture (fit Flask well)</td>
<td>9 same</td>
<td>same</td>
<td>same</td>
<td>worse</td>
</tr>
<tr>
<td>Intuitive interface setting parameters</td>
<td>9 same</td>
<td>better</td>
<td>worse</td>
<td>worse</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability</th>
<th>Lab Use</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Long lasting electronics</td>
<td>5 same</td>
<td>same</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td>Durable sensors</td>
<td>5 same</td>
<td>worse</td>
<td>better</td>
<td>better</td>
</tr>
<tr>
<td>Seal that survives (&gt;1,000,000) cycles</td>
<td>5 same</td>
<td>same</td>
<td>better</td>
<td>better</td>
</tr>
<tr>
<td>Corrosion resistant material</td>
<td>3 same</td>
<td>same</td>
<td>better</td>
<td>same</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cost</th>
<th>Lab Use</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost Effective Materials</td>
<td>3 same</td>
<td>worse</td>
<td>worse</td>
<td>worse</td>
</tr>
<tr>
<td>Open source Circuit board</td>
<td>3 same</td>
<td>better</td>
<td>better</td>
<td>better</td>
</tr>
<tr>
<td>Easy to Assemble</td>
<td>1 same</td>
<td>worse</td>
<td>worse</td>
<td>worse</td>
</tr>
<tr>
<td>Inexpensive display</td>
<td>3 same</td>
<td>better</td>
<td>better</td>
<td>better</td>
</tr>
<tr>
<td>Fewer Custom Parts</td>
<td>3 same</td>
<td>better</td>
<td>better</td>
<td>better</td>
</tr>
<tr>
<td>In house manufacturing</td>
<td>3 same</td>
<td>better</td>
<td>better</td>
<td>better</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Within Year</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy sourcing From the U.S.</td>
<td>9 same</td>
<td>same</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td>Quick Manufacturing Time</td>
<td>3 same</td>
<td>worse</td>
<td>worse</td>
<td>worse</td>
</tr>
<tr>
<td>Ease of Testing</td>
<td>3 same</td>
<td>same</td>
<td>same</td>
<td>same</td>
</tr>
</tbody>
</table>

| Net Score | 0 | 44 | 38 | 7 |

Conceptual Models and Analysis

Cardboard Prototype

A cardboard cutout prototype was made to assess the dimensioning and component sizing for the incubator structure, and to see the incubator sizing on the lab bench. The prototype did not include the two doors, only the outer, inner, and sampling chambers, which can be seen in Figures 15 & 16. This prototype was placed in the lab where the incubator is now located with an included QR code survey that was used to survey the opinion of individuals in the lab on the overall size and usability, the survey questions and responses can be referenced in Appendix D.
Reviewing the survey questionnaire, the main concern was understanding how many flasks should be able to fit in the chamber. This feedback helped us be intentional about fitting 2-4 flasks comfortably. Although the primary cell container will be smaller petri dishes.

**CAD Development**

*Final Incubator CAD Model*

The final design was updated and modeled in SolidWorks 2020. This model was used to create the initial design the incubator structure, to assess how much material to order, and to machine the final incubator structure. Figure 17 & 18 show the final updated status of the incubator CAD model. This model was
edited as necessary for the machining on the incubator as changes arose throughout the project. The final built structure underwent a few deviations from the CAD model as conditions changed, these changes are noted in the manufacturing section of the report. The CAD model does not feature all the required fasteners, the adjustable feet, finalized shelf design, rotated door with cutouts, and nylon spacers. These design iterations, additional features were added in during manufacturing and will be noted and seen in the Chamber Manufacturing & Final Chamber Assembly sections of the document. Additional files were created to water jet our materials and these images, mechanical drawings, and additional CAD model images, and can be found in Appendix F.

Figure 17. Exploded view of the incubator structure.
The door design remained conceptually the same, but throughout the year was modeled, dimensioned, and all the details were added in. Two glass door latches were attached to the front panel and polycarbonate door for the inner door assembly. A latch, which requires two holes to be drilled is secured on the polycarbonate door face, and surrounding the door is the gasket. For the outer door there are two corner brackets that interact with two magnets on the outer door. These magnets are held in place with two 3D printed attachments that connect with the outer door via a male/male standoff that screws into heat set inserts on both the door and the 3D printed part. This magnet attachment will keep the outer door shut but will not be airtight. The door design can be seen in Figure 19 below.
The original design of the sensor box seen in Figure 20 below, had a tube outlet and inlet arbitrarily in the side of the box with the sensors contained inside. The sensors also were sealed into the sensor box, which did not allow for the sensors to be replaced or monitored. Air flow through the sensors was also a concern, as the air at the inlet was stagnant. For the sensors to properly function, they need a constant flow of air over the sensing surface.

The updated sensor box design includes an airtight section and a non-airtight section, where the sensors (CO₂ and O₂) can be removed/replaced. The box now sits on top of the incubator structure, where our fan
directly blows air into the inlet opening and moves the mixed air across the sensors. The air will then flow out through the other circle outlet on the other side of the chamber. Iteration 2, shown in Figure 21 below, used 4 heat-set inserts and 4 screws to attach the outer plate to allow access to the electronics. Every other element is epoxied together.

![Figure 21. Second Iteration of sensor box design with incubator assembly.](image)

The final iteration of the sensor box uses two shelf brackets, nuts, and screws to attach the top of the sensor box. The iteration allowed for easier machining, drilling through the material thickness was not ideal and could have led to cracking or other issues. The two brackets also allow for easy removal of the lid. In addition, a cut out was added for the pressure relief valve on the second layer of the chamber. Adding the pressure relief valve here was the most optimal placement in terms of manufacturability and ease of component replacement. This final design iteration is show in Figure 22 below.
The original design for the fan attachment and gas inlet placement is shown in Figure 23 below. The design had the gas inlets coming from three cut outs in the top of the box and the fan was attached directly to the roof of the structure. The placement of these inlets was arbitrary, and the fan was oriented to blow downward into the main box environment.

The update and final design iteration had the gas inlets intentionally placed on the back panel, the fan orientation was flipped 180° to blow up into the sensor box and was suspended via standoffs approximately 1/2 inch from the inlet of the sensor box. The standoffs were screwed directly into threaded holes in the aluminum plate. This system was redesigned so the gases after being injected into the system will be sucked into the fan, mixed, then blown upwards towards the sensor box. The gas inlets entered through the
polycarbonate and are connected to the aluminum through an adaptor & and hex nut, shown in Figure 24.

In Figure 25, the orientation of the fan, inlets, and sensor box can be observed.

**Figure 24.** Final design of fan assembly allowing for gas mixing.

**Figure 25.** Final design of fan assembly showing relationship to sensor box and gas inlets.
3D Printed Parts CAD Models

All 3D printed parts used for the project were modeled in SolidWorks 2020. These files were converted to STL files and were sent to various on campus printers. The following items were created: solenoid stands, display stands, perf board holder, magnet holders, and the hinge spacers. For the designing of these parts the solenoids, displays, perf boards, magnets, and hinges were all hand measured using a caliper and ruler. The measured dimensions created the framework for the printed parts. The final printed designs are shown in the Figures 26-31 below.

Figure 26. Display stand QTY 2.

Figure 27. Display stand assembly front and back view.
Figure 28. Solenoid stand QTY 3.

Figure 29. Hinge spacer QTY 2.

Figure 30. Magnet holder QTY 2.
Control System Breadboard Prototype

Relay Control

All systems requiring modular control utilize an SPDT relay. These systems include temperature, CO₂, O₂, and N₂ control. Figure 32 illustrates the basic setup for these circuits.

Figure 32. Circuit setup for solenoids and heating pad control.
This configuration allows easy control of the 12V power supply for both the solenoids and the heating pad. The relay allowed a transistor to trigger the relay switch with 5V from the Arduino if a digital pin was set at HIGH. Triggering this switch let the 12V supply through the circuit to actuate the solenoid valves or supply the heating pads. The switch could be shut off by setting the digital pin LOW, which closes the solenoids and deactivates the heating pads. This created an easy method for controlling the solenoids and heating pads.

Oxygen Control

A hurdle to overcome during the course of this project was oxygen control. Previous attempts were hindered by the data type the sensor outputted. The sensing data was outputted as a string, so logic statements comparing to an integer were not able to work. To fix this issue, the data needed to be converted from a string to an integer. This was achieved by searching the string for any numerical values and making that its own string. Because the sensor purchased can detect more than just O₂ concentrations, that data needed to be extracted from the string. This was done by creating a substring starting at index 13 of the numerical string to index 16, which effectively pulled out the O₂ concentration data. From there, the substring was converted to an integer using various functions in Arduino. The only stipulation was that the O₂ concentration data was outputted as 2000 instead of 20.00, for example, since searching for numerical values removes the decimal point. This allowed the O₂ sensor data to be called for comparison to a set point, so the relay could respond in kind. The full code may be found in Appendix N. The control itself was set to open the O₂ solenoid if oxygen concentration fell below a setpoint and opened the N₂ solenoid if it was above. Within a threshold of the setpoint, both solenoids would open and close rapidly, as to not overshooot the setpoint.

The physical circuit setup for both the O₂ solenoid circuit and the N₂ solenoid circuit can be seen in Figure 32 because they both utilize relay control. The oxygen sensor was attached to the Arduino following the pin layout seen in Figure 33. Pins 1 and 2 were connected to the 5V and ground pins on the Arduino, respectively and pins 3 and 4 were connected to pins 11 and 12, respectively.
The CO$_2$ sensor was simple to implement without any modifications to the sensing values since there is a dedicated Arduino library for sensors like these called the COZIR library. Functioning similar to the oxygen and nitrogen control, the CO$_2$ control has a setpoint. If the value sensed was under the setpoint, the CO$_2$ solenoid would actuate. Once the value reaches a threshold, it would begin to actuate rapidly to avoid overshoot. The temperature control works in a similar way, through averaging readings from four sensors in different locations throughout the incubator for a more accurate reading. Through sensor testing it was discovered that the temperature sensors ordered have opposite connections to normal sensors of the type, due to a manufacturer defect. We believe this to be the reason the logic statements had to be opposite as well in order for the relay control to work. These modifications can be seen in the code in Appendix M.

Physically, the circuit setup was the same relay setup as seen in Figure 32. Pins 1, 3, 5, and 7 from the CO$_2$ sensor were connected to the Arduino, according to the pin layout seen in Figure 34. Pins 1 and 3 were connected to ground and 5V, respectively and pins 5 and 7 were connected to pins 2 and 3 on the Arduino. The temperature sensors were daisy chained together to take readings. The configurations for two temperature sensors may be seen in Figure 35. These sensors used 5V and ground from the Arduino and transmit data through pin 6.
Weight = ~6g

PIN-OUT DESCRIPTION: SprintIR®-W (Either Version)

<table>
<thead>
<tr>
<th>PIN</th>
<th>NAME</th>
<th>TYPE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GND</td>
<td>Supply</td>
<td>Sensor ground</td>
</tr>
<tr>
<td>2</td>
<td>NC</td>
<td>Unused</td>
<td>Do Not Connect</td>
</tr>
<tr>
<td>3</td>
<td>VDD</td>
<td>Supply</td>
<td>Sensor supply voltage</td>
</tr>
<tr>
<td>4</td>
<td>GND</td>
<td>Supply</td>
<td>Sensor ground</td>
</tr>
<tr>
<td>5</td>
<td>Rx_In</td>
<td>Digital Input</td>
<td>UART Receive Input</td>
</tr>
<tr>
<td>6</td>
<td>GND</td>
<td>Supply</td>
<td>Sensor ground</td>
</tr>
<tr>
<td>7</td>
<td>Tx_Out</td>
<td>Digital Output</td>
<td>UART Transmit Output</td>
</tr>
<tr>
<td>8</td>
<td>NITROGEN_ZERO</td>
<td>Digital Input</td>
<td>Set low to initiate a Zero in Nitrogen Setting Cycle</td>
</tr>
<tr>
<td>9</td>
<td>ANALOGUE_OUTPUT</td>
<td>Analogue Output</td>
<td>CO₂ Level (Optional)</td>
</tr>
<tr>
<td>10</td>
<td>FRESH_AIR_ZERO</td>
<td>Digital Input</td>
<td>Set low to initiate a Zero in Fresh Air Setting Cycle</td>
</tr>
</tbody>
</table>

**Figure 34.** CO₂ sensor pin layout.

**Figure 35.** Temperature sensor circuit with two sensors.
Material Selection

Incubator Materials

Outer Material
For the outer material, stainless steel, resin, and plastic were all considered in the initial concept development. Based on the decision matrix, stainless steel or plastic were the top two considered material choices as the outer material for the incubator. Due to the difficulty of machining and the expensive overall cost, stainless steel was eliminated as an option. Upon farther research on various plastics, polycarbonate was selected as our final material. This material was ultimately selected because of its machinability, low cost, and compatibility with heat, IPA and water use, the two solvents the material would frequently come into contact with for cleaning and sterilization. The final material ordered was a ¼” thick and a thin .08” thick clear polycarbonate for the outer structure, sensor box, and both the inner and outer door.

Insulation
From the results of the design decision matrix featured in the concept development section, sheet insulation was chosen as the insulation material over a foam or spray insulation. The sheet insulation was selected for its ease of machining, installation, and use in prior incubator designs. The final material selected was a 1” inch thick glass wool sheet. Glass wool was within budget, was easy to access, and would not be damaged by a humid environment. The insulation material was implemented in both the outer door and to line the main incubator structure.

Inner Material
The final choice for the inner material was aluminum. Aluminum and copper were the two original materials considered for the inner chamber due to the best thermal conductivity and corrosion resistance. Aluminum was ultimately selected because of the low cost, superior thermal conductivity, sterilizability and cleanability, and compatibility with water and IPA. A thinner aluminum was also used to construct the inner shelf used for culturing cells and housing the petri dishes used for humidity. The final material ordered was two sheets of 0.08” thick aluminum for the shelf construction and a 1/4” thick sheet for the inner chamber.
Off-the-Shelf Components

For the chamber construction, various off-the-shelf (OTS) components were utilized for the door design, air circulation, incubator feet, sealing gaskets, adhesion, and latching. All pricing and vendors of the OTS components can be referenced in Final Budget & in the Bill of Materials. All data sheets and additional material specs can be found in Appendix H. All images of materials’ function in the system are included in both the CAD Development & Final Chamber Assembly section.

For the inner door assembly, two compression glass hinges, gasket material, and a latch with compatible O-rings and fasteners were ordered. These hinges attached to the front panel, Part IN111-A, with two screws and, on the other end, used two screws with silicone caps to compress the door material. The original design of the incubator used a glass sheet, so minimizing any drilling into the material was imperative. This material selection led to the choice to use compression hinges. After multiple failed attempts at assembling our latch to the glass sheet, the design pivoted to use a thin sheet of polycarbonate for its ease of machining. To create a seal between the polycarbonate door and the main front panel 5/16 in. x ¼ in. x 17 ft. white D-Center EPDM medium gap weather seal tape was purchased. This weather seal material was heat and moisture resistant and provided enough elasticity to create compression and seal the door onto the panel when latched. The last major OTS component sourced for the inner door assembly was a stainless-steel surface mounting draw latch sourced from McMaster, which applies a downward pressure on the door and gasket material allowing for a tight seal. We decided to use a draw latch for the lower cost, smaller overall dimensions that were compatible with the incubator’s overall dimensions and applied downward pressure. Two small O-rings were selected for the fastening of the latch onto the polycarbonate inner door, and two larger (thicker) O-rings were selected to elevate and eliminate gaps that may cause leaking for the paired hook end of the latch on the front panel surface. These O-rings were measured and fit tested for the most optimal seal.

For the outer door assembly, heat inserts with a paired solder iron attachment, standoffs, corner brackets, magnets, various fasteners, and two stainless steel hinges to connect the door to the main structure were utilized. The heat inserts were selected to allow easy threading of the standoffs into the plastic door. The gap between the front panel and the outer door was measured, and the necessary standoff was ordered to allow for a direct connection between the magnets and the corner bracket. The corner brackets chosen were
a stainless steel that were large enough to come in contact with the magnet fasteners and were set at a 90°
age, which worked well with the incubator structural design. The magnets selected for the outer door
closer were smaller 1” disks that were strong enough to create a connection between the door and the front
panel. The hinges purchased were selected based on the weight they could comfortably support, the
compatible corrosion resistance material, the squeak free technology, and for the dimensions that were
compatible with the door and incubator structure design.

For the inner chamber magnets, a fan, standoffs, tube adaptors/nuts, a spacer for wiring, adjustable feet, a
pressure relief valve w/ a compatible O-ring, and four 1” spacers to elevate the inner chamber were
selected. The inner chamber magnets are the same 1” diameter disk magnets used for the outer door
assembly. These magnets were tested with the aluminum and heating pads for optimal attachment. These
magnets were all tested with IPA and showed no corrosion or loss in magnetic property, so they were
selected to function in the inner chamber as well as for the outer door closure. The fan selected provided
easy attachment to our control system set up and the dimensions allowed ample room within the chamber.
The spacer chosen was 1” and an electrically insulating material, G-10/FR4 Garolite to ensure electrical
safety while wiring through the insulation. The 1” spacer selected to raise the inner aluminum chamber
were nylon to allow for easy placement, not add additional weight, and the low price. The adjustable feet
selected were a combination product of felt, plastic, and steel. These feet were selected because they were
lightweight, easy to install and use, and came at a low price. The pressure relief valve chosen was made of
a brass material, and this component actuated whenever the box was pressurized. This valve was selected
based on its size, price, and functionality. An O-ring was fitted to the relief valve, in order to press fit the
valve into the system to ensure a tight connection. All fasteners & hex nuts selected were all stainless steel
and fit checked with corresponding OTS components.

3D Printed Material

In addition to the main chamber materials and OTS hardware there were ten 3D printed parts utilized in the
design: solenoid holders (QTY 3), display stands (QTY 2), hinge spacers (QTY 2), magnet holders (QTY 2), and a perf board holder (QTY 1). All 3D printing was performed by David Laiho in Engineering IV.
The solenoid holders, display stands, hinge spaces, and perf board holder were all printed using PLA in
white, grey, and orange using the Ultimaker 3. The magnet holders were printed using a Vero Yellow V.
material colored yellow/orange, the data sheet for this material can be referenced in Appendix H. All 3D
printed parts are utilized in the external environment (not in the inner chamber), meaning humidity, hot
temperatures, and constant sterilization were not a large concern for these parts. The main functionality for
the 3D printed parts was to accommodate a custom geometry. These parts can all been viewed in Figure 42-
Figure 44, in the prototype manufacturing section under Final Chamber Assembly.

Control System Materials

Gas Control

All components were purchased off-the-shelf for the control system. A full list of materials used in the
control system may be found in Appendix I.2. For these systems, most materials were chosen based off a
tutorial for a low-budget, DIY CO\textsubscript{2} incubator (Pelling, 2021). One of the most important components for
gas control were the gas sensors for O\textsubscript{2} and CO\textsubscript{2}. The O\textsubscript{2} sensor chosen was the LuminOx LOX-O2 25%
Oxygen Sensor. This sensor was capable of detecting oxygen from 0-25% concentration, which fell within
the range desired for this project. It was also simple to connect to an Arduino for measurement of O\textsubscript{2}
concentrations. The SprintIR-WF-20 was the sensor chosen for CO\textsubscript{2} sensing. This sensor detects CO\textsubscript{2}
concentrations from 0-20%. Though the chamber will be held at 5% CO\textsubscript{2}, it was necessary to have a sensor
capable of detecting a higher than 5% in case the CO\textsubscript{2} was ever over 5%. This sensor is also easily
connected to an Arduino board for measurement. Both sensors can operate under 5V, which can be
provided by an Arduino, making them ideal for this project.
The most important part of the control system itself is the relay, which allows for the control of the
solenoids and heating pads based on data collected from the sensors. The relay was sourced from Spark
Fun and has a minimum switching voltage of 5V. This is easily provided by the Arduino, making it
compatible with out control system. It is rated for up to 30 VDC, which is more than enough for the 12
VDC power source used to supply power to the solenoids, heating pads, and fan.
The main determinants for the solenoid valve were inlet/outlet size and actuating voltage. The solenoids
chosen actuate at 12VDC, which can be sourced from a wall outlet with an adapter. The inlet/outlet size
was chosen based on the tubing available and adapter sizes. The fan was chosen on a similar basis, ensuring
it can operate at 12 VDC. The chosen fan has a speed of 3000 RPM, with a maximum airflow of 0.327 m³/min and a maximum air pressure of 2.21 mmH₂O, all suitable specs for the purposes of this project. The displays chosen for this project came with a backpack from Adafruit that makes using them with an Arduino extremely simple. It turns a 13-pin interface into 2 pins along with power and ground. Coding was also simplified to single command rather than working with matrices. The rest of the components for the control system were smaller scale and commonly used in many projects. These include 1K and 4.7K ohm resistors, diodes, 2N2222 transistors, 18 and 22 AWG wire, jumper cables, and breadboards. The specific values of the resistors, the type of diode, and the transistor were based off Andrew Pelling’s incubator tutorial since they were proven to work with his gas CO₂ control system (Pelling, 2021). Data sheets for all components listed above may be found in Appendix H.

Temperature Control

For temperature sensing, two DS18B20 sensors, which are small and simple sensors that are easy to obtain, are utilized in the design. This sensor was selected based on examples of other home incubators that had successfully developed their temperature control systems using this device. The DS18B20 temperature sensors also were easy to implement into the Arduino code, which allowed for easy control. For the heat source, we considered a few various mechanisms: using an external source and blowing hot air into the chamber, heating pads, or some type of heating coil. We decided to utilize heating pads into the design for the slow heating – to not overshoot the set point – the low cost, and the ease of use with Arduino. We chose the heating pad, 5V DC Heating Pad 600MA 5X15CM from SparkFun Electronics, based on a recommendation from the Andrew Pelling DIY CO₂ incubator tutorial (Pelling, 2021). The flat profile of the heating pads was desirable for convection heating the aluminum inner chamber walls, and to have minimal interference with the chamber space. For the heating system, larger gauge wires were utilized to distribute the necessary current and to not overheat the system. The datasheets for both the DS18BB20 sensors and the 5V DC Heating Pad can be found in Appendix H.
Prototype Manufacturing

Incubator Manufacturing

Chamber Manufacturing Protocol

All manufacturing for the hypoxic chamber was performed at Mustang 60, the Aero Hanger, and in an off-campus garage. The detailed manufacturing protocol can be found in Appendix I.1. This protocol contains all necessary equipment, drill bit sizes & speeds, and utilizes the dimensions shown in the mechanical drawings in Appendix F. The manufacturing process started with water jetting out the base materials of polycarbonate and aluminum, followed with drilling all necessary holes for the inner aluminum structure and outer polycarbonate box. Each hole was measured and fit tested if there was a corresponding OTS part or direct junction with a differing part. The aluminum chamber was epoxied first, then the sensor box, then the outer door and outer polycarbonate structure, and lastly the inner door assembly. The final piece machined and assembled was the stand-alone removable shelf. During manufacturing two major iterations occurred that deviated from the initial design plan: the inner door assembly had to be rotated 180˚ to accommodate a conflict between the outer door hinges and the front panel and the inner door material changed from glass to polycarbonate after two failed attempts at drilling the thin glass material without shattering. Major milestone images can be found in the following section: Final Chamber Assembly.

Final Chamber Assembly

For the incubator structure manufacturing, the assembly was split into sub-assemblies: the inner chamber, outer chamber, sensor box, inner door, outer door, and removeable shelf. After the machining and assembling for all sub-assemblies was completed, the additional hardware & insulation were installed, the subassemblies were epoxied together, and then the electronic & gas components were added in. Additional manufacturing images can be found in Appendix J.

Inner chamber

For the inner aluminum chamber, all additional holes for the electrical outlet, three gas outlets, sensor box outlet/inlet and four threaded holes were measured and drilled by hand after water jetting the stock material. For the inner chamber, the initial aluminum was cut at a misaligned angle during water jetting so
additional filing was required. Using paint cans to prop the walls and various square measuring alignment tools, the aluminum was epoxied together in three stages. Four 1” nylon spaces were then epoxied to the bottom of the chamber to allow spacing for insulation and a 1” polycarbonate spacer was epoxied to the back of the electrical outlet cutout for ease of moving wiring through the insulation. Four magnets were epoxied to the outer surface of both side walls for heating pad attachment. The placement for these magnets were premeasured and aligned using the heating pads. Three tube adaptors with connected tubing and with respective hex nuts were fastened into position as the last step. In Figure 36 below, the completed inner chamber unassembled without the adaptors or spacers is shown. To see more detailed images, refer to Appendix J.1.

**Figure 36.** Inner aluminum chamber without OTS attachments.

**Sensor Box**

For the sensor box pre-assembly, two small holes for the CO₂ sensor, one large hole for the O₂ sensor, and four holes for the side brackets were drilled before epoxying the box together. Post assembly of the sensor box an additional hole was drilled for the pressure relief valve. A side slit on the top panel for the wiring was cut and expanded as necessary. All three of these components: O₂ & CO₂ sensor and pressure relief
valve were precisely measured, and press fit into the sensor box. Shown in Figure 37 below is the final sensor box without the side brackets screwed into place or the sensors.

![Figure 37. Sensor box including pressure relief valve.](image)

**Outer Chamber**

For the outer polycarbonate chamber, the main parts were all water jet from the stock sheet. Except for the square cut out from the top panel, all holes were hand drilled: outer door hinge, adjustable feet, corner brackets, electrical outlets, and gas outlets. All holes were hand measured and drilled using the drill press. The feet of polycarbonate chamber were epoxied into place on the bottom panel. The walls of the chamber were leveled and aligned using a carpenter square. The outer chamber was dried similarly to the aluminum chamber: in multiple stages and propped up in position with heavy paint cans, shown in Figure 38.
The front panel of the inner door assembly had the main slot water jet. The two side cut outs were hand cut with a vertical band saw, and the holes for the latch and hinges were measured and drilled. Two half boxes were cut with the vertical band saw and sanded for the side cut outs. The door itself was originally made of glass and cut and sanded in the craft center. For the latch, two holes were needed to be drilled. The holes in the glass material were attempted to be drilled with a special glass drill bit and with a small circle drill bit, both drilling techniques lead to cracking in the glass material, either completely or partially. The design pivoted to use a thin clear polycarbonate for the inner door material. This door was cut using the vertical band saw and the filets were made via hand sanding the corners. To re-enforce and stiffen the polycarbonate sheet four pieces of ¼” thick polycarbonate were cut and epoxied onto the panel. The two half boxes around the side cut outs were then epoxied and dried. Two O-rings were used to fasten the latch onto the panel, and two thicker spacers were used with the fastener for the other half of the latch on the front panel. The gasket material was applied around the cut out and the door was tightened onto the hinges. After testing the door seal and evaluating the fit, the front panel was adhered to the aluminum inner chamber. The adhered front panel with attached hardware is shown in Figure 39 below.
Outer Door Assembly

For the outer door assembly, the main components were all water jet from the stock polycarbonate. Two holes were drilled for connecting the magnet holders and six holes were measured and drilled for the hinges. The epoxying of the outer door had three stages: the top/bottom onto the back panel, the front panel, then the sides once hex nuts were epoxied on. The necessary insulation was sized, cut, and fitted before the front panel was secured into position. The heat set inserts were melted into place, then the magnet holders/standoffs were secured into place. The door hinges were then fastened to the door and the chamber, and the gasket material was added to the edges of the inner door wall. The final door assembly is shown below in Figure 40 and additional images can be references in Appendix J.5.
Removable Shelf

For the removable shelf, first, the top plate was sized to the inner chamber, measured & aligned using a square and cut using the foot sheet metal shear. The holes were measured, hand drilled, and deburred individually. The legs of the shelf were measured, cut using a hand saw and filed until identical and level. The bottom shelf was sides and cut using the foot sheet metal shear. The legs were epoxied and dried, then the bottom shelf was epoxied and cured. The final shelf is shown in Figure 41 below, additional images can be referenced in Appendix J.6.

Figure 40. Outer door final assembly.

Figure 41. Final assembled removable shelf fully leveled.
Full Chamber Assembly

For the full chamber assembly, First the insulation was sized, cut, and fit checked with the outer chamber. Next the feet of the incubator were installed. The tubing was fed through the outer chamber and the inner chamber was put into position and the insulation was fit snug on all surfaces. Next, the front panel was epoxied in place and after the sensor box was epoxied. The outer door hinges and corner brackets were then fastened into place, this step proved the most difficult for alignment. All additional 3D printed parts were assembled with the per boards, displays and the solenoids, shown in Figures 42-44. The fan, heating pads, and sensor were placed in the box and the electrical wiring cover was placed on the back of the incubator, shown in Figure 45 & 46. A close up of the gas inlets can be seen in Figure 47.

Figure 42. 3D Printed per board holder.
Figure 43. 3D Printed display stands.

Figure 44. 3D printed solenoid holders.

Figure 45. Back view of completed incubator with wire covering.
All four perf boards were manufactured based on the breadboard prototype setup. For this procedure, the central components were soldered first, seen in Figure 48 & 49. These include the resistor, the diode, the 2N2222 transistor, and the SPDT relay. Once those connections were made, following the diagram seen in
Figure 50, a wire allowing for Arduino control was placed on the board, connected to the free end of the resistor. After this, header pins were added to the boards for power and ground connections from the Arduino. Then, power and ground connections were made following the diagram in Figure 50. Female ended jumper cables were then soldered onto two of the boards to allow power sharing from the Arduino. Next, header pins were added to one board for the 12 V connection. Power and ground from this source were connected to all boards following the diagram seen in Figure 50. All wire-to-wire solder joints were covered with heat shrink tubing for insulation. The materials and detailed protocols for this manufacturing may be found in Appendix I.2. Additional photos of the control system development process can be found in Appendix G.
Figure 49. All four basic relay circuits set up.

Figure 50. Perf board final setup.

Electronics, Chamber, and Gas Integration

After the chamber and perf board manufacturing were completed, the control system and chamber could then be assembled. First, the temperature sensors had extended male-ended jumper cables soldered to the
pins for ease of use with the breadboard circuit for temperature sensing. Heat shrink tubing was added to the solder joints and the pins to mitigate any issue with humidity. The wires were fed through the designated wire channel and attached to the circuit. Next, 18 AWG wire was soldered to the ends of the heating pads and solenoids, making sure to place heat-shrink tubing on the wires before soldering and feed the heating pad wires through the channel before soldering. Next, eight extended female-to-male jumper cables were made for the O₂ and CO₂ sensors using 22 AWG wire, adding heat shrink tubing on the joints. The full list of materials and protocol may be found in Appendix I.3.

To set up the gas lines, the appropriate regulators were added to their respective gasses. Tubing adapters were attached to the regulators and ¼” ID tubing was attached from there. In-line HEPA filters were added to preserve the sterile environment of the incubator. After the filters, the solenoids were attached via adapters. More tubing was added from the outlet of the solenoids to reach the inlet of incubator. Pictures of this integration may be found below in Figure 51 - 54. Additional photos can be found in Appendix K.

Figure 51. Current control system setup.
**Figure 52.** Control system with solenoids.

**Figure 53.** Gas system setup. O₂ is the left tank, N₂ is the middle tank, and CO₂ is the right tank.
Test Protocol Development

Material Testing

IPA & Water Testing

The inner and outer chamber material, aluminum and polycarbonate, respectively, were tested for compatibility with water and 70% IPA. These two liquids were selected because the materials will frequently be exposed to them from humidity and sterilization. For IPA & water testing two small sample sections of aluminum and polycarbonate were cut. These sections of the material were immersed in the respective liquids for 24 hours. The materials were visually examined before and after immersion. Following a visual examination for discoloration and corrosion, the samples were dropped 10 times from 4ft off the ground and bent at the material midpoint using hand strength. Although the materials will not be
dropped or bent in the incubator’s daily intended use, this method was a simple measure for assessing changes in material strength, hardness, and surface finish. The material compatibility testing setup can be seen in Figure 55 below. The detailed protocol for the IPA & water testing is in Appendix L.1.

![Figure 55. Material testing setup.](image)

**Leak Testing**

*Soapy & Generic Water Testing*

Creating airtight seams at all junction points for the sensor box, door, and aluminum chamber was essential for maintaining the gas environment of the incubator and to minimize gas waste. To assess any leaking in junction points of the incubator a generic water test was performed. To access gas leaking a soapy water test was performed. A generic water bottle was filled with water and mixed with a teaspoon of soap. The soapy water was poured onto the seam in question and observed for bubbles and dripping. A generic water test was performed on all three sub-assemblies: sensor box, inner door, & aluminum chamber and a soapy water test was performed on the inner aluminum chamber. The detailed protocol can be referenced in Appendix L.2.
Long-term Seal Testing

Once the chamber was completely manufactured and assembled with control system, a long-term seal testing was performed to assess at what rate gas and heat would leak from the system. Knowing the leaking rate was important for optimizing the control system settings and for awareness on overall gas use. For long term seal testing, the full incubator with the assembled control system was necessary; the O₂ & CO₂ gas systems and the heating system were all utilized. The system was set and stabilized to 5.5% O₂, the control system was switched off, and then O₂ concentration was collected over a four-hour period. The leaking rate was recorded. To assess the rate of heat transfer with insulation use, the chamber was heated to a high value and left over a 3 hour time period. The temperature values directly on the inside bottom wall and external wall of the chamber were recorded to study heat transfer through a conduction flat wall model. The detailed protocol can be referenced in Appendix L.2.

Control System Testing

Sensor Calibration

Protocols were developed for gas sensor calibration in the event it was needed based off manufacturer recommendations. To determine if the sensors needed calibration, they were placed in ambient air and compared to accepted values. They were then exposed to exhaled air to ensure they reacted to a change of concentration. The sensing capabilities of the O₂ sensor were tested by simply connecting the pins to the Arduino. A pin schematic may be seen in Figure 18 above. Pin 1 was connected to 5V on the Arduino, pin 2 to ground, pin 3 to port 10, and pin 4 to port 13. The ambient air O₂ concentration and exhaled breath concentration were recorded. The CO₂ sensor was similarly connected and tested. The temperature sensors were connected as mentioned in the CO₂ and Temperature Control section. These sensors were also tested in ambient conditions and in contact with a hand. The results of these tests may be seen in Table 3, below. All codes used for this testing may be found in Appendix N & O. The results of this testing may be seen in the next section. The calibration protocols were similar for the O₂ and CO₂ sensors. They would be placed in an airtight bag which would then be vacuumed to suck all air out. The bag would then be flooded with a pre-set mixed air. If the value read did not match the value of the mixed gas, then the sensor would be
calibrated using pure nitrogen as a 0% set point. The full protocols for these tests for both O\textsubscript{2} and CO\textsubscript{2} may be found in Appendix L.3.

**Control System Response**

Throughout the course of manufacturing the control system, it was important to confirm that the sensors could detect changes in their measurands, and the control system could respond to that change. Relay response was vital to this project because relays are the basis of our control system. To ensure the relays would respond to the sensors, first individual relays were tested for responses with success. The code used for this may be found in Appendix P. After this, we tested multiple relays on one Arduino since that was necessary for this project. We also ensured that everything requiring 12V to power worked when powered with 12V before subjecting them to relay control. Full control system response was not tested until everything was soldered together, but with the incremental testing we had done, we were confident it would work together.

**Environmental Control**

For the environmental control test, the main optimization test, the full incubator system was needed. The purpose of this test was to discover which settings in the control system are most optimal for maintaining the gas environment. Both Arduinos were uploaded with the proper code (Appendices N & O), and all three gas systems are turned on with all three of the pressure regulators set to the lowest possible pressure. Within the code, each sensor has a low setpoint and a high setpoint within an acceptable range. For example, the O\textsubscript{2} low setpoint was 4.5% and the high setpoint was 5.5% so the target of 5% O\textsubscript{2} could be maintained. The low range and high range values for all three gases were kept constant throughout the experiment and the interval time ranges for the solenoids opening/closing were varied 1 & 3ms for the N\textsubscript{2} and O\textsubscript{2} and 6 & 10ms for the CO\textsubscript{2}. There were two phases to the test: one where the door was open 2 full minutes to reset the space and a second where the door was opened 30 seconds. Each phase lasted 10 minutes total. All O\textsubscript{2} values were recorded on CoolTerm, and CO\textsubscript{2} values were recorded in Excel. Each test produces 4 files for a total of 32 total files. The data was then analyzed Minitab using factorial analysis.
with three factors that have two levels each, corresponding to gas and solenoid on time for each. A table of factors and levels may be seen in Table VI. The detailed testing protocol can be referenced in Appendix L.3.

Table VI. Table of factors and levels for factorial analysis

<table>
<thead>
<tr>
<th>Factor - Gas</th>
<th>Level – Solenoid on Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂</td>
<td>1 ms</td>
</tr>
<tr>
<td>O₂</td>
<td>1 ms</td>
</tr>
<tr>
<td>CO₂</td>
<td>6 ms</td>
</tr>
</tbody>
</table>

CO₂ Optimization

After the completion of the environmental control, it was necessary to optimize the code for CO₂ based on the results. Testing was performed in a similar manner to the environmental control testing, but only the CO₂ setpoint and threshold were being altered. In order to reach an ideal goal value of 5% CO₂ the solenoid on value/setpoint was altered including: 4, 3.9, 3.8, and 3.7%. These setpoints lower than the 5% ideal value were chosen based on the overshoot experienced in previous tests. Once an optimal on value/setpoint was found, the box was left for an hour to determine if the ideal range of 4.5%-5.5% could be maintained. The averaging value was also changed from 3 to 6 datapoints to avoid any actuation from an outlying data point. Once this was complete, the box was left overnight for the same purpose. The primary response of this test was the actual sensed CO₂ concentration, specifically determining if the incubator could maintain CO₂ concentration close to 5% over time. Results of these tests are described in the CO₂ optimization test results section.

Cell Incubation

CO₂ Cell Incubation and Temperature Validation

For this experiment, 3T3 cells were unfrozen and grown in a T-75 flask in a standard incubator for 2 days. After this, cells were passed into two 48 well plates, one for a standard CO₂ incubator and one for our incubator. The oxygen range for our incubator in this experiment was set to 19.5-20.5% so results could be
compared to a standard incubator. Cells were counted using a hemocytometer and a light microscope to calculate the number of cells going into each well. Unfortunately, there was a small number of cells due to non-adherence when initially culturing. The cell estimate came out to around 150,000 cells, and only about 1,000 cells went into each well. Due to time constraints, there was no opportunity to re-do this experiment, so we continued with the limited number of cells. A detailed procedure for passing and counting the cells may be found in Appendix L.3.

After 2 days of incubation in the 48-well plates, the cells were removed from their respective incubators and examined under a light microscope for growth at 10x. If cells were present, they were counted visually. Images of the wells were captured using an inverted light microscope using the same exposure time of 1.470ms. For our incubator, O₂, CO₂, and temperature were tracked throughout the experiment. The primary endpoint for this experiment was cell growth, while the environmental data being tracked was secondary. The results of and images taken this experiment may be found in the Cell Incubation Analysis section below.

**Testing Data and Analysis**

**Material Testing**

*IPA & Water Testing*

The materials were visually examined before and after immersion in water and 70% IPA, which can be seen in Figure 56 & Figure 57 below. No visual changes were noted after 24 hours for either material in either liquid. Attempts were made to bend the materials after testing with no significant change in any material property.
Leak Testing

*Inner Chamber Seal*

To assess the inner chamber seal, a generic and soapy water test was performed on both the aluminum inner box and the sensor chamber after all seams were epoxied. During the first test on the aluminum chamber, where plain water was poured into the chamber, multiple leaks were found on the two side seams. Following the test, the side seams were re-epoxied to enforce the seal between the walls. After a full 24-hour cure, another leak test was performed and there was no water leaking through any junction point. The sensor box also showed areas of leaking on the first water test, but after re-applying epoxy to weak sections of the seams, the sensor box also had no water leaking. Marine grade JB Weld Water Weld Epoxy Putty was applied to the bottom sides of the aluminum box as well for additional insurance. A soapy water test was performed in both sub-assemblies and no bubbles were observed on any seam.

*Door Seal*
For the door seal, the initial assessment was done with the front panel alone, unassembled from the main incubator assembly. The first inspection was done assessing the compression of the gasket material visually. With the switch of materials from glass to polycarbonate, the seal was no longer tight. The thinner polycarbonate material was significantly more elastic/flexible than glass, which is an extremely stiff material with no significant flexion. When latched the polycarbonate had significant bending, creating gapping on the sides of the door, the results of this initial examination are shown below in Figure 58 & Figure 59.

![Figure 58](image1.png)

**Figure 58.** Front view of initial door seal displaying bending at latch point.

![Figure 59](image2.png)

**Figure 59.** Side view of initial door seal displaying side gapping.

After this initial failed seal test, support material of ¼” thick polycarbonate was cut to fit along the sides of the door and was epoxied into position to mitigate material bending. After adding support material, the polycarbonate door made significant contact with all sections of the gasket material. A water test was
preformed along the edges of the door, via pouring water onto the door and was rotated around the seams. Any water leaking occurred at corners of the gasket material, but no leaking occurred on any sides of the door. When the door seal was confirmed, the front panel was then epoxied onto the aluminum chamber. The final door seal assembled on the chamber is shown below in Figure 60.

![Figure 60](image_url)

**Figure 60.** Front view of inner door assembly displaying support material and seal.

*Long-term Seal Testing*

Two long-term seal tests were performed over a four- and three-hour period respectively. These tests examined the rate of gas leaking and the insulating abilities of the chamber via looking at the rate of change with oxygen percentage values and rate of heat transfer over the set time period. For the gas leaking test, a linear fit was used to assess the % rate of change over time. A linear fit was used because leak point area, volume of the chamber, velocity of gas flow, and pressure remain constant throughout the test. We assume pressure remains relatively constant with use of our pressure relief valve, but a pressure gauge was not utilized during this testing. The starting percent O₂ value was 5.5% and the ending value was 8.73% resulting in a 0.78% change in O₂ per hour. The O₂ concentration was recorded every second. In Figure 61,
the full O₂ data over the four-hour test with a line of best fit are shown. The rate of oxygen increase in the chamber was .000217% per second. Overall, the chamber itself without a control system was able to maintain a hypoxic environment with a slow & linear leak. This data also shows us there should only be a small input of gas from the control system per hour.

![Graph showing O₂ concentration over four hours with a line of best fit.](image)

**Figure 61.** Long term leak/seal test: percent O₂ over four-hour time period. O₂ concentration is in percent and the sample interval is 1 measured value per 1 second.

R-value (resistance) is a materials capacity to resist heat flow from one side to the other side. It measures the effectiveness of insulation, with higher R values depicting more effective insulation and greater resistance to heat flow. These values are additive with each material layer. This incubator is a three-layered chamber: aluminum 1/4”, glass wool 1”, and polycarbonate 1/4”. The R value was calculated using equation 1 where the thickness of a material divided by its thermal conductivity (these values were pulled from the material datasheets) and the plane area.

\[
\text{EQ 1. } \frac{t}{kA} = \frac{l}{\lambda} = R \left( \frac{^\circ C}{W} \right)
\]

For this structure, the main body of the chamber has a theoretical R value of 6.27°C/W, where the glass wool has a R value of 3.7, polycarbonate has a value of 1.67 and the aluminum has a R value of 0.9. The actual real insulation abilities will differ with the glass door layer as well as sensor box sections of the chamber. An R value of 6.27 when comparing to other chambers of this size should be sufficient. To find
the insulation conduction heat transfer rate, the structure was heated to 70°C and the internal temperature change was measured over a three-hour period.

For the insulation test, the systems had an initial internal temperature of 70°C and a final internal temperature of 32.5°C. The external temperature directly on the outer polycarbonate wall remained between 24.5-26°C. At higher temperatures, the loss in heat occurred more rapidly than at lower temperatures where the rate in heat loss was lower. The thermal decay and heat transfer rate of the chamber in intended use may be slightly different than what is shown through this testing due to the normally humid nature of the chamber; during this testing the chamber was not under humid conditions. However, this testing did provide insight on the heat transfer and insulating ability of material composition of the chamber. With use of the control system the insulation was fully sufficient.

Using a steady state conduction flat wall model the temperature difference between the internal and external environments was used with the R value to find the rate of heat transfer occurring, refer to equation 2 below.

\[
EQ \ 2. \quad Q = \frac{(T_2 - T_1)}{x/KA} = \frac{(T_2 - T_1)}{R} \quad (W)
\]

In future studies, an exponential decay model could be analyzed through using a convection and conduction model, incorporating air into the model. The user could also calculate the rate of heat transfer applied by the heating system to find the level of energy stored in the chamber at various time points using equation 3.

\[
EQ \ 3. \quad E_{in} - E_{out} + E_{gen} = E_{stored}
\]

**Figure 62.** A. Change in temperature (°C) over 3 hours with no additional heat applied. Temperature is in °C and the sample interval is 1 measured value per 3 seconds. B. Rate of heat transfer (W) across the incubator wall over time. Q is in Watts and the sample interval is 1 measured value per 3 seconds.
Both long term seal tests showed leaking and non-perfect insulation. The gas leaking occurring was extremely minimal compared to opening a door, which returns hypoxic conditions 5% O$_2$ back to room conditions 20% O$_2$ in approximately 1-3 minutes. The rate of heat transfer slows over time and the temperature drops over time, but the controlled heating system with the additional power source applies enough power to maintain a constant temperature, which is show in future control system testing. This leaking of both gas and heat are acceptable for use with a control system.

Control System Testing

Sensor Calibration

Sensors were tested according to the protocol described above and the following results were measured and recorded, seen in Table VII.

Table VII. Sensor testing results.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Ambient Condition</th>
<th>Manipulated Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>20.5%</td>
<td>19.77%</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>0.09%</td>
<td>3.44%</td>
</tr>
<tr>
<td>Temperature</td>
<td>25.5°C</td>
<td>29°C</td>
</tr>
</tbody>
</table>

Sensors were tested in ambient conditions, meaning room air and room temperature. They were then manipulated by either blowing on the sensor for the O$_2$ and CO$_2$ sensors or grasping it with fingers for the temperature sensor. This testing verified that the sensors were working accurately since the ambient conditions conformed to typical values. Therefore, it was determined that calibration was not necessary for these sensors. It also confirmed that they were able detect changes in their respective measurands accurately.

Control System Response

All electronic components were tested to confirm functioning electronic components, board set up, and code following the protocol in the previous section. The components were hooked up to power and tested separately. Results of this testing may be seen in Table VIII Table VIII below.
Table VIII. Control system response testing results.

<table>
<thead>
<tr>
<th>Electronic Component</th>
<th>Responding to</th>
<th>Responds? (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relay x4</td>
<td>Arduino</td>
<td>Yes</td>
</tr>
<tr>
<td>Solenoid x3</td>
<td>12 V power</td>
<td>Yes</td>
</tr>
<tr>
<td>Heating pad x2</td>
<td>12 V power</td>
<td>Yes</td>
</tr>
<tr>
<td>Solenoid x3</td>
<td>Relay</td>
<td>Yes</td>
</tr>
<tr>
<td>Heating pad x2</td>
<td>Relay</td>
<td>Yes</td>
</tr>
</tbody>
</table>

It was also necessary to determine if the sampling rates of the sensors chosen were adequate for the response time needed. To do this, linear portions of data were taken for O₂ and CO₂ concentrations and fit to a linear trendline. These graphs can be seen in Figure 63, below. It can be seen that oxygen changes at a rate of about 0.18% per second and carbon dioxide changes at a rate of about 0.013% every 3 seconds, which is .0042% per second. Our engineering specs have tolerances of ±0.5%. Since the rate of change for both gasses were less than 0.5%, we determined that the sensors were adequate.

![Figure 63](image)

**Figure 63.** Linear fits for sections of O₂ data (left) and CO₂ data (right). O₂ is in percent and samples are taken every second. CO₂ is in percent and samples are taken every 3 seconds.

**Environmental Control**

To refine the raw test data, the data was examined for instances where conditions would leave the optimal range. For both O₂ and CO₂, this range was 4.5%-5.5%. Each of these instances where the sensor values left the target range were highlighted and the amount of time the gas concentration was out of spec was noted. The time it took to for the control system to return to the target range initially was recorded, as well as the time it remained within the range. In addition to recording the time length, a count of the number of instances the data went out of range was also recorded. From there, the total time was calculated, and any
data measured by time was normalized using this value since every test had a different total time. The normalization made the data comparable. Variables produced by refinement included the time to reach the setpoint, the time within spec and time out of spec for each test, which were used for comparison between tests. For CO₂, the maximum overshoot was noted as well. This data was collected to ensure the incubator met with engineering specifications created. The most relevant to these tests are the ability to maintain a sealed incubator environment, O₂ concentration below 5% for 48 hours, incubator CO₂ concentration within 5%, and incubator temperature within 0.5°C.

A full factorial design in Minitab was used to analyze this data, with eight responses for CO₂, being time to setpoint, time in spec, time out of spec, and max overshoot all for a stabilized run and a run with the door open. O₂ data had the same responses, minus the maximum overshoot. A table of factors and levels may be referenced in [testing protocol] as Table VI. The goal of this data analysis was to determine the optimal solenoid on time for each gas based on responses from the testing like time out of spec. From the factorial regression, both N₂ and O₂, as well as their interaction term were significant factors for time out of spec for CO₂ in both conditions, as seen in Figure 64. The interaction term being significant means that the effects on time out of spec for CO₂ concentration of the N₂ solenoid on time and the O₂ solenoid on time depend on one another. CO₂ solenoid on time had no significant effect on any responses and therefore was left out of the rest of this analysis. The O₂ concentration data had no significant effects from any of the factors, therefore the rest of the analysis will only address CO₂ concentration data. The raw data as well as refined data for both O₂ and CO₂ concentrations may be found in [Appendix M].

![Pareto Chart](image)

**Figure 64.** Pareto charts from Minitab showing N₂ (B), O₂ (C), and their interaction term (BC) as significant factors in time out of spec for both conditions.
Time in spec for the door opened conditions was also significantly affected by N$_2$ and O$_2$. Their interaction term was included in the refinement as well because it was very close to significance, as seen in Figure 65.

![Pareto Chart of the Effects](image)

**Figure 65.** Pareto chart showing the significant factors N$_2$ (B), and O$_2$ (C) for time in spec for door open.

From here, the regression analysis for these three responses were refined to only include N$_2$, O$_2$, and their interaction term. The Minitab setup may be found in [Appendix M.3](#). This refined analysis allowed for contour plots to predict the best setting of those tested for N$_2$ and O$_2$. These may be seen in Figure 66 - Figure 68.

![Contour Plot of Time out of Spec – Stationary: N2 vs O2](image)

**Figure 66.** Contour plot for time out of spec for CO$_2$ concentration, stationary. For both N$_2$ and O$_2$, -1 refers to a 1ms solenoid on time and 1 refers to a 3ms solenoid on time.
Figure 67. Contour plot for time out of spec for CO\(_2\) concentration, door open. For both N\(_2\) and O\(_2\), -1 refers to a 1ms solenoid on time and 1 refers to a 3ms solenoid on time.

Figure 68. Contour plot for time in spec for CO\(_2\) concentration, door open. For both N\(_2\) and O\(_2\), -1 refers to a 1ms solenoid on time and 1 refers to a 3ms solenoid on time.

The goal was to minimize time out of spec and maximize time in spec for the CO\(_2\) data. Time out of spec was minimized when N\(_2\) has a solenoid on time of 3ms and O\(_2\) has a solenoid on time of 1ms. This can be seen in the light green shaded area of Figure 67, which suggests that the percent of time out of spec will be less than 40% with these settings. Time in spec was maximized with these same conditions. The dark green shaded area of Figure 68 showed that the percent of time in spec will be greater than 50% with these
settings. The other plot, Figure 66, suggests both N₂ and O₂ have their solenoid on time set at 3ms. This can be seen in the light green shaded area of the plot suggesting that time out of spec will be less than 52% with those settings. Because two separate statistical analyses suggested N₂ solenoid on time at 3ms and O₂ solenoid on time at 1ms, these settings were chosen as the primary settings for the incubator. These solenoid on times provided the best results of the tested settings. Because CO₂ solenoid on time was not a significant factor, the 6ms threshold setting was chosen for the CO₂ solenoid based on the raw data.

**CO₂ Optimization**

Of the setpoints tested (3.7, 3.8, 3.9, and 4%), 4% stayed in spec the longest; this setting kept CO₂ within the ideal range of 4.5%-5.5%. With 1-hour long testing, once reaching the ideal range, CO₂ stayed within the confines well, as seen in Figure 69. The O₂ concentration stayed well within the range of 4.5%-5.5% once it reached its setpoint, as seen in Figure 69. This result met the engineering specifications of reaching and maintaining O₂ concentrations below 5%. Although, there was a stipulation with the setpoint of 4 though. Initially, it would overshoot the high range value of 5.5%. Once it did, the door had to be opened to allow ambient air in to lower the CO₂ concentration. Once 3% was reached, the door could be closed again, and the chamber will re-gas and should settle at an appropriate CO₂ concentration. Although CO₂ may eventually fall due to leakage, this method allowed for stabilization around the target value more rapidly than without the interference and allows the incubator to better meet the engineering specification of maintaining CO₂ concentration within 5%. Therefore, this method was employed for the overnight test. As seen in Figure 70, the CO₂ concentration overnight did spike up to 20% but the chamber was able to correct itself and come back to a more reasonable level. The most likely reason for this overshoot is an inadequate CO₂ pressure regulator. The current regulator has a wide range of pressure, which doesn’t allow for the fine-tune adjustments this chamber needs. This issue will be addressed further in the discussion section. The O₂ data for this test stayed within spec during the duration, as seen in Figure 70. The second spike in O₂ was when the door was opened to get CO₂ down to 3%.
Figure 69. 1-hour testing data for CO$_2$ concentration (left) and O$_2$ concentration (right). CO$_2$ concentrations is in percent and samples are taken every 3 seconds. O$_2$ concentration is in percent and samples are taken every second.

Figure 70. Overnight testing data for CO2 concentration (left) and O2 concentration (right). CO$_2$ concentrations is in percent and samples are taken every 3 seconds. O$_2$ concentration is in percent and samples are taken every second.

Cell Incubation

CO$_2$ Cell Incubation and Temperature Validation

Throughout the course of cell testing, CO$_2$ concentration did not remain stable. During day 1 of testing, CO$_2$ concentration remained in spec (4.5%-5.5%) and stable for around 6.5 hours before rising, eventually reaching the sensor limit of 20 at about 10 hours, as seen in Figure 71. After around 15 hours, in incubator was checked on and the CO$_2$ concentration was lowered by letting ambient air into the incubator. This remained in spec and stable for around 2 hours, before rising to 20% again. At 20 hours, the incubator was checked for a final time and brought back in spec using the same method as before. This was in spec for
around two hours again before rising, which can be seen in Figure 71. The experiment was terminated after about 35 hours total. The oxygen concentration remained stable around 20%, as seen in Figure 72. Figure 73 shows temperature data during the period of the test. As can be seen in the figure, once the temperature reached its setpoint of 37 °C, it remained stable at that point. Both oxygen and temperature graphs show outlying noise, most likely due to instrumentation. Based on this testing, it was concluded that the chamber can successfully maintain temperature within 0.5 °C, another of the engineering specifications.

**Figure 71.** CO2 concentration on day 1 (left) and day 2 (right) of cell testing. CO2 concentrations is in percent and samples are taken every 3 seconds.

**Figure 72.** O2 concentration on day 1 (left) and day 2 (right) of cell testing. O2 concentration is in percent and samples are taken every second.
Figure 73. Temperature on day one (left) and day two (right) of cell testing. Temperature is in °C and samples are taken every 3 seconds.

The cell testing did not provide any useful results. As seen in Figure 74 & 75, cells did not grow in either incubator. This is most likely due to the lack of cells initially since neither incubator had successful growth. Though the images may look blurry, they were in focus. There was something either on the objective lens or the camera lens which appear in every picture taken that make the photos look blurry. It is possible there could have been contamination in the original sample as well. This limitation will be discussed in a further section. Figure 76 shows evidence of humidification, which is necessary for cell growth and meets the engineering specification of maintaining a humid environment at 37°C.

Figure 74. Well plate from standard incubator after two days.
**Figure 75.** Well plate from experimental incubator after two days.

**Figure 76.** Evidence of humidification.

**Discussion and Future Directions**

**Engineering Specifications and Success Criteria**

Of the testable engineering specifications, the incubator passed most. Leak testing showed its ability to maintain an incubator environment without the control system making changes to the environment. The overnight testing as well as cell testing proved that the incubator could reach the desired O$_2$ concentration
in less than three hours and able to maintain it for an extended period of time. The incubator was not capable of maintaining less than a 0.1% input tolerance, but it can stay well within ±0.5% of the setpoint, as evidenced by the overnight and cell testing. Cell testing also verified that the incubator was able to maintain a set temperature within ±0.5°C and maintain a humid environment at 37°C. The main issue with the incubator as it stands is the inability to maintain CO₂ concentration at 5% ±0.5%. Solutions to this issue are discussed below. Engineering specifications such as withstanding temperatures up to 121°C and withstanding pressures up to 16 psig were inferred from manufacturing data from the materials selected. As for the ability to fit 2 T-75 flasks, the incubator was dimensioned to ensure at least 2 T-75 flasks would be able to fit inside. Ease of cleaning was tested before cell testing since the environment had to be sterilized. It may be a bit laborious to remove the internal electronic components each time for cleaning but overall, the process did not take too long. The electrical components have been used all throughout the testing process without need for charging or changing, which meets another specification. Finally, the components lasting 2 years or longer was unable to be tested, but data sheets suggest this specification will be met. The data sheets referenced in this section may be found in Appendix H. By meeting most of the engineering specifications set, much of the success criteria was met as well. Although the project went over the original $2000 budget, we were able to create a functional, controllable gas system, as evidenced by the many rounds of testing and optimization. The incubator can set and control O₂ levels but cannot maintain 5% CO₂ for an extended period of time in its current state. The temperature system is also functional and controllable, shown by the overnight tests maintaining 37°C. The chamber is sealable, but not completely airtight. However, through leak testing, we were able to quantify this and conclude that the control system can correct any changes in the environment due to leakage. The chamber does create a hypoxic environment; however, we were unable to confirm its cell culturing capabilities due to limitations in the testing.

Limitations

As mentioned previously, an inadequate pressure regulator for the CO₂ gas was a major issue. The wide pressure range was not suitable for this project since getting small pressure values is difficult with a wide range. This issue affected our testing and optimization phase of the project. During testing, it was difficult
to get CO₂ in spec and keep it there and the same is true for the optimization phase. CO₂ not being in spec would adversely affect any cells being incubated because it would affect the pH of the media they are being grown in. This would be a concern for cells that are sensitive to pH. Solving this issue is imperative before any more cell testing occurs.

The invalid results of the cell testing are also a limitation of this project. The fact that neither incubator had cells grow means that our incubator may still be able to house cells, but it is uncertain at this point. Combined with the CO₂ overshooting for most of the experiment, this test did not provide useable data for cell testing.

Another limitation of this project is that a computer is always required to run the incubator. One is also necessary if the code needs to be changed. The user must interact with raw code to change setpoints, which is not ideal on a user interface front. Having to reupload the code while ensuring the code is being sent to the proper board is cumbersome for the user. Solutions for these issues are presented in the next section.

Future Directions

Control & Gas System Iteration

The main concern from our data collection is the overshoot of the CO₂ gas system, these results can be referenced in the CO₂-optimization & overnight hypoxia test. The current system was able to recognize the designated set point and up-regulate CO₂ concentration, as desired but overshot the setpoint most of the time. While maintaining hypoxia settings, the nitrogen input helps downregulate the CO₂ concentration in the chamber, shown in the overnight hypoxia test. However, the effects were not enough to stabilize CO₂ concentration around 5%. The main issue was that the flow rate was too high at the lowest possible regulator setting with the current setup. For the future of this project, we would recommend purchasing a lower pressure regulator that can control the CO₂ gas flow to an extremely low flow rate or increasing resistance with a flow regulator. Flow rate and resistance are inversely related so increasing resistance will decrease flow rate. Decreasing cross-sectional area is one way to increase resistance, so implementing a flow regulator to decrease cross-sectional area would decrease flow rate. Decreasing the CO₂ flow rate would account for less overshoot and could eliminate the need for a new regulator. Additionally, new methods could be implemented with the code and could continue to be optimized with the current high
flow. Additional tests would need to be performed with the selected alteration to the system to assess if the 
CO₂ overshoot would be lowered to stay within spec. Once the selected change is implemented, another 
cell incubation test should be run to ensure that CO₂ stays within spec and the chamber is capable of 
incubating cells.

*User Interface Streamlining*

The current user must use a computer & the raw Arduino code to change to a new set point for all of the 
gas systems and the heating system. For a future project, the user interface could be streamlined to set input 
values without needing to open and upload the raw code or to not need a computer at all. The display set up 
currently uses either the serial monitor, Excel data stream & CoolTerm, or the small LED displays. In the 
future, one interactive display could be used to set inputs and view data to make the user experience easier, 
need less training to operate the incubator, and to avoid any mistakes uploading code.
REFERENCES/WORKS CITED/BIBLIOGRAPHY


Hung, S.-C., Pochampally, R. R., Chen, S.-C., Hsu, S.-C., & Prockop, D. J. (2007). Angiogenic Effects of Human Multipotent Stromal Cell Conditioned Medium Activate the PI3K-Akt Pathway in Hypoxic


**APPENDICES**

A. **Core Customer Charts**

A.1 **SUCCESS CRITERIA**

*Table IX. Overall success criteria.*

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Within budget</td>
<td>• Market-ready device</td>
</tr>
<tr>
<td>• Functional, controllable gas system (O2, CO2, N2, H2O)</td>
<td>• Streamlined user interface</td>
</tr>
<tr>
<td>• Set/control O2 levels, while maintaining 5% CO2</td>
<td>• Validation that angiogenesis can occur in hypoxic incubator</td>
</tr>
<tr>
<td>• Functional, controllable temp system</td>
<td></td>
</tr>
<tr>
<td>• Gas and heat uniform distribution</td>
<td></td>
</tr>
<tr>
<td>• Chamber is sealable and airtight</td>
<td></td>
</tr>
<tr>
<td>• Successfully creates hypoxic environment in which to culture cells</td>
<td></td>
</tr>
</tbody>
</table>

A.2 **MAJOR DELIVERABLE CHARTS**

*Table X. Major sub-deliverable 1: Literature review.*

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Annotating relevant sources</td>
<td>• In depth look into each paper</td>
</tr>
<tr>
<td>• 50+ sources</td>
<td>• Irrelevant papers/research</td>
</tr>
<tr>
<td>• Wide scope covering all aspects of the project</td>
<td></td>
</tr>
<tr>
<td>• Pulling specific details from papers</td>
<td></td>
</tr>
<tr>
<td>• Clinical relevance</td>
<td></td>
</tr>
<tr>
<td>• Patent review</td>
<td></td>
</tr>
</tbody>
</table>

*Table XI. Major Sub-deliverable 2: Incubation chamber structure.*

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
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</thead>
<tbody>
<tr>
<td>• Sealed chamber</td>
<td>• Gas system</td>
</tr>
<tr>
<td>• Material selection</td>
<td>• Heating system</td>
</tr>
<tr>
<td>• Designed CAD File</td>
<td>• Control system</td>
</tr>
<tr>
<td>• Detailed BOM</td>
<td>• Electrical components</td>
</tr>
<tr>
<td>• Prototyping &amp; Assembly</td>
<td></td>
</tr>
<tr>
<td>• Temperature control</td>
<td></td>
</tr>
<tr>
<td>• Aseptic environment</td>
<td></td>
</tr>
<tr>
<td>• Mechanical drawings</td>
<td></td>
</tr>
<tr>
<td>• Testing: seals, thermal regulation, etc.</td>
<td></td>
</tr>
</tbody>
</table>
### Table XII. Major sub-deliverable 3: Gas system.

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
</tr>
</thead>
<tbody>
<tr>
<td>• O2 level control</td>
<td>• Humidity control</td>
</tr>
<tr>
<td>• CO2 level control</td>
<td>• Temperature control</td>
</tr>
<tr>
<td>• Inert gas control</td>
<td>• Incubation structure</td>
</tr>
<tr>
<td>• Uniform gas mixing</td>
<td></td>
</tr>
<tr>
<td>• User interface: read gas levels</td>
<td></td>
</tr>
<tr>
<td>• User interface: input desired O2 level</td>
<td></td>
</tr>
<tr>
<td>(setpoints)</td>
<td></td>
</tr>
<tr>
<td>• Control/understanding of other gas levels</td>
<td></td>
</tr>
<tr>
<td>• Testing: control systems</td>
<td></td>
</tr>
</tbody>
</table>

### Table XIII. Major sub-deliverable 4: Temperature and humidity system.

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Temp control</td>
<td>• Cooling system</td>
</tr>
<tr>
<td>• Water pan for humidity</td>
<td>• Gas system</td>
</tr>
<tr>
<td>• Uniform temperature distribution</td>
<td>• Chamber structure</td>
</tr>
<tr>
<td>• Heating system</td>
<td></td>
</tr>
<tr>
<td>• User interface (must be able to read</td>
<td></td>
</tr>
<tr>
<td>temperature level)</td>
<td></td>
</tr>
<tr>
<td>• Testing: thermal regulation</td>
<td></td>
</tr>
</tbody>
</table>

### Table XIV. Major Sub-deliverable 5: System integration and assembly testing.

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Functioning subsystems (independently)</td>
<td>• Integration of gas and temperature control</td>
</tr>
<tr>
<td>• Functioning subsystems (full assembly)</td>
<td>• Mechanical testing of our materials (cyclic fatigue, stress tests, etc.)</td>
</tr>
<tr>
<td>• Testing subsystems and assembly as</td>
<td>• Biocompatibility testing</td>
</tr>
<tr>
<td>incubator for cell culturing</td>
<td></td>
</tr>
<tr>
<td>• Evaluation testing via maintaining consistent gas and</td>
<td></td>
</tr>
<tr>
<td>temperature reads</td>
<td></td>
</tr>
</tbody>
</table>
### Table XV. Major sub-deliverable 6: User interface.

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Temp control/display</td>
<td>• Streamlined controls</td>
</tr>
<tr>
<td>• Gas concentration control/display</td>
<td>• Expensive displays</td>
</tr>
<tr>
<td>• Easy to open and close incubator door</td>
<td></td>
</tr>
<tr>
<td>• Interactions with control systems</td>
<td></td>
</tr>
<tr>
<td>• Ability to maintain</td>
<td></td>
</tr>
<tr>
<td>• Easy to clean</td>
<td></td>
</tr>
<tr>
<td>• Autoclavable</td>
<td></td>
</tr>
</tbody>
</table>

### Table XVI. Major sub-deliverable 7: User documentation.

<table>
<thead>
<tr>
<th>Includes</th>
<th>Does Not Include</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Instructions for use (user manual)</td>
<td>• Mechanical drawings</td>
</tr>
<tr>
<td>• Maintenance manual</td>
<td>• Detailed BOM</td>
</tr>
<tr>
<td>• Testing documentation</td>
<td>• Literature review</td>
</tr>
<tr>
<td>• User instructions for code</td>
<td></td>
</tr>
</tbody>
</table>

### B. Project Management Tools

#### B.1 FLEXIBILITY MATRIX

Resources were ranked the least flexible due to our limited starting budget in comparison with other O₂ incubator systems on the market. Scope was moderate because we know what the project requirements were, but the design was flexible. The most flexible consideration was the schedule, due to our abilities to alter our personal schedules to prioritize the project and finish accordingly.

### Table XVII. Flexibility matrix.

<table>
<thead>
<tr>
<th></th>
<th>Least</th>
<th>Moderate</th>
<th>Most</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scope</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Schedule</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Resources</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
B.2 RISK MANAGEMENT

Table XVIII. Risk assessment.

<table>
<thead>
<tr>
<th>Potential Issue</th>
<th>Alternative Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas control system non-functional</td>
<td>Manual controlled valves, apply different coding techniques, try different products: sensors/valves, sensing methods</td>
</tr>
<tr>
<td>Long lead time ordering OTS parts/materials</td>
<td>Order early, research lead times early, source local materials, borrow materials from university</td>
</tr>
<tr>
<td>Poor seal on incubation chamber</td>
<td>Design control systems for possible leaking</td>
</tr>
<tr>
<td>Malfunctioning temperature control</td>
<td>Apply alternative heating source</td>
</tr>
<tr>
<td>Delayed integration / prototyping</td>
<td>Develop robust systems for functioning subassemblies</td>
</tr>
</tbody>
</table>

B.3 BUDGET

Table XIX. Initial budget.

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Product Number</th>
<th>Purpose</th>
<th>Associated Task</th>
<th>Planned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unit</td>
</tr>
<tr>
<td>Luminox-O2 sensor</td>
<td>LOX-O2</td>
<td>Sensing O2 levels</td>
<td>O2, CO2 control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Quantity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$259.00</td>
</tr>
<tr>
<td>Arduino Uno Rev. 3 board</td>
<td>A000066</td>
<td>Controller for O2 circuit</td>
<td>O2, CO2 control &amp; temperature control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$23.00</td>
</tr>
<tr>
<td>Breadboard Pack</td>
<td>64</td>
<td>Create connections for O2 circuit</td>
<td>O2, CO2 control &amp; temperature control</td>
<td>EA 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$11.49</td>
</tr>
<tr>
<td>4-channel logic converter</td>
<td>757</td>
<td>Convert signals in O2 circuit</td>
<td>O2, CO2 control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$4.00</td>
</tr>
<tr>
<td>iTemco 36 AWG wire spool</td>
<td>MW0542</td>
<td>Make electrical connections</td>
<td>O2, CO2 control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$5.40</td>
</tr>
<tr>
<td>Acrylic mounting plate</td>
<td>275</td>
<td>Mount the O2 circuit (may not need)</td>
<td>O2, CO2 control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$5.00</td>
</tr>
<tr>
<td>Heat shrink tubing</td>
<td>4559</td>
<td>Protect wires</td>
<td>O2, CO2 control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$9.95</td>
</tr>
<tr>
<td>Breadboard jumper wires</td>
<td>153</td>
<td>Make electrical connections</td>
<td>O2, CO2 control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$6.00</td>
</tr>
<tr>
<td>CO2 sensor</td>
<td>TBD</td>
<td>Sensing CO2 levels</td>
<td>O2, CO2 control</td>
<td>EA 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$169.00</td>
</tr>
<tr>
<td>Item Description</td>
<td>Product Number</td>
<td>Purpose</td>
<td>Associated Task</td>
<td>Actual Price (Unit: $)</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------------</td>
<td>--------------------------------------------</td>
<td>-----------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Incubator material</td>
<td>TBD</td>
<td>Make incubator</td>
<td>Incubator structure</td>
<td>EA</td>
</tr>
<tr>
<td>Heating element</td>
<td>TBD</td>
<td>Apply heat to maintain desired heat level</td>
<td>Temp control</td>
<td>EA</td>
</tr>
<tr>
<td>Fan</td>
<td>4808</td>
<td>circulate both heat and gas levels</td>
<td>Uniform gas/heat mixing</td>
<td>EA</td>
</tr>
<tr>
<td>Pressure Regulator - N2</td>
<td>TBD</td>
<td>Maintain proper pressure for Nitrogen levels</td>
<td>O2, CO2 control</td>
<td>EA</td>
</tr>
<tr>
<td>N2 gas cylinder</td>
<td>TBD</td>
<td>N2 supply for controlling gas levels</td>
<td>O2, CO2 control</td>
<td>EA</td>
</tr>
<tr>
<td>O2 gas cylinder</td>
<td>TBD</td>
<td>O2 supply for controlling gas levels</td>
<td>O2, CO2 control</td>
<td>EA</td>
</tr>
<tr>
<td>CO2 gas cylinder</td>
<td>TBD</td>
<td>CO2 supply for controlling gas levels</td>
<td>O2, CO2 control</td>
<td>EA</td>
</tr>
<tr>
<td>Tubing</td>
<td>TBD</td>
<td>to transfer gas to incubator</td>
<td>O2, CO2 control</td>
<td>EA</td>
</tr>
<tr>
<td>Thermometer</td>
<td>TBD</td>
<td>sense temperature levels</td>
<td>temp control</td>
<td>EA</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Description</td>
<td>Model/Part Number</td>
<td>Notes</td>
<td>Control type</td>
<td>EA</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------------------</td>
<td>--------------------------------------------</td>
<td>--------------</td>
<td>----</td>
</tr>
<tr>
<td>AWG wire spool</td>
<td>275</td>
<td>connections O2, CO2 control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Heat shrink tubing</td>
<td>4559</td>
<td>Protect wires O2, CO2 control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Breadboard jumper wires</td>
<td>153</td>
<td>Make electrical connections O2, CO2 control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>CO2 sensor</td>
<td>2091-SPRINTIR-WF-20-ND</td>
<td>Sensing CO2 levels O2, CO2 control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>SPDT Relay</td>
<td>COM-00100</td>
<td>Control solenoid/heating pads Temp/gas control</td>
<td>T</td>
<td>6</td>
</tr>
<tr>
<td>TIP120 Transistor</td>
<td>497-2539-5-ND</td>
<td>Circuits Temp/gas control</td>
<td>T</td>
<td>5</td>
</tr>
<tr>
<td>5V DC Heating Pad</td>
<td>1568-1798-ND</td>
<td>Heating incubator Temp control</td>
<td>T</td>
<td>4</td>
</tr>
<tr>
<td>12V Power Supply</td>
<td>N/A</td>
<td>Power circuits Temp/gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Resistor Kit</td>
<td>N/A</td>
<td>Circuits Temp/gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Capacitor Kit</td>
<td>N/A</td>
<td>Circuits Temp/gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Diode Pack</td>
<td>N/A</td>
<td>Circuits Temp/gas control</td>
<td>EA</td>
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</tr>
<tr>
<td>Dupont Wire Pack</td>
<td>N/A</td>
<td>Circuits Temp/gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Perf Board Kit</td>
<td>N/A</td>
<td>Circuits Temp/gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>18 Gauge Wire</td>
<td>N/A</td>
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<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>2N2222A Transistors</td>
<td>38236</td>
<td>Circuits Temp/gas control</td>
<td>T</td>
<td>5</td>
</tr>
<tr>
<td>Alligator Clip Wires</td>
<td>N/A</td>
<td>Circuits Temp/gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Fan</td>
<td>AFB0612LB</td>
<td>circulate both heat and gas levels Uniform gas/heat mixing</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Heat Sink</td>
<td>ATS-61700W-C2-R0</td>
<td>Distribute heat Temp control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Temp Sensors</td>
<td>SEN-14049</td>
<td>Sensing temp Temp control</td>
<td>T</td>
<td>4</td>
</tr>
<tr>
<td>4 Digit Display</td>
<td>1497-1487-ND</td>
<td>Display CO2 concentration Gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>7 Segment Display with Backpack</td>
<td>1528-1473-ND</td>
<td>Display CO2, temp, and O2 values Gas control</td>
<td>T</td>
<td>4</td>
</tr>
<tr>
<td>Solenoid Valves</td>
<td>N/A</td>
<td>Control gas Gas control</td>
<td>T</td>
<td>3</td>
</tr>
<tr>
<td>Pressure Relief Valve</td>
<td>4772K4</td>
<td>Release pressure in system Gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Tubing -25 ft</td>
<td>5233K58</td>
<td>Transfer gas Gas control</td>
<td>T</td>
<td>2</td>
</tr>
<tr>
<td>Tube-Tube Adapter Pack</td>
<td>5372K513</td>
<td>Connect tubing Gas control</td>
<td>EA</td>
<td>1</td>
</tr>
<tr>
<td>Item Description</td>
<td>Part Number</td>
<td>Description</td>
<td>Department</td>
<td>Quantity</td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>-----------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>---------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Solenoid Adapter Pack</td>
<td>5372K123</td>
<td>Connect solenoids to tubing</td>
<td>Gas control</td>
<td>EA</td>
</tr>
<tr>
<td>Barbed Adapter Pack</td>
<td>5372K114</td>
<td>Connect tubing to incubator</td>
<td>Gas control</td>
<td>T</td>
</tr>
<tr>
<td>#36 O-ring</td>
<td>37155967506</td>
<td>Leak proofing connections</td>
<td>Gas control</td>
<td>EA</td>
</tr>
<tr>
<td>Regulator Adapters</td>
<td>N/A</td>
<td>Adapt regulators to tubing</td>
<td>Gas control</td>
<td>T</td>
</tr>
<tr>
<td>Pressure Regulator - N2</td>
<td>3687N115</td>
<td>Maintain proper pressure for Nitrogen</td>
<td>Gas control</td>
<td>EA</td>
</tr>
<tr>
<td>Pressure Regulator - CO2</td>
<td>7897A39</td>
<td>Maintain proper pressure for CO2</td>
<td>Gas control</td>
<td>EA</td>
</tr>
<tr>
<td>Electrical Tape</td>
<td>783250809691</td>
<td>Attach circuit components</td>
<td>Circuitry</td>
<td>EA</td>
</tr>
<tr>
<td>De-soldering Kit</td>
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<td>Circuitry</td>
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**Total** $2,449.96
In the fall, we developed initial concept designs, engineering specs, started our CAD model, and did extensive past product, control system, and biological relevance research. Following fall quarter, at the beginning of January, we selected a design to move forward with and worked on creating a detailed CAD model. This included selecting and ordering all our circuit and incubator structure materials, making a cardboard prototype, and started the build of our initial circuits. Spring quarter we finalized our circuit control systems for both heating and gas, constructed our incubator, updated designs, ordered additional materials, and started the writing of many protocols and testing plans. By the end of quarter and through the early summer the incubator structure build was completed, all 3D printed parts and inner shelf build were completed, our circuits were all soldered on perf boards and were tested separately and together, and all optimizing, testing, and statistical analysis of our control system within the incubator structure were finished. The last task we have listed is our project defense.
Table XXI. Full project Gantt chart.

<table>
<thead>
<tr>
<th>#</th>
<th>Task</th>
<th>Duration</th>
<th>Start Date</th>
<th>End Date</th>
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<tr>
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<td>Sun 10/17/21</td>
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<td>Thu 10/28/21</td>
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<td>Sun 11/7/21</td>
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<td>Step up heating system &amp; fan in chamber</td>
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<td>Sun 4/25/21</td>
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<td>2 days</td>
<td>Mon 5/23/22</td>
<td>Tue 5/24/22</td>
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<td>Mon 5/23/22</td>
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<td>Thu 6/10/22</td>
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<tr>
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<td>15 days</td>
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<td>Fri 7/8/22</td>
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<tr>
<td>Make Statistical Models for all Testings Results (accuracy)</td>
<td>7 days</td>
<td>Fri 7/1/22</td>
<td>Mon 7/11/22</td>
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<tr>
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<td>11 days</td>
<td>Mon 6/27/22</td>
<td>Mon 7/11/22</td>
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<td>3 days</td>
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<td>Thu 7/21/22</td>
<td>Thu 7/26/22</td>
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<tr>
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<td>Thu 8/9/22</td>
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<td>5 days</td>
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100
B.5 PENTA CHART

Currently, most hypoxic incubators use mixed gas, so the goal of this project was to create a control system that would allow for any desired concentrations of N₂, O₂, and CO₂ to be used in tumor research. A more in-depth description of the approach can be seen in the Penta Chart, Figure 78.

Figure 77. Network diagram.

Figure 78. Penta chart.
C. House of Quality

![House of Quality Diagram]

D. Prototype Survey

Questionnaire:

1. How is the overall sizing of the chamber?
   a. Response: It depends on the size/quantity of what you want in it? It’s enough to fit one T25 comfortably

2. Do you feel there is enough space to move things around comfortably?
   a. Response: just barely for like 1-2 flasks. Depending on what this box with a tail is supposed to represent

3. Any glaring issues you notice?
   a. Response: I have no idea how big it should be because idk what size and how many flasks you are trying to house

4. Feel free to leave any other comments, thank you!!
### E. Bill of Materials

**Table XXII.** Bill of materials used in incubator structure.

<table>
<thead>
<tr>
<th>ITEM #</th>
<th>PART NUMBER</th>
<th>PART NAME</th>
<th>MATERIAL</th>
<th>QTY</th>
<th>VENDOR</th>
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<tbody>
<tr>
<td>1</td>
<td>SM11-A</td>
<td>SENSOR BOX, BOTTOM PANEL</td>
<td>POLYCARBONATE, CLEAR</td>
<td>1</td>
<td>MTS (CUSTOM)</td>
</tr>
<tr>
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<td>SM21-A</td>
<td>SENSOR BOX, SIDE PANEL 1</td>
<td>POLYCARBONATE, CLEAR</td>
<td>1</td>
<td>MTS (CUSTOM)</td>
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<tr>
<td>3</td>
<td>SM31-A</td>
<td>SENSOR BOX, SIDE PANEL 2</td>
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<td>1</td>
<td>MTS (CUSTOM)</td>
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<td>4</td>
<td>SM41-A</td>
<td>SENSOR BOX, FRONT/BACK PANEL</td>
<td>POLYCARBONATE, CLEAR</td>
<td>2</td>
<td>MTS (CUSTOM)</td>
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<tr>
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<td>PLA</td>
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<td>90’ GLASS DOOR HINGES</td>
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<td>RUBBER</td>
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</table>
F. Detailed Drawings: Incubator chamber
F.1 WATER JET DXF FILES

Figure 80. DXF files used to water jet cut stock: polycarbonate left, aluminum right.

F.2 POLYCARBONATE MECHANICAL DRAWINGS
NOTES:
1. PART IS TO BE WATER JET
2. SURFACE FINISH: MEDIUM
3. ALL SURFACE EDGES AND HOLES ARE TO BE SMOOTHED AND DERSIGNED IF NECESSARY

THIRD ANGLE PROJECTION
NOTES:
1. MATERIAL: POLYCARBONATE, CLEAR
2. FINISH: MEDIUM SURFACE TREATMENT
3. ALL WELD SURFACES TO BE SMOOTHED

THIRD ANGLE PROJECTION

SM31-A

MEASUREMENTS
1/2
1.5
0.5
0.25
0.15
0.50
1

POLY CARBONATE MASTERS PROJECT
(503) 816-4145

8

CAL POLY BMED MASTERS PROJECT
(503) 816-4145

8

SM41-A

THIRD ANGLE PROJECTION
F.3 ALUMINUM MECHANICAL DRAWINGS

NOTES:
1. SURFACE TREATMENT: MEDIUM
2. ALL HOLES SHOULD BE收入, ALL SIDES Sanded

3. 3X 12.95 THRU
   0
2X 60.0
90.0
2X

ANGULAR:
X.X   0.5
8
ALM21 A
THIRD ANGLE PROJECTION

3. 3X 20.00 THRU
20.00
19.00
0

ANGULAR:
X.X   1
CAL POLY BMED INCUBATOR PROJECT
(503)-816-4145
8
ALM11 A
THIRD ANGLE PROJECTION
F.4 ASSEMBLY DRAWINGS
G. Control System Progression

Figure 81. Breadboard relay circuit.

Figure 82. Basic temperature sensor circuit.
Figure 83. Temperature relay test setup.

Figure 84. Perf board map.
**Figure 85.** Backside of perf board.

**H. Data Sheets: Off-the-shelf Components**

Click the [link](#) provided to access folder of all necessary data sheets from components used throughout this project.
I. Detailed Manufacturing Protocols

I.1 CHAMBER MANUFACTURING

Protocol

1. Create DXF files, which can be referenced in Appendix A, in SolidWorks 2020 in order to cut out the necessary parts from the stock Aluminum and Polycarbonate 1/4in sheets.
   a. The incubator inner and outer walls, sensor box, and outer door were cut out via the water jet using these DXF files, the overall dimensions of these cuts are shown in the mechanical drawings in Appendix A.

2. For all parts indicated in procedure below refer to CAD file INCU123 for necessary dimensions. Drill all holes with a drill press, all special speed/drilling requirements will be noted if necessary for successful cut.

3. Part IN111-A: Front Panel
   a. Drill four holes, drill bit size 17, for the glass hinge attachment
   b. Drill two holes, drill bit size 17, for the glass latch attachment

4. Part IN11-A: Bottom Outer Panel
   a. Drill four holes, drill bit size 17/64, for the four adjustable feet
   b. Drill two holes, drill bit size 17, for the bottom corner bracket

5. Part IN71-A: Top Panel, Chamber Input
   a. Drill two holes, drill bit size 17, for the top corner bracket

6. Part IN91-A: Outer Size Wall
   a. On one side wall, drill four holes, drill bit size 17, for the top and bottom corner brackets
   b. On the second side wall, drill four holes, drill bit size 17, for the outer door hinges

7. Part IN181-A: Outer Back Panel
   a. Drill three holes, drill bit size 17/64 for CO₂ and O₂ gas inlets
   b. Drill two 1/2in holes, drill bit size 1/2, for wire outlet and additional outlet hole

8. Part DO31-A: Door side panel
   a. Drill four holes, drill bit size 17, for outer door hinge

9. Part DO1-A: Main Door Panels
   a. On one door panel, drill 2 holes, drill bit size 15, for heat inserts used to attach magnet assembly

10. Part SM61-A: Sensor Box, Top Panel
    a. Drill two holes, drill bit size 17, for bracket attachment
    b. Cut out slot via table saw and hole drill, size 15, for sensor wires

11. Part SM31-A: Sensor Box, Side Panels
    a. Drill one hole, drill bit size 17, on each side panel for bracket attachment

12. Part SM51-A: Sensor Box, Middle Panel
    a. Drill one hole, drill bit size 15, for CO₂ insert
    b. Drill two holes, drill bit size 16, for O₂ insert
    c. Sand holes until sensors fit snugly in inserts

13. Part IN41-A: Incubator Walls
    a. On one aluminum wall, drill three holes, drill bit size 27/32, for CO₂ and O₂ inlets
       i. Have drill press set to low speed and apply consistent pressure
    b. On same aluminum wall, drill two holes, drill bit size 1/2, for electronics outlet
    c. Use deburring tool to clean holes after drilling and remove any additional material to have smooth surface finish

14. Part IN51-A: Incubator Top
    a. Drill two holes, drill bit size 1”, for the sensor box inlet and outlet
       i. Use lowest speed possible for the drill press for optimal cutting
       ii. Use deburring tool to clean holes after drilling and remove any additional material to have smooth surface finish
b. Drill four holes, drill bit size 36, then thread all holes with #6-32 threads using hand threading tool and aluminum cutting oil, for standoffs for fan assembly

15. Cut Insulation using box cutter and wear a mask (placed in-between inner and outer incubator boxes and outer door); six total parts

16. Shelf Assembly
   a. Cut four equal sized sections out of the L bracket of aluminum for shelf feet
   b. Cut 3mm thickness aluminum sheet into desired dimension
   c. Drill 1/2in holes 5x7 (35 total) using drill bit size ½ into aluminum sheet
   d. Epoxy shelf feet onto aluminum sheet for shelf

17. Sensor Box Assembly
   a. Sand all connecting edges of sensor box polycarbonate
   b. Epoxy all sensor box parts together
   c. Epoxy hex nuts onto inner side of L brackets
   d. Place O₂ and CO₂ sensors inside middle panel
   e. Use 4 #6-32 316 screws to faster the corner brackets and top panel

18. Aluminum Chamber Assembly
   a. File all edges of the aluminum for flat surface finish
   b. Sand all connecting edges of the aluminum box
   c. Epoxy all aluminum box parts together
   d. Attach four #6-32 F/M standoffs to top panel
   e. Attach fan and four #6-32 screws to top panel
   f. Attach three adaptors to the gas inlet holes and fasten with appropriate hex nuts
   g. Cut two 1” length corner brackets and epoxy to back corners of aluminum box
   h. Epoxy magnets in desired locations to outer surface of aluminum box for heating pad attachment
   i. Epoxy wire sleeve to back of aluminum chamber behind 1/2” hole
   j. Epoxy sensor box directly to top panel; align with inlet and outlet

19. Outer Door Assembly
   a. Use soldering iron and appropriate tip to install two #6-32 heat set inserts
   b. Epoxy top/bottom edges and front door panel to back door panel
   c. Insert insulation
   d. Epoxy hex nuts onto back of holes for door hinge & epoxy two side panels of door
   e. Use #8-32 screws to faster hinges to the side door panel
   f. Screw #6-32 M/M standoffs to the installed heat set inserts
   g. 3D print magnet holders
      i. Epoxy magnets to bottom of holders
      ii. Use soldering iron and appropriate tip to install two #6-32 heat set inserts
   h. Attach magnet holders to the back door panel
   i. Cut out gasket material to proper dimension
      i. Epoxy gasket to outer edge of door

20. Polycarbonate Chamber Assembly
   a. Attach feet to bottom panel
   b. Epoxy all pieces, except for the front inner door panel and top panel
   c. Line all sides of box with insulation
   d. Place aluminum chamber in proper position
   e. Attach outer door hinge using #8-32 screws and corresponding nuts
   f. Cut out gasket material to proper dimension
      i. Epoxy gasket to edge of door opening
   g. Attach glass door hinges, latch, and glass sheet to the front panel using #6-32 screws and corresponding nuts
   h. Epoxy the front panel into position
   i. Fit top panel over sensor box and epoxy into place
   j. Attach corner brackets using #8-32 screws and corresponding nuts to top, bottom, and side panel

21. Place self, tray, electronics into incubator, and thread any wires out the wire outlet hole

22. Place 3D printed solenoid shelfs on back side of incubator & attaching to the gas inlets
23. Use washers and spacers were necessary and allow 8 hours for epoxy to dry
24. All hardware indicated are stainless steel 316
25. Fill in any cracks with water seal aluminum and blue tack

I.2 PERFORM BOARD MANUFACTURING

Materials

- SPDT Relays x4
- 2N2222 Transistor x4
- 1Kohm resistor x4
- Diode x4
- 18 AWG wire
- 22 AWG wire
- Header pins
- 5x7 cm perf boards x4
- Heat-shrink tubing
- Hair dryer/heat gun
- Soldering kit

Protocol (All steps apply to every board unless otherwise stated)
1. Solder both ends of the resistor according to Figure 32 using through hole soldering.
2. Solder both ends of the diode according to the figure using through hole soldering.
3. Solder the transistor using through hole soldering, the collector pin going towards the diode, the base pin going towards the resistor, and the emitter pin going the opposite direction as the others to be used later (see figure).
4. Solder the 5 pins of the relay according to the figure using through hole soldering.
5. Connect the ends of the diode to the bottom two pins according to the figure using wire or solder material.
6. Solder the free end of the resistor to a length of 22 AWG wire.
   a. Remove and strip one end of a male-male jumper cable and attach to 22 AWG wire using wire to wire soldering. Add heat shrink tubing over solder point.
7. Connect 2 header pins to the top right of the perf board using through hole soldering (will be the Arduino power and ground connections).
8. Solder a connection from the 5V Arduino connection to the front left relay pin according to the diagram using a length of wire.
9. Solder a connection from the ground Arduino connection to the free pin of the transistor using a length of wire.
10. For two of the four perf boards, remove one end of two female to female jumper cables and strip the wire to connect the Arduino 5V and ground connections, refer to Figure 50 (4 wires total).
11. For 1 of the four perf boards, solder 2 header pins on the top left of the board. These will be the 12 V power and ground connections
12. Solder a length of wire from the 12 V ground to top left pin on the relay.
13. Solder a length of wire from the middle right pin on the relay to a solenoid wire or ground wire on heating pads.
14. Solder a length of wire from the 12 V power to a solenoid wire or power wire on heating pads.
15. Cover wire-to-wire solder joints with heat-shrink tubing using a hair dryer or heat gun.
16. Connect the 12 V power and ground to all other perf boards as seen in the interconnection diagram.

I.3 INTEGRATION PROTOCOL

Materials

- Completed chamber
- Completed perf boards
- 22 AWG wire
- 18 AWG wire
- Oxygen sensor
- CO₂ sensor
- 12V solenoids x3
- Heating pads x2
- Temperature sensors x2
- Displays x2
- 12V fan
- Heat-shrink tubing
- Hair dryer/heat gun
- Soldering kit

**Protocol**

1. Place perf boards behind the chamber and feed wires attaching to the fan, heating pads, and temperature sensors through the designated wire hole.
   a. Solder a length of wire to a female ended jumper cable 6 times using 22 AWG wire.
   b. Attach free end to each pin of the two temperature sensors.
   c. Use the heat shrink tubing to cover the solder joints.
2. Assemble temperature sensor circuit on a breadboard as shown in Figure 35.
3. Solder 18 AWG wire to the positive and ground wires of the heating pads, making sure to place heat-shrink tubing on the wires before soldering. Also ensure the wires are fed through the wire channel so that the heating pads are in the box when soldering.
4. Solder 18 AWG wire to the ends of the solenoid wires (positive and ground do not matter), making sure to place heat-shrink tubing on the wires before soldering.
5. Solder 18 AWG wire to the positive and ground wires of the fan, making sure to place heat-shrink tubing on the wires before soldering.
6. Shrink all tubing with a hair dryer or heat gun.
7. Solder a length of 22 AWG wire in the middle of a female-to-male jumper wire 8 times. These are for the O$_2$ and CO$_2$ sensors, so they need to be long.
   a. Add heat shrink tubing to the solder joints with a hair dryer or heat gun.

**J. Additional Manufacturing Images**

**J.1 INNER CHAMBER**

![Figure 86. Cutouts of the aluminum inner chamber.](image-url)
Figure 87. Tube adaptor with hex nut on gas inlet cutout.

Figure 88. Fan assembly on top plate of aluminum chamber.

Figure 89. Alignment of sensor box on top plate of aluminum chamber.
Figure 90. Drying epoxy for aluminum chamber assembly: 90˚ made using carpenter square & held in place with paint cans.

J.2 SENSOR BOX

Figure 91. Applying and drying epoxy for sensor box.
Figure 92. Sensor box with press fit components: pressure relief valve includes O-ring.

J.3 INNER DOOR

Figure 93. Latches fastened on inner door assembly displaying O-rings & spacers.

Figure 94. Inner door assembly/front panel unassembled.
Figure 95. Polycarbonate half boxes for clearance with outer door hinges.

Figure 96. Polycarbonate inner door support material.
Figure 97. Plastic heat set insert in outer door used for threading standoffs.

Figure 98. Magnet attachment installed on outer door.
J.5 OUTER CHAMBER

Figure 99. Holes drilled out for outer chamber pre epoxying.

Figure 100. Epoxied outer chamber with feet and nylon spacer.
Figure 101. Shelf top plate: each hole measured, drilled, and deburred.

Figure 102. Each leg was hand sawed, filed, & leveled.
Figure 103. Four legs were epoxied first, the second bottom plate was leveled and epoxied second.

J.7  FULL CHAMBER

Figure 104. Measuring and cutting insulation material.
Figure 105. Fitting inner insulation material.

Figure 106. Inner chamber with heating pad, fan, temperature sensors, and removable shelf.
K. Additional Integration Photos

Figure 107. O₂ Regulator.

Figure 108. N₂ Regulator.
Figure 109. CO₂ Regulator.

Figure 110. In-line HEPA filter.
Figure 111. Sensors in place in sensor box.

Figure 112. Display in stand.
Figure 113. Functioning display.

Figure 114. 12 V power supply.

L. Detailed Test and Analysis Protocols

L.1 MATERIAL TESTING

L.1.1 IPA & Water Testing

Materials

- 70% IPA
- Tap water room temp
- Two beakers
- Parafilm
- Two samples aluminum
Two samples polycarbonate

**Protocol**

1. Cut two small rectangles of stock aluminum & polycarbonate < 3in. X 1in. via the water jet, vertical band saw, or hand saw.
   a. Ensure samples are of each material are the size.
2. Locate two beakers.
3. Fill one beaker with water and the second with 70% IPA.
4. Photograph, observe, and bend the sample materials before the start of the test.
5. Place one aluminum & one polycarbonate sample in the IPA beaker. Place the remaining two samples in the tap water beaker.
6. Cover both beakers with parafilm.
7. Wait 24 hours.
8. Remove samples from baths and record any visual observations.
9. Bend each sample (if possible) and drop each sample to assess any changes in mechanical strength.
10. Look for signs of brittle failure, cracking, and decolorization.
11. If no changes are observed visually and there are no changes in properties; materials pass IPA & water examination.

**L.2 LEAK TESTING**

**L.2.1 Soapy & Generic Water Testing**

**Materials**
- Soap
- Water
- Water bottle
- Testing material

**Protocol**

1. Epoxy necessary materials together.
2. Fill water bottle with water.
3. Pour water onto dried epoxied seams.
4. Visually assess any dripping from junction point & physically feel along seam for any moisture.
5. Mark any points of leaking.
6. Re-epoxy along sections of leaking.
7. Once all edges of material is epoxied together and seams are tested with water, add 1 tea spoon of soap to filled water bottle.
8. Apply soapy water to edges of chamber assess if any gas leaking is occurring via visualizing bubbling.
9. If no bubbling occurs, structure passes with no gas leaking.
10. If bubbling occurs, re-epoxy along regions where bubbling was found.

**L.2.2 Long Term Seal**

**Materials**
- O₂ Sensor
- CO₂ Sensor
- Hair Dryer
- Temperature Sensors
- Computer with Arduino installed
- 2 USB cables to connect Arduino
- Full control system code
- Full incubator installed into gas system
- Excel Data Stream plug in
• CoolTerm data recording plug in

Protocol
1. Turn on computer and open CoolTerm, Excel Data Stream, and Arduino
2. Open control system code for O₂ and CO₂, which can be referenced in Appendix K and L.
3. Plug both the Arduino Uno and Arduino Mega into computer.
4. Upload the O₂ control code to the Arduino Mega & connect the Mega to the CoolTerm plug in using port 4.
5. Upload the CO₂ control code to the Arduino Uno & open the serial monitor to ensure proper data collection.
6. Connect the Arduino Uno to the Excel Data Stream plug in using port 3.
7. Place one temperature sensor directly against inner chamber wall and one sensor directly on the external wall.
8. Use hair dryer on high to quick heat internal chamber to around 70˚C.
9. Shut incubator door.
10. Turn on the external power supply next to the incubator.
11. Allow the O₂ and CO₂ values to reach an equilibrium.
   a. Let the control system run until it reaches hypoxia (4.5-5.5% O₂) and remains at a constant value for 1 minute.
12. Start recording data on both the Excel Data Stream and on CoolTerm.
13. Turn off the external power supply
   a. This will turn off heating pads and will keep solenoids in a closed state while allowing sensors to maintain collecting data.
14. Leave both incubator doors fully closed
15. Allow data to collect for four hours, stop the collection at the end of the four-hour time period.
16. Upload O₂ Text data into excel.
17. Create graph of %O₂ over total time.
18. Upload temperature data into excel.
19. Create graph of change in temperature over total time.
20. Find line of best fit for %O₂ graph.
21. Record the slope value to find the rate of gas leaking.
22. Use change in temperature to find the rate of heat transfer over time.

L.3 CONTROL SYSTEM TESTING

L.3.1 Sensor Reactivity Testing

Materials
• O₂ sensor basic circuit
• CO₂ sensor basic circuit
• Temperature sensor basic circuit
• Computer with Arduino installed
• USB cable for Arduino
• Code for each sensor provided

Protocol
1. Connect sensing circuit of choice to computer using USB cable
2. Open the serial monitor in Arduino to view sensing values and ensure they are proper for the conditions of the room, and record the value (ambient O₂ ~ 21%)
3. Induce a change the component you are testing, examples below. Make a record of which method you used
   a. Blow on the O₂ sensor, values should decrease
   b. Blow on CO₂ sensor, values should increase
   c. Place fingers around temperature sensor, values should increase
4. Record the change and estimate the reactivity time

L.3.2 Sensor Calibration O₂

Materials
- O₂ Sensor, computer with Arduino installed
- USB cable to connect Arduino
- Basic code to receive sensor information
- Tubing
- O₂ regulator
- Vacuum bag or Ziplock
- Fixed gas amount (mixed gas chamber)

**Protocol**
1. Build circuit directly connecting O₂ sensor to Arduino.
2. Plug Arduino into computer and open serial monitor in Arduino to view sensing values
3. Reference Appendix B for the code used to retrieve sensor values
4. Cut out one/two small circular holes in bag
5. Open bag and place O₂ sensor in enclosure threading wires through one of the small holes in the bag or through the bag opening
6. Re-seal bag opening and plug any cracks with blue tack
7. Connect tubing from gas chamber to regulator and to the enclosed bag using barbed fittings and press fits
8. Vacuum any/all air out of the bag
9. Flood bag with pre-set mixed air
10. Assess if sensor values matched the pre-set mixed air concentration
11. If values DO NOT match continue to calibration protocol
   a. Build circuit directly connecting O₂ sensor to Arduino.
   b. Plug Arduino into computer and open serial monitor in Arduino to view sensing values
   c. Reference Appendix B for the code used to retrieve sensor values
   d. Cut out one/two small circular holes in bag
   e. Open bag and place O₂ sensor in enclosure threading wires through one of the small holes in the bag or through the bag opening
   f. Re-seal bag opening and plug any cracks with blue tack
   g. Connect tubing from nitrogen gas chamber to the regulator and to the enclosed bag using barbed fittings and press fits
   h. Vacuum any/all air out of the bag
   i. Flood bag with nitrogen gas
   j. Run calibration code indicated in Appendix B
   k. Set sensor for a value of 0% O₂

**L.3.3 Sensor Calibration CO₂**

**Materials**
- CO₂ Sensor
- Computer with Arduino installed
- USB cable to connect Arduino
- Basic code to receive sensor information
- Tubing
- CO₂ regulator
- CO₂ incubator

**Protocol**
1. Build circuit directly connecting CO₂ sensor to Arduino.
2. Plug Arduino into computer and open serial monitor in Arduino to view sensing values
3. Reference Appendix B for the code used to retrieve sensor values
4. Place CO₂ sensor in aseptic dish and insert into CO₂ incubator enclosure threading wires through a small opening in chamber
5. Close and seal incubator
6. Assess if sensor values match the 5% CO₂ conditions of the incubator
7. If values DO NOT match continue to calibration protocol
   a. Build circuit directly connecting CO₂ sensor to Arduino.
   b. Plug Arduino into computer and open serial monitor in Arduino to view sensing values
c. Reference Appendix B for the code used to retrieve sensor values
d. Cut out one/two small circular holes in bag
e. Open bag and place CO₂ sensor in enclosure threading wires through one of the small holes in the bag or through the bag opening
f. Re-seal bag opening and plug any cracks with blue tack
g. Connect tubing from nitrogen gas chamber to the regulator and to the enclosed bag using barbed fittings and press fits
h. Vacuum any/all air out of the bag
i. Flood bag with nitrogen gas
j. Run calibration code indicated in Appendix B
k. Set sensor for a value of 0% CO₂

L.3.4 Environmental control optimization

Materials
- O₂ Sensor
- CO₂ Sensor
- All gas regulators and pressure relief valve
- Computer with Arduino installed
- 2 USB cables to connect Arduino
- Full control system code
- Full incubator installed into gas system
- Excel Data Stream plug in
- CoolTerm data recording plug in

Engineering Specs
- Desired Range O₂: 4.5 - 5.5%
- Desired Range CO₂: 4.5-5.5%

Controlled Settings
- Gas regulators are set to the lowest possible PSI, while still maintaining an open flow.
- Low Range CO₂: 4.5%
- Low Range O₂: 4.5%
- High Range O₂: 5.4%
- Multiplier value for threshold: N₂: 1.2, CO₂: 0.3, O₂: 0.8

Explanatory Variables
- Threshold interval time lengths:
  o N₂ & O₂: 1ms & 3ms
  o CO₂: 6ms & 10ms

Protocol
1. Turn on computer and open CoolTerm, Excel Data Stream, and Arduino
2. Open control system code for O₂ and CO₂, which can be referenced in Appendix K and L.
3. Plug both the Arduino Uno and Arduino Mega into computer.
4. Upload the O₂ control code to the Arduino Mega & connect the Mega to the CoolTerm plug in using port 4.
5. Upload the CO₂ control code to the Arduino Uno & open the serial monitor to ensure proper data collection.
6. Close the serial monitor and connect the Arduino Uno to the Excel Data Stream plug in using port 3.
7. Turn on the external power supply next to the incubator.
8. Open door for two minutes to neutralize incubation chamber.
9. At the end of two minutes shut door click record data on excel and CoolTerm
10. Stop both recordings after 10 total minutes.
11. Start new recording.
12. Open door for 30 seconds; close door.
13. Wait for 10 additional minutes; stop recording at the end of the interval.
14. Save files to Test Data Folder.
15. Note any observations during test cycles.
16. Change N₂, O₂, or CO₂ settings for the next test in the control system code.
17. Repeat steps 4-16 until all interval settings are tested.

18. EACH TEST will produce 4 files 32 files total (16 for each test)
   a. Test 1.1: File name (2): StationaryO₂₁N₂₁ & StationaryCO₂₁
   b. Test 1.2: File name (2): DoorO₂₁N₂₁ & DoorCO₂₁
   c. Test 2.1: File name (2): StationaryO₂₁N₂₁ & StationaryCO₂₃
   d. Test 2.2: File name (2): DoorO₂₁N₂₁ & DoorCO₂₃
   e. Test 3.1: File name (2): StationaryO₂₃N₂₁ & StationaryCO₂₁
   f. Test 3.2: File name (2): DoorO₂₃N₂₁ & DoorCO₂₁
   g. Test 4.1: File name (2): StationaryO₂₁N₂₃ & StationaryCO₂₁
   h. Test 4.2: File name (2): DoorO₂₁N₂₃ & DoorCO₂₁
   i. Test 5.1: File name (2): StationaryO₂₁N₂₃ & StationaryCO₂₃
   j. Test 5.2: File name (2): DoorO₂₁N₂₃ & DoorCO₂₃
   k. Test 6.1: File name (2): StationaryO₂₃N₂₁ & StationaryCO₂₃
   l. Test 6.2: File name (2): DoorO₂₃N₂₁ & DoorCO₂₃
   m. Test 7.1: File name (2): StationaryO₂₃N₂₃ & StationaryCO₂₁
   n. Test 7.2: File name (2): DoorO₂₃N₂₃ & DoorCO₂₁
   o. Test 8.1: File name (2): StationaryO₂₃N₂₃ & StationaryCO₂₃
   p. Test 8.2: File name (2): DoorO₂₃N₂₃ & DoorCO₂₃

19. Upload all data into Excel & Minitab for data analysis.

Reference Appendix XX for the code used

L.3.5 CO₂ optimization

Materials
- O₂ Sensor
- CO₂ Sensor
- All gas regulators and pressure relief valve
- Computer with Arduino installed
- 2 USB cables to connect Arduino
- Full control system code
- Full incubator installed into gas system
- Excel Data Stream plug in
- CoolTerm data recording plug in

Engineering Specs
- Desired Range O₂: 4.5 - 5.5%
- Desired Range CO₂: 4.5-5.5%

Controlled Settings
- Gas regulators are set to the lowest possible PSI, while still maintaining an open flow.
- Low Range O₂: 4.5%
- High Range O₂: 5.4%
- Multiplier value for threshold: N₂: 1.2, CO₂: 0.3, O₂: 0.8
- Threshold interval time lengths:
  - N₂: 3ms
  - O₂: 1ms
  - CO₂: 6ms

Explanatory Variables
- Low Range CO₂: 3.7%, 3.8%, 3.9% & 4.0%

Protocol
1. Turn on computer and open CoolTerm, Excel Data Stream, and Arduino
2. Open control system code for O₂ and CO₂, which can be referenced in Appendix K and L.
3. Plug both the Arduino Uno and Arduino Mega into computer.
4. Upload the O₂ control code to the Arduino Mega & connect the Mega to the CoolTerm plug in using port 4.
5. Upload the CO₂ control code to the Arduino Uno & open the serial monitor to ensure proper data collection.
6. Close serial monitor and connect the Arduino Uno to the Excel Data Stream plug in using port 3.
7. Turn on the external power supply next to the incubator.
8. Open door for two minutes to neutralize incubation chamber.
9. At the end of two minute shut door click record data on excel and CoolTerm.
10. If CO₂ overshoots, move to step 12.
11. If CO₂ stabilizes within spec, note the setpoint and track data for at least 20 minutes to ensure CO₂ stability.
12. Save files to Test Data Folder.
13. Note any observations during test cycles.
14. Change settings for the next test in the control system code.
15. Repeat steps 4-16 until all setpoints are tested.

L3.6 CO₂ cell incubation and temperature validation

Materials
- 3T3 cells in T-75 flask
- 3 mL trypsin
- 5 mL PBS
- 12 mL cell media
- 50 µL trypan blue
- Warming bath at 37°C
- 15 mL conical
- Pipette aid
- Pipette tips
- 100 µL micropipette and tips
- Hemocytometer
- Light microscope

Protocol
All steps listed with an “*” must be performed in a biosafety cabinet (BSC) to avoid contamination. Gloves must be worn at all times during this procedure, decontaminating them with 70% IPA anytime contact with a non-sterile object is made.
1. Defrost trypsin in the warming bath.
2. Obtain cell media and transfer 12 mL to a 15 mL conical. *
3. Place conical of media in warming bath.
4. Obtain the flask of cells you wish to pass and aspirate the old media in the BSC. *
5. Transfer the PBS to the flask to remove excess media and aspirate. *
6. Once the trypsin is warm, add 3 mL to flask and wait for cells to detach. *
7. Add 12 mL of media to the flask to deactivate the trypsin, ensuring to thoroughly mix the trypsin and media. *
8. Transfer the mixture to a 15 mL conical. *
9. Transfer 50 µL of the cell mixture to a small vial using a micropipette. *
10. Add 50 µL of trypan blue to the mixture.
11. Transfer 10 µL of the dyed cells to a hemocytometer.
12. Count the cells under a light microscope using the following formula:

\[
\text{Cells counted} = \frac{\text{# cells counted}}{4} \times 10,000 \times 5
\]

Cells are counted in the four corner squares of the hemocytometer.
13. Calculate number of cells to add to each well plate.
14. Transfer appropriate amount of cell mixture to each well plate. *
15. Label both well plates, one for the standard incubator and one for the experimental incubator. *
16. Move the well plates to appropriate incubators and leave overnight.
17. Check cells the next day for growth under a light microscope.
18. Count cells if there are an appropriate number, if not, incubate for another 24 hours.
More data can be provided upon request.

### M.2  REFINED DATA
### Table XXIII. Refined data for CO\textsubscript{2} - stationary

<table>
<thead>
<tr>
<th>Stationary</th>
<th>Time to Setpoint (s)</th>
<th>Time in spec (s)</th>
<th># of times out of spec after reaching setpoint</th>
<th>Time out of Spec (s)</th>
<th>Max Overshoot (%)</th>
<th>Total time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 1ms, O\textsubscript{2} - 1ms</td>
<td>12</td>
<td>180</td>
<td>4</td>
<td>441</td>
<td>6.98</td>
<td>633</td>
</tr>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 1ms, O\textsubscript{2} - 3ms</td>
<td>0</td>
<td>162</td>
<td>3</td>
<td>372</td>
<td>11.07</td>
<td>534</td>
</tr>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 3ms, O\textsubscript{2} - 1ms</td>
<td>0</td>
<td>198</td>
<td>4</td>
<td>420</td>
<td>8.06</td>
<td>618</td>
</tr>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 3ms, O\textsubscript{2} - 3ms</td>
<td>27</td>
<td>210</td>
<td>3</td>
<td>264</td>
<td>8.44</td>
<td>501</td>
</tr>
<tr>
<td>CO\textsubscript{2} - 6ms, N\textsubscript{2} - 1ms, O\textsubscript{2} - 1ms</td>
<td>36</td>
<td>156</td>
<td>4</td>
<td>447</td>
<td>8.13</td>
<td>639</td>
</tr>
<tr>
<td>CO\textsubscript{2} - 6ms, N\textsubscript{2} - 1ms, O\textsubscript{2} - 3ms</td>
<td>108</td>
<td>99</td>
<td>3</td>
<td>483</td>
<td>9.56</td>
<td>690</td>
</tr>
<tr>
<td>CO\textsubscript{2} - 6ms, N\textsubscript{2} - 3ms, O\textsubscript{2} - 1ms</td>
<td>39</td>
<td>180</td>
<td>4</td>
<td>435</td>
<td>6.99</td>
<td>654</td>
</tr>
<tr>
<td>CO\textsubscript{2} - 6ms, N\textsubscript{2} - 3ms, O\textsubscript{2} - 3ms</td>
<td>24</td>
<td>267</td>
<td>2</td>
<td>294</td>
<td>7.63</td>
<td>585</td>
</tr>
<tr>
<td><strong>Normalized by total time</strong></td>
<td><strong>From setpoint of 5%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 1ms, O\textsubscript{2} - 1ms</td>
<td>0.018957346</td>
<td>0.28436019</td>
<td>0.696682464</td>
<td>1.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 1ms, O\textsubscript{2} - 3ms</td>
<td>0</td>
<td>0.30337078</td>
<td>6.07</td>
<td>0.696629213</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 3ms, O\textsubscript{2} - 1ms</td>
<td>0</td>
<td>0.32038835</td>
<td>0.67961165</td>
<td>3.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO\textsubscript{2} - 10ms, N\textsubscript{2} - 3ms, O\textsubscript{2} - 3ms</td>
<td>0.053892216</td>
<td>0.41916167</td>
<td>0.526946108</td>
<td>3.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table XXIV. Refined data for CO₂ - door open

<table>
<thead>
<tr>
<th>Door Open</th>
<th>Time to Setpoint (s)</th>
<th>Time in spec (s)</th>
<th># of times out of spec after reaching setpoint</th>
<th>Time out of Spec (s)</th>
<th>Max Overshoot (%)</th>
<th>Total time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ - 10ms, N₂ - 1ms, O₂ - 1ms</td>
<td>87</td>
<td>75</td>
<td>2</td>
<td>492</td>
<td>8.73</td>
<td>654</td>
</tr>
<tr>
<td>CO₂ - 10ms, N₂ - 1ms, O₂ - 3ms</td>
<td>111</td>
<td>57</td>
<td>2</td>
<td>504</td>
<td>7.47</td>
<td>672</td>
</tr>
<tr>
<td>CO₂ - 10ms, N₂ - 3ms, O₂ - 1ms</td>
<td>66</td>
<td>309</td>
<td>3</td>
<td>242</td>
<td>8.04</td>
<td>617</td>
</tr>
<tr>
<td>CO₂ - 10ms, N₂ - 3ms, O₂ - 3ms</td>
<td>90</td>
<td>90</td>
<td>2</td>
<td>459</td>
<td>6.26</td>
<td>639</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 1ms, O₂ - 1ms</td>
<td>81</td>
<td>78</td>
<td>2</td>
<td>471</td>
<td>9.9</td>
<td>630</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 1ms, O₂ - 3ms</td>
<td>99</td>
<td>90</td>
<td>2</td>
<td>486</td>
<td>11.49</td>
<td>675</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 3ms, O₂ - 1ms</td>
<td>54</td>
<td>420</td>
<td>3</td>
<td>171</td>
<td>6.24</td>
<td>645</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 3ms, O₂ - 3ms</td>
<td>78</td>
<td>57</td>
<td>2</td>
<td>480</td>
<td>6.29</td>
<td>615</td>
</tr>
</tbody>
</table>

Normalized by total time from setpoint of 5%

<table>
<thead>
<tr>
<th>Door Open</th>
<th>Time to Setpoint (s)</th>
<th>Time in spec (s)</th>
<th># of times out of spec after reaching setpoint</th>
<th>Time out of Spec (s)</th>
<th>Max Overshoot (%)</th>
<th>Total time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ - 10ms, N₂ - 1ms, O₂ - 1ms</td>
<td>0.133027523</td>
<td>0.11467889</td>
<td>9</td>
<td>0.752293578</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>CO₂ - 10ms, N₂ - 1ms, O₂ - 3ms</td>
<td>0.165178571</td>
<td>0.08482142</td>
<td>9</td>
<td>0.75</td>
<td>2.47</td>
<td></td>
</tr>
<tr>
<td>CO₂ - 10ms, N₂ - 3ms, O₂ - 3ms</td>
<td>0.106969206</td>
<td>0.50081037</td>
<td>0.392220421</td>
<td>3.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stationary</td>
<td>Time to Setpoint (s)</td>
<td>Time in spec (s)</td>
<td># of times out of Spec after reaching setpoint</td>
<td>Time out of Spec (s)</td>
<td>Max Overshoot (%)</td>
<td>Total time (s)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------------</td>
<td>------------------</td>
<td>-----------------------------------------------</td>
<td>---------------------</td>
<td>-------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>CO2 - 10ms, N2 - 3ms, O2 - 1ms</td>
<td>261</td>
<td>333</td>
<td>1</td>
<td>54</td>
<td>N/A</td>
<td>648</td>
</tr>
<tr>
<td>CO2 - 6ms, N2 - 1ms, O2 - 1ms</td>
<td>213</td>
<td>342</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>555</td>
</tr>
<tr>
<td>CO2 - 6ms, N2 - 3ms, O2 - 1ms</td>
<td>246</td>
<td>419</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>665</td>
</tr>
<tr>
<td>CO2 - 10ms, N2 - 3ms, O2 - 3ms</td>
<td>145</td>
<td>342</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>487</td>
</tr>
<tr>
<td>CO2 - 6ms, N2 - 1ms, O2 - 1ms</td>
<td>305</td>
<td>360</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>665</td>
</tr>
<tr>
<td>CO2 - 6ms, N2 - 1ms, O2 - 3ms</td>
<td>308</td>
<td>260</td>
<td>4</td>
<td>66</td>
<td>N/A</td>
<td>634</td>
</tr>
<tr>
<td>CO2 - 6ms, N2 - 3ms, O2 - 1ms</td>
<td>211</td>
<td>423</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>634</td>
</tr>
<tr>
<td>CO2 - 6ms, N2 - 3ms, O2 - 3ms</td>
<td>235</td>
<td>373</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>608</td>
</tr>
</tbody>
</table>

**Normalized by Total Time**

<table>
<thead>
<tr>
<th>Stationary</th>
<th>Time to Setpoint (s)</th>
<th>Time in spec (s)</th>
<th># of times out of Spec after reaching setpoint</th>
<th>Time out of Spec (s)</th>
<th>Max Overshoot (%)</th>
<th>Total time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO2 - 10ms, N2 - 1ms, O2 - 1ms</td>
<td>0.4027777778</td>
<td>0.51388888 89</td>
<td>0.0833333333</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO2 - 10ms, N2 - 1ms, O2 - 3ms</td>
<td>0.383783784</td>
<td>0.6162162 16</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table XXVI. Refined data for O₂ - door open

<table>
<thead>
<tr>
<th>Door Open</th>
<th>Time to Setpoint (s)</th>
<th>Time in spec (s)</th>
<th># of times out of spec after reaching setpoint</th>
<th>Time out of Spec (s)</th>
<th>Max Overshoot (%)</th>
<th>Total time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ - 10ms, N₂ - 3ms, O₂ - 1ms</td>
<td>146</td>
<td>494</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>640</td>
</tr>
<tr>
<td>CO₂ - 10ms, N₂ - 3ms, O₂ - 3ms</td>
<td>173</td>
<td>461</td>
<td>1</td>
<td>19</td>
<td>N/A</td>
<td>653</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 1ms, O₂ - 1ms</td>
<td>140</td>
<td>453</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>593</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 1ms, O₂ - 3ms</td>
<td>159</td>
<td>474</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>633</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 3ms, O₂ - 1ms</td>
<td>152</td>
<td>468</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>620</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 3ms, O₂ - 3ms</td>
<td>222</td>
<td>428</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>650</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 1ms, O₂ - 3ms</td>
<td>146</td>
<td>451</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>597</td>
</tr>
<tr>
<td>CO₂ - 6ms, N₂ - 3ms, O₂ - 3ms</td>
<td>178</td>
<td>425</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>603</td>
</tr>
</tbody>
</table>

**Normalized by Total Time**

<table>
<thead>
<tr>
<th>Door Open</th>
<th>Time to Setpoint (s)</th>
<th>Time in spec (s)</th>
<th># of times out of spec after reaching setpoint</th>
<th>Time out of Spec (s)</th>
<th>Max Overshoot (%)</th>
<th>Total time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ - 10ms, N₂ - 1ms, O₂ - 1ms</td>
<td>0.228125</td>
<td>0.771875</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>640</td>
</tr>
<tr>
<td>CO₂ - 10ms, N₂ - 1ms, O₂ - 3ms</td>
<td>0.264931087</td>
<td>0.705972435</td>
<td>0</td>
<td>0</td>
<td>0.029096478</td>
<td>650</td>
</tr>
</tbody>
</table>
### Table XXVII. Factorial setup – CO₂ and O₂ data.

<table>
<thead>
<tr>
<th>StdOrder</th>
<th>RunOrder</th>
<th>CenterPt</th>
<th>Blocks</th>
<th>CO₂</th>
<th>N₂</th>
<th>O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>6</td>
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<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
</tr>
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<td>7</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table XXVIII. CO₂ data - stationary.

<table>
<thead>
<tr>
<th>Time to Setpoint_S</th>
<th>Time in Spec_S</th>
<th>Time out of Spec_S</th>
<th>Max Overshoot_S</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.018957346</td>
<td>0.28436019</td>
<td>0.696682464</td>
<td>1.98</td>
</tr>
<tr>
<td>0.053892216</td>
<td>0.419161677</td>
<td>0.526946108</td>
<td>3.44</td>
</tr>
<tr>
<td>0.056338028</td>
<td>0.244131455</td>
<td>0.699530516</td>
<td>3.13</td>
</tr>
<tr>
<td>0.156521739</td>
<td>0.143478261</td>
<td>0.7</td>
<td>4.56</td>
</tr>
<tr>
<td>0.059633028</td>
<td>0.275229358</td>
<td>0.665137615</td>
<td>1.99</td>
</tr>
<tr>
<td>0.041025641</td>
<td>0.456410256</td>
<td>0.502564103</td>
<td>2.63</td>
</tr>
</tbody>
</table>

### Table XXIX. CO₂ data – door open.

<table>
<thead>
<tr>
<th>Time to Setpoint_D</th>
<th>Time in Spec_D</th>
<th>Time out of Spec_D</th>
<th>Max Overshoot_D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.133027523</td>
<td>0.114678899</td>
<td>0.752293578</td>
<td>3.73</td>
</tr>
</tbody>
</table>
Table XXX. O₂ data – stationary.

<table>
<thead>
<tr>
<th>Time to Setpoint_S_O2</th>
<th>Time in Spec_S_O2</th>
<th>Time out of Spec_S_O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.402777778</td>
<td>0.513888889</td>
<td>0.083333333</td>
</tr>
<tr>
<td>0.383783784</td>
<td>0.616216216</td>
<td>0</td>
</tr>
<tr>
<td>0.369924812</td>
<td>0.630075188</td>
<td>0</td>
</tr>
<tr>
<td>0.297741273</td>
<td>0.702258727</td>
<td>0</td>
</tr>
<tr>
<td>0.458646617</td>
<td>0.541353383</td>
<td>0</td>
</tr>
<tr>
<td>0.485804416</td>
<td>0.410094637</td>
<td>0.104109466</td>
</tr>
<tr>
<td>0.332807571</td>
<td>0.667192429</td>
<td>0</td>
</tr>
<tr>
<td>0.386513158</td>
<td>0.613486842</td>
<td>0</td>
</tr>
</tbody>
</table>

Table XXXI. O₂ data – door open.

<table>
<thead>
<tr>
<th>Time to Setpoint_D_O2</th>
<th>Time in Spec_D_O2</th>
<th>Time out of Spec_D_O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.228125</td>
<td>0.771875</td>
<td>0</td>
</tr>
<tr>
<td>0.264931087</td>
<td>0.705972435</td>
<td>0.029096478</td>
</tr>
<tr>
<td>0.23608769</td>
<td>0.76391231</td>
<td>0</td>
</tr>
<tr>
<td>0.251184834</td>
<td>0.748815166</td>
<td>0</td>
</tr>
<tr>
<td>0.24516129</td>
<td>0.75483871</td>
<td>0</td>
</tr>
<tr>
<td>0.341538462</td>
<td>0.658461538</td>
<td>0</td>
</tr>
<tr>
<td>0.244556114</td>
<td>0.755443886</td>
<td>0</td>
</tr>
<tr>
<td>0.295190713</td>
<td>0.704809287</td>
<td>0</td>
</tr>
</tbody>
</table>

N. Code/scripts CO₂ and Temperature Control

/*
   ///////////////////////////////////////////////////////////////////////
   Feedback Loop Testing
   ///////////////////////////////////////////////////////////////////////
Arduino UNO Pin Connections:
Pin 2,3: Tx (yellow)/Rx (orange) from CO₂ Sensor
Pin #6: Temperature Sensors
Pin #7: Heating Pad Relay
Pin #8: CO₂ Solenoid Relay
*/

// float CO₂SetpointHighRange = 5; // High Range for Setpoint CO₂ level in %
float CO₂SetpointLowRange = 4; // Low Range for Setpoint CO₂ Level in %
float CO2Threshold = .3; // Threshold to switch to stepping control in %/100
int SolenoidOffTime = 6; // Duration time OFF in milliseconds

float TSetpoint = 37; // Setpoint Temperature in Celsius
float TempThreshold = 0.99; // Threshold to switch to stepping control in %/100
int HeatOnTime = 1; // Duration time in milliseconds

// CO2 Sensors
#include "SoftwareSerial.h"
#include "cozir.h"

// DS18B20 Temp Sensors
#include "OneWire.h"
#include "DallasTemperature.h"

// Adafruit 7-segment Display for updating CO2 and Temperature
#include <Wire.h>
#include <Adafruit_LEDBackpack.h>
#include <Adafruit_GFX.h>

int Heater = 7; // Relay for controlling power to heaters
int Solenoidco2 = 8; // Relay for controlling 12V solenoid valve

////////* SPRINTIR COZIR CO2 SENSOR *////////
SoftwareSerial nss(2,3); // Rx,Tx - Pin 5,7 on sensor
COZIR czr(nss);
float SingleCO2, CO2 = 0;
float multiplier = 0.001; // 10/10000 (Hardware multiplier/ppm conversion).
float reading = 0;

#define ONE_WIRE_BUS 6 // Sensors on digital pin 6 of the Arduino
OneWire oneWire(ONE_WIRE_BUS); // Setup a oneWire instance to communicate with ANY OneWire devices
DallasTemperature sensors(&oneWire); // Pass our oneWire reference to Dallas Temperature.
float T1, T2, AvgT, SingleT = 0;

///////// MATRIX LED DISPLAY SETUP //////////
Adafruit_7segment matrix = Adafruit_7segment();
long previousMillis = 0; // will store last time LED was updated
int update = 0;

void setup()
{
  Serial.begin(9600); // Start serial port
czr.SetOperatingMode(CZR_POLLING); // Start the CO2 sensor and put into POLLING mode
matrix.begin(0x70); // Start Matrix
matrix.print(8888); // Print '8888' on matrix
matrix.writeDisplay();
pinMode(Solenoidco2, OUTPUT); // Sets pin for controlling solenoid relay
digitalWrite(Solenoidco2, LOW); // Set LOW (solenoid closed off)
sensors.begin(); // Start up the temperature sensor library
pinMode(Heater, OUTPUT); // Sets pin for controlling heater relay
digitalWrite(Heater, LOW); // Set LOW (heater off)
delay(2000); // Wait
}
void loop()
{

    // Read CO2 sensor 3 times and determine the average.
    for (int i = 0; i<6; i++) {
        SingleCO2 += czr.CO2()*multiplier;
    }
    CO2 = SingleCO2/6;
    SingleCO2 = 0;

    // Open/close the solenoid valve based on the current CO2 reading
    // Solenoid closes for a duration of 'SolenoidOffTime' and then opens
    if (CO2 > CO2SetpointLowRange && CO2 > CO2Threshold*CO2SetpointLowRange) {
        digitalWrite(Solenoidco2, HIGH);
    } else if (CO2 < CO2SetpointLowRange && CO2 > CO2Threshold*CO2SetpointLowRange) {
        digitalWrite(Solenoidco2, HIGH);
        delay(SolenoidOffTime);
        digitalWrite(Solenoidco2, LOW);
    } else if (CO2 < CO2SetpointLowRange) {
        digitalWrite(Solenoidco2, LOW);
    }

    // Read the average temperature over both sensors 3 times, then find the average.
    for (int i = 0; i<3; i++) {
        sensors.requestTemperatures();
        T1=sensors.getTempCByIndex(0);
        T2=sensors.getTempCByIndex(1);
        SingleT += (T1+T2)/2
    }
    AvgT = SingleT/3;
    SingleT = 0;

    // Turn on/off heater based on temperature reading
    // Heater turns on for a duration of 'TempOnTime' and then turns off
    if (AvgT > TSetpoint && AvgT > TempThreshold*TSetpoint) {
        digitalWrite(Heater, HIGH);
    } else if (AvgT < TSetpoint && AvgT > TempThreshold*TSetpoint) {
        digitalWrite(Heater, HIGH);
        delay(HeatOnTime);
        digitalWrite(Heater, LOW);
    } else if (AvgT < TSetpoint) {
        digitalWrite(Heater, LOW);
    }

    updateMatrix(); // Update the Matrix display,
    SerialPrintResults();
}

void updateMatrix()
{
    unsigned long currentMillis = millis();

    // Alternate the display between Temperature and CO2 every 2 seconds
if (currentMillis - previousMillis > 2000) {
    previousMillis = currentMillis;
    if (update == 0) {
        matrix.print(AvgT, 1);
        matrix.writeDisplay();
        update = 1;
    } else if (update == 1) {
        matrix.print(CO2, 1);
        matrix.writeDisplay();
        update = 0;
    }
}

// UNCOMMENT to print results over Serial port for debugging

// For debugging only

// For debugging only
void SerialPrintResults() {
    Serial.print("CO2, ");
    Serial.print(CO2);
    Serial.print(", ");
    Serial.print("Ave T, ");
    Serial.print(AvgT);
    Serial.print(", ");
    Serial.print("T0, ");
    Serial.print(T1);
    Serial.print(", ");
    Serial.print("T1, ");
    Serial.print(T2);
    Serial.println(", ");
}

O. Code/scripts O₂ and N₂ Control
/*
Arduino UNO Pin Connections:
Pin 11,12: Tx (yellow)/Rx (blue) from O₂ Sensor
Pin #8: O2 Solenoid Relay
Pin #9: N2 Solenoid Relay
*/

// O₂ Sensor
#include <SoftwareSerial.h>

// Adafruit 7-segment Display for updating CO₂ and Temperature
#include <Wire.h>
#include <Adafruit_LEDBackpack.h>
#include <Adafruit_GFX.h>

float O2SetpointHighRange= 540;  // Setpoint O₂ level in %, no decimal place (this is 7.5%)
float O2SetpointLowRange = 450;  // Setpoint O₂ level in %, no decimal place (this is 6.5%)
float O2Threshold= 0.8;  // Threshold to switch to stepping control in %/100
float N2Threshold= 1.3;  // Threshold to switch to stepping control for N₂ in %/100
int SolenoidOnTimeO2 = 1;  // Duration time in milliseconds
int SolenoidOnTimeN2 = 3; // Duration time in milliseconds
int Solenoido2 = 8; // Relay for controlling O2
int Solenoidn2 = 9; // Relay for controlling N2

///////// MATRIX LED DISPLAY SETUP /////////
Adafruit_7segment matrix = Adafruit_7segment();
long previousMillis = 0; // will store last time LED was updated
int update = 0;

SoftwareSerial mySerial(11,12);
String inString=""; // hold the string
String subString=""; // O2 substring
int O2Data; //variable to store string

void setup() {
  Serial.begin(9600);
  mySerial.begin(9600);
  matrix.begin(0x70); // Start Matrix
  matrix.print(8888); // Print '8888' on matrix
  matrix.writeDisplay();
  pinMode(Solenoido2, OUTPUT); // Sets pin for controlling solenoid relay
  digitalWrite(Solenoido2, LOW); // Set LOW (solenoid closed off)
  pinMode(Solenoidn2, OUTPUT); // Sets pin for controlling solenoid relay
  digitalWrite(Solenoidn2, LOW); // Set LOW (solenoid closed off)
  delay(2000); // Wait
}

void loop () {

  // O2 conversion
  if(mySerial.available()){
    //Serial.write(mySerial.read());
    int inChar=mySerial.read();
    if(isDigit(inChar))inString+=(char)inChar;
    subString = inString.substring(13,17);
    //isDigit Analyse if a char is a digit (that is a number)
    // Returns true is thisChar is a number
    if(inChar=='\n'){
      O2Data=subString.toInt();
      matrix.print(O2Data, 1);
      matrix.writeDisplay();
      Serial.print("O2,");
      Serial.println(O2Data);
      //Serial.print("String: ");
      //Serial.println(subString);
      inString=""; //clears string for new input
      subString="";
    }

  //O2 feedback loop - low O2
  if (O2Data > O2SetpointLowRange && O2Data > O2Threshold*O2SetpointLowRange) {
    digitalWrite(Solenoido2, HIGH);
  } else if (O2Data < O2SetpointLowRange && O2Data > O2Threshold*O2SetpointLowRange) {
    digitalWrite(Solenoido2, HIGH);
}
delay(SolenoidOnTimeO2);
digitalWrite(Solenoido2, LOW);
} else if (O2Data < O2SetpointLowRange) {
digitalWrite(Solenoido2, LOW);
}

// N2 feedback loop - high O2

if (O2Data < O2SetpointHighRange && O2Data < N2Threshold*O2SetpointHighRange) {
digitalWrite(Solenoidn2, HIGH);
} else if (O2Data > O2SetpointHighRange && O2Data < N2Threshold*O2SetpointHighRange) {
digitalWrite(Solenoidn2, HIGH);
delay(SolenoidOnTimeN2);
digitalWrite(Solenoidn2, LOW);
} else if (O2Data > O2SetpointHighRange) {
digitalWrite(Solenoidn2, LOW);
}

P. Additional Code

P.1 SIMPLE OXYGEN SENSOR CODE

#include <SoftwareSerial.h>
SoftwareSerial mySerial(10, 11); // RX (O2 Pin 3), TX (O2 Pin 4)
void setup() {
  Serial.begin(9600);
  mySerial.begin(9600);
}
void loop() {
  if (mySerial.available()) {
    Serial.write(mySerial.read());
  }
}

P.2 SIMPLE CARBON DIOXIDE SENSOR CODE

/*
    ///////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////
    Simple program to read from a COZIR SprintIR CO2 sensor and report over serial.
    Sensor powered by Arduino 5V. Tx/Rx on Arduino Pins 2/3.
    CO2 Meter (SprintIR 0-20% GC-0017):
    http://www.co2meter.com/collections/co2-sensors/products/sprintir-100-percent-co2-sensor
    Also need the latest COZIR library, version 1.0 used for this code.
    Online forum has links to library and lots of information:
    http://forum.arduino.cc/index.php?topic=91467.0
    ///////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////
*/

#include "cozir.h"
#include "SoftwareSerial.h"
SoftwareSerial nss(2, 3); // Tx, Rx from the sensor to Pins 2, 3 on Arduino
COZIR czr(nss);
float c, reading = 0;
float multiplier = 0.001; // 0.001 = 10/10000 (Hardware multiplier/ppm conversion)
    // For more details see sensor specificaiton sheet

void setup()
{
    Serial.begin(9600);
    czr.SetOperatingMode(CZR_POLLING);
    delay(100);
}

void loop()
{
    c = czr.CO2(); // read the sensor, values output as ppm
    reading = c*multiplier; // convert ppm reading to percentage
    Serial.print("CO2 Content: ");
    Serial.print(reading);
    Serial.println("");
    Serial.println();
    delay(50);
}

P.3 SIMPLE TEMPERATURE SENSOR CODE

#include <OneWire.h>
#include <DallasTemperature.h>
define ONE_WIRE_BUS 12 // Sensor on digital Pin 12 of the Arduino
OneWire oneWire(ONE_WIRE_BUS); // Setup a oneWire instance to communicate with ANY OneWire devices
DallasTemperature sensors(&oneWire); // Pass our oneWire reference to Dallas Temperature.
float T1, T2, Tavg;

void setup()
{
    Serial.begin(9600); // start serial port
    sensors.begin(); // Start up the OneWire library
}

void loop()
{
    sensors.requestTemperatures(); // Send the command to get temperatures
    T1=sensors.getTempCByIndex(0); // Sensor 0
    T2=sensors.getTempCByIndex(1); // Sensor 1
    Tavg = (T1+T2)/2;
    Serial.print("Temperature for Sensor 0 is: ");
    Serial.println(T1);
    Serial.print("Temperature for Sensor 1 is: ");
    Serial.println(T2);
    Serial.print("Average Temperature: ");
    Serial.println(Tavg);
    Serial.println();
    delay(500);
}

Q. Operation Manual
1. Check code to ensure desired setpoints are active.
a. If not, change setpoint and reupload the code.
b. CO\textsubscript{2} and temperature code should be uploaded to the Arduino UNO.
c. O\textsubscript{2} code should be uploaded to the Arduino MEGA.
d. The displays should start displaying data as soon as the Arduinos are plugged in. If they don’t open the serial monitor and initialize the data by typing into the bar up top and hitting enter. The data should display a few moments later.

2. If you are recording data:
a. Excel:
   i. Open excel and navigate to data streamer tab.
   ii. Click “connect a device” and connect either the UNO or MEGA.
   iii. To see live data, click “start data.”
   iv. To record data, click “record data.”
   v. To end recording, click “stop recording.”
   vi. Name your file and save it to the desired location.
   vii. Disconnect the device when done viewing data.
b. CoolTerm:
   i. Open CoolTerm.
   ii. Click “connect” to connect the UNO or MEGA.
   iii. Live data should show up right away.
   iv. To record data, navigate to the “connection” drop-down menu.
   v. Click “capture to text/binary file” then “record.”
   vi. Name your file and the data will start recording.
   vii. To end the recording, navigate back to “connection” then to “capture to text/binary file” then click stop.
   viii. The data will be saved wherever you allocated it when the recording started.
   ix. Disconnect the device when finished viewing data.

3. Ensure both doors are closed before starting gassing procedure.

4. Flip the power switch on the power source to provide 12V to the solenoids and heating pads. Gas will start flowing and the heating pads will turn on.

5. Wait for gases to reach their setpoints.

6. If CO\textsubscript{2} overshoots the maximum range:
a. Open the inner door and wait for CO\textsubscript{2} concentration to reach 3%.
b. Close the doors and wait for the gases to reach their setpoint.
c. If CO\textsubscript{2} stabilizes, move on. If not, repeat.

7. Once gases are stable, add cells. If the incubator is sitting at room temperature, the heating pads will take about 3 hours to heat the incubator to 37 °C.

8. Incubate for desired period of time.

R. Maintenance Manual

1. Cleaning & sterilizing inner chamber
   a. Before cell incubation entire inner chamber must be cleaned and sterilized
   b. Remove shelf from chamber.
      i. Wash shelf with soap and water.
      ii. Spray or soak shelf with 70% IPA to sterilize.
         1. Shelf can also be autoclaved to sterilize.
   c. Remove magnets (QTY 8) from both heating pads.
      i. Wash magnets with soap and water.
      ii. Spray or soak magnets with 70% IPA to sterilize.
   d. Move electronics to center of chamber
   e. Spray/wipe walls, roof, floor of incubator with 70% IPA.
   f. Spray/wipe heating pads with 70% IPA.
   g. Wipe down door with soap and water and 70% IPA.
   h. Clean any outside components with IPA or soapy water that appear dusty, dirty, or that will be entering the chamber

2. Before cell incubation, check humidity tray.
a. Fill humidity tray before long term use.

3. Component replacement
   a. CO₂, O₂, and temperature sensors can be removed & unplugged from sensor box if
      replacement is necessary
      i. If sensor values are incorrect in room air; try calibration
      ii. If after calibration sensors are still incorrect or nonfunctional; replace
   b. If system is nonfunctional check linked data sheets to access if electronics need
      replacement/are at the end of the product life.
   c. Replace gasket material if dirty or ripped; pull off old gasket, cut and measure new
      material from stock supply.

4. Gas replacement
   a. When gas cylinders are empty, remove regulators from gas cylinder.
      i. No gas flow when system is turned on
   b. Remove old tank and bring in new tank
   c. Install regulator on new gas tank
   d. Turn on system to where both O₂ and CO₂ are out of spec
      i. While N₂ and CO₂ solenoids are open adjust regulator to the lowest possible
         flow
      ii. If O₂ cylinder needs to be replaced, set O₂ set point above 20% to open solenoid.
      iii. While solenoid is open adjust regulator to the lowest possible flow.