FLORIDA INTERNATIONAL UNIVERSITY

SENIOR DESIGN PROJECT REPORT

Manufacturing Process of a SCOBY Bio-Thread

Submitted in partial fulfillment of the
requirements for the degree of

BACHELOR OF SCIENCE
in
BIOMEDICAL ENGINEERING

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I have read this report, evaluated its contents (see attached evaluation form), and recommend that it be approved.

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This Senior Design Project Report and its Faculty Evaluation is approved.

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1. Recognition of Need/Opportunity

What clinical problem is being addressed? Is anything currently being used to address this problem? What opportunities exist to improve clinical outcomes, cost management, or ease of use? What are the particular needs of the customer? How have these needs been identified and defined?

Clinical Problem

Among the population about 6.5 million people experience Chronic Wounds.[1] A chronic wound is one that does not progress through the healing process properly. These wounds have usually been open for more than a month, have lengthy healing times and lead to serious infections. Currently, bacterial cellulose seems to be a potential material that can aid the healing process. After the completion of this project, there will be a biocompatible, biodegradable solution primarily composed of Cellulose that will aid in the wound healing process of Chronic Wounds. This solution will contain the ideal wound dressing conditions of applicability, flexibility, strength, biocompatibility, and stability.[2] This thread will also be one of the first successful manufactured processes of bacterial cellulose.

Clinical Research

The wound healing process can be divided into three main phases: inflammatory, proliferative and remodeling. The inflammatory stage begins after hemostasis has occurred within the wound. In a healthy wound this stage will last around 3 days. The main goal is to clear any pathogens and foreign material in the wound. Macrophages play a large role within this stage and will digest damaged tissue. Neutrophils will also secrete growth factors while Cytokines promote tissue proliferation. As the wound moves into the proliferation stage the production of collagen and ground tissue occurs. Due to this there is a rapid growth of endothelial cells. Angiogenesis will occur in order to create a strong vascular network to supply the wound. The last stage is remodeling and here is where the wound begins to appear in a more normal and stable state. Type III Collagen will be restored to type I.

During the inflammation stage in the healing process is where the wound will fester and not continue to decrease in severity. Around the four-week mark is when the wound transitions into the classification of a chronic wound. Underlying diseases such as Peripheral Vascular Disease, Immunosuppression and Diabetes will increase the chances of developing a chronic wound. As previously stated, about 6.5 million people experience chronic wounds.[1] The increasing prevalence of obesity and diabetes within the population are increasing the needs for wound care. Diabetes patients have a 15-25% chance of developing a chronic wound. If these wounds get too severe the patient will have to go through an amputation. Especially if the wound is on the foot.[3]

Between 19 and 34 percent of diabetes patients will develop an ulcer, which is considered a Chronic Wound. Diabetes patients will develop these wounds due the high blood sugar levels that are examined within these patients. The high blood sugar weakens the blood vessel walls which will lead to poor circulation and blood supply inhibiting the healing of the wound [3]. Currently these wounds are being treated with Hydrogel, Hydrocolloid and Alginate wound dressings.

Current Techniques

Hydrogels are currently the superior wound dressing to treat chronic wounds [4]. They are a hydrophilic material that can come in a sheet or a free-flowing amorphous form. Hydrogel dressings are capable of healing chronic wounds by providing a moist wound bed and enhancing autolytic debridement. However, a concern with Hydrogel dressings is that they absorb fluids slowly and will not be compatible for bleeding wounds. This will also require the use of a second dressing. An additional concern with Hydrogels is the possibility of Maceration. Since Hydrogels promote a moist healing environment, maceration can occur which happens when the skin is exposed to moisture for too long and becomes soft, wet, and soggy. Both concerns will cause further complications within the wound and will overall decrease the healing process. Future improvements include adding a drug or therapeutic agent which could possibly lead to increased wound healing [5].

Hydrocolloids are a two-layer hydrophilic wound dressing. The inner layer absorbs exudate and creates a moist environment for the wound. Exudate is a bodily fluid composed of cells, proteins, and solid materials that commonly leaks from areas of inflation. The removal of exudate allows for more clotting and absorption of necrotic tissue [6]. The second layer focuses on sealing the wound to protect against outside debris as well as bacterial contamination [4]. Since hydrocolloids are composed of two layers, they often do not need the implementation of a second dressing. As the hydrocolloid dressing absorbs the exudate the dressing will swell and form a mound over the wound. These dressings allow for autolytic debridement, the removal of necrotic tissues and devitalized tissues. These dressings also stimulate the body's endogenous enzymes through the creation of a moist environment. All aiding in improving the healing time for these wounds.

Alginates are a third option for treatment of chronic wounds. These dressings are used specifically in high exudative or bleeding wounds [4] due to their high absorbency and hemostatic properties. Depending on the liquid, alginates can absorb up to 20 times their rate. Alginates are nonwoven fibers derived from brown seaweed. They naturally contain alginic acid and are covered in calcium and sodium salts. The sodium and calcium ions that are present within these dressings interact with serum to create a hydrophilic gel creating moisture within the wound bed. They are not suitable for dry wounds. Alginates can come in a sheet form for superficial wounds as well as a rope to allow for packing of deep wounds.

Clinical Need

Cellulose in particular Bacterial Cellulose is considered a prominent material that can help revolutionize the wound care industry. It has previously demonstrated chemical and mechanical properties that can be useful within a wound dressing and overall aid the healing process. Those properties include high tensile strength, flexibility, water absorption, gas and liquid permeability and biocompatibility [2]. These properties have allowed for cellulose to cause epithelial regeneration when applied to a wound. The nanostructure of cellulose is a 3D network structure that is already similar to the extracellular matrix allowing for high levels of biocompatibility. Bacterial Cellulose enhances exudate absorption. Removing the necrotic tissue will stop the wound from spreading and eventually head more towards healing. These properties give bacterial cellulose a high potential to be implemented into the wound care industry.

Market Research

When examined from a financial standpoint the wound dressing market is an ever growing industry. By 2024 the wound dressing market is expected to reach 15-22 billion USD[7] . It is also predicted that the market will exceed 22 billion USD after 2024. North America and Europe have the world's largest demand for wound dressings. Some of the top wound care companies include 3M (USA), Smith + Nephew (UK) and Mölnlycke Health Care AB (SE)[3] .

Not only are the wound dressings themselves in a competitive market but the care and treatment of these wounds has also cost billions of dollars. Medicare projections show that the treatment of wounds for their patients costs between 28.1 billion USD and 96.8 billion[7]. Within this data outpatient costs consisted of a majority of the costs.

In the case of Bacterial Cellulose our proposed SCOBY Bio-Thread will fall under the category of advanced wound dressings alongside the previously mentioned hydrocolloids, hydrogels and aligantes. Within the previously mentioned statistics it is important to note that advance wound care takes up a majority of the market. Our product would be considered an advanced dressing. The overall growth within the market is expected to occur based off of the increasing rate of diabetes, geriatric population and other chronic diseases. Both of these diseases lead to the development of Chronic Wounds.

It is also important to note that the completion of this project will be one step closer to properly commercializing Bacterial Cellulose. Since the 1980s companies have tried to commercialize Bacterial Cellulose for uses within and outside of the medical field. But due to a lack of designing an efficient fermentation system has been restricting the overall commercialization.

Conclusion

The treatment of Chronic wounds can be enhanced with the implementation of a wound dressing composed of a SCOBY Bio-Thread. This thread has potential in the clinical aspect by being able to to promote wound healing as or more effective than the current techniques. As well as from a marketing standpoint. The wound care industry is a prosperous industry that is continuing to grow and can benefit from a device of this kind.

2. Problem Formulation:

Includes the conversion of customer requirements to functional, performance, and interface design specifications, and all engineering standards that must be met and other design constraints (should be multiple).

a. Project Objectives:

Is the objective a device, system, component, or process? What can be specifically accomplished in the allotted time and with the allotted resources?

Our objective was to develop a cellulose-based SCOBY (Symbiotic Culture of Yeast and Bacteria) Bioactive-Thread towards wound dressing applications. In order to produce the thread a process and a mold will need to be designed. Within an allotted time of four months the group designed a four-step process along with the construction of a silicone mold basin for the growth of the bio-thread. The process and mold together allowed for the completion of the market requirements and design inputs. The process design is necessary since the SCOBY is produced through the fermentation of Kombucha tea. The following variables; material dimensions, fermentation time, drying time and temperature were all optimized to allow for the proper production of the SCOBY. The designed process can be applied to mass production and the physical molds can be altered to fit the desired dimensions of thread. This was accomplished within a budget of $500 USD by April of 2022.

b. Design Specifications

How were the customer needs converted into specific design specifications? What are the criteria for success in meeting those specifications? How will they be measured?

Table 2.1 Market Requirements, Design Inputs and Verification Tests

Market Requirement	Design Input	Verification Test
1. The Bio-thread will contain bioactive compounds.	The final product will have cellulose.	Schulze's Reagent. A purple color change occurs in the presence of cellulose
	Rationale: Cellulose is a biocompatible material that has the capability of promoting wound healing.	
2. An uninterrupted Bio-thread with dimensions that will facilitate large scale manufacturing	The Bio-thread must present repeatability with a length of 152.4 +/- 15.24 cm and a diameter of 2 +/- 0.2 mm to allow for future large scale production.	Measure the final length of the Bio-thread using a tape, measure the width two dimensionally across each foot of the Bio-thread using calipers, determine the standard deviation of the width and compare it to the standard deviation across the entire length.
	Rationale: Future large scale production.	
3. The Bio-thread must be strong and flexible for future use in wound dressings.	The Bio-thread must endure a tensile stress of 32 MPa and fit around a spool with a diameter of 1.0 cm.	Tensile Strength testing: The Bio-thread must endure a minimum tensile stress of 32 MPa cycles to ensure strength. Flexibility testing: The bio-thread must spool around a spool with 1.0 cm diameter without fracturing.
	Rationale: To allow for future use in wound dressings.	
4. The SCOBY must not be acidic	The pH of the SCOBY must not be below 4.7	pH meter for soft solids. Measure the pH at every 3 inches and calculate the standard deviation.
	Rationale: The average pH of natural skin is 4.7[9]. Since the SCOBY is in contact with various acids, it may adopt an acidic concentration, which will need to be measured to ensure it will not harm the skin.	

c. Constraints and other considerations

What economic, environmental, social, political, ethical, safety, manufacturability, or sustainability constraints exist?

Environmental

The SCOBY must be fermented within average room temperature. Thorough research it was determined that room temperature is considered to be between 22°C-28°C. Within the facility at which the SCOBY was grown the room temperature was around 26°C. The building's thermostat was set to 26°C and a thermometer was placed next to the tube molds to monitor the temperature. If the SCOBY were to be fermented at a temperature above 28°C there is a greater risk for mold developing.

Manufacturability

The length of the thread is determined by the dimensions of the silicone tube mold that it is grown in. The larger thread that is desired will require a mold and proper storage. The original workspace was unable to fit the 6.25 foot tubes. Since this design proved to be the most feasible arrangements were made to meet the space requirement.

Since SCOBY is an organic material there is a higher risk of bacteria and mold growing if not properly sanitized between batches. All the equipment was cleaned with antibacterial soap and water and then rinsed with isopropyl alcohol to ensure the next batch was being started in a clean environment.

3. Solution Formulation: Conceptualization and Creativity (Alternative Solutions)

What are all the possible ways the objectives could have been accomplished?
What methods were used to foster and optimize creativity?
What engineering solutions were formulated or adapted to meet the customer needs?

The objective could have been accomplished through various designs, which were proposed as design concepts.

Table 3.1 Summary of Design Concepts

Design Concept #	Base Structure	Number of Tubes	Tube Configuration
1	3 plastic bins with dimensions 23.4" x 16.9" x 6.7''	3	Snaked at the base of the bins, only requiring two loops inside. The caps on the ends of the tubes would stand outside of the bins through holes with a diameter of 16 mm.
2	4 5-gallon buckets with dimensions 12'' x 13'' x 14.5''	3	Wrapped around the outside of the buckets.
3	Wooden base with dimensions 6 ft x 1.5 ft x 1''	3	Extended, with the distance between each tube measuring 4.5 in

			and the distance between the end of the tubes and the edge of the base measuring 1.5 in.

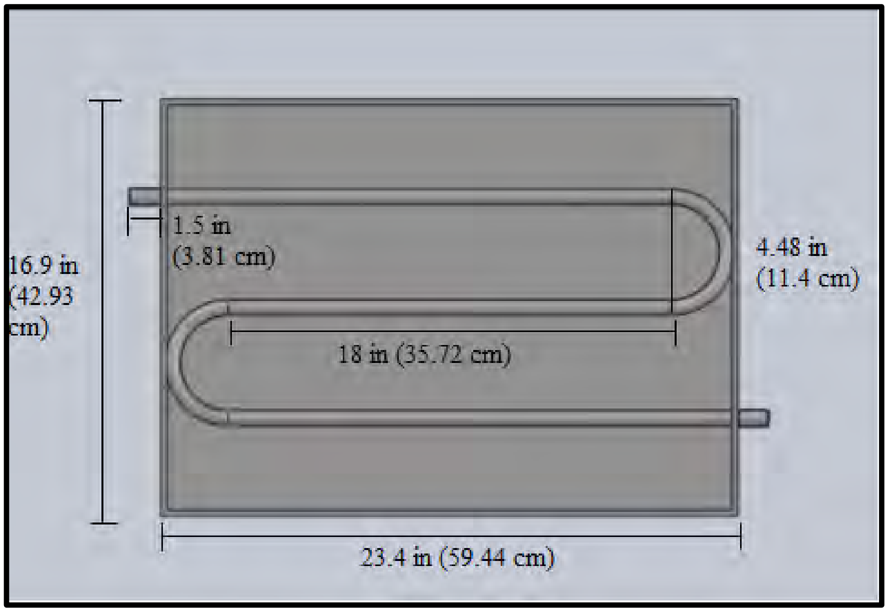

Figure 3.1 Design Concept 1

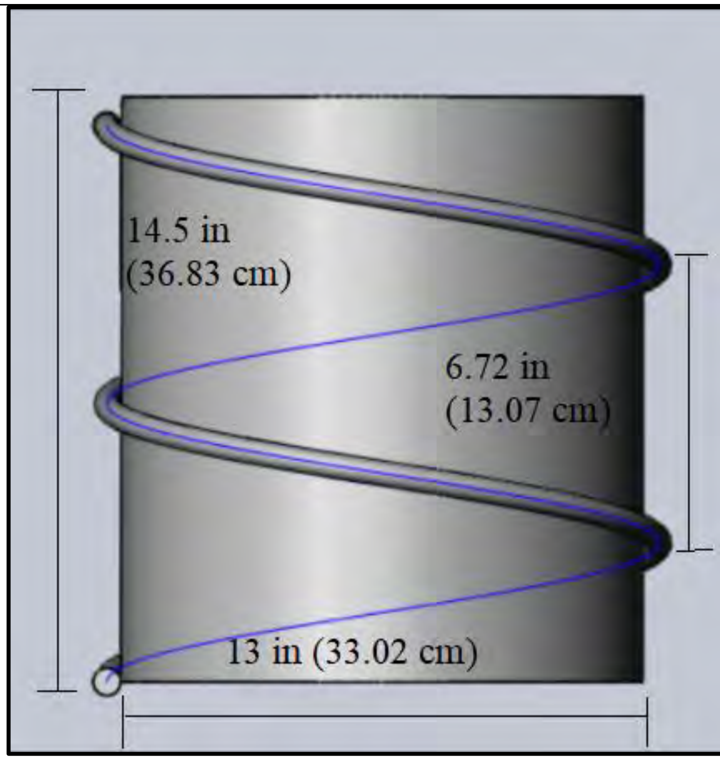

Figure 3.2 Design Concept 2

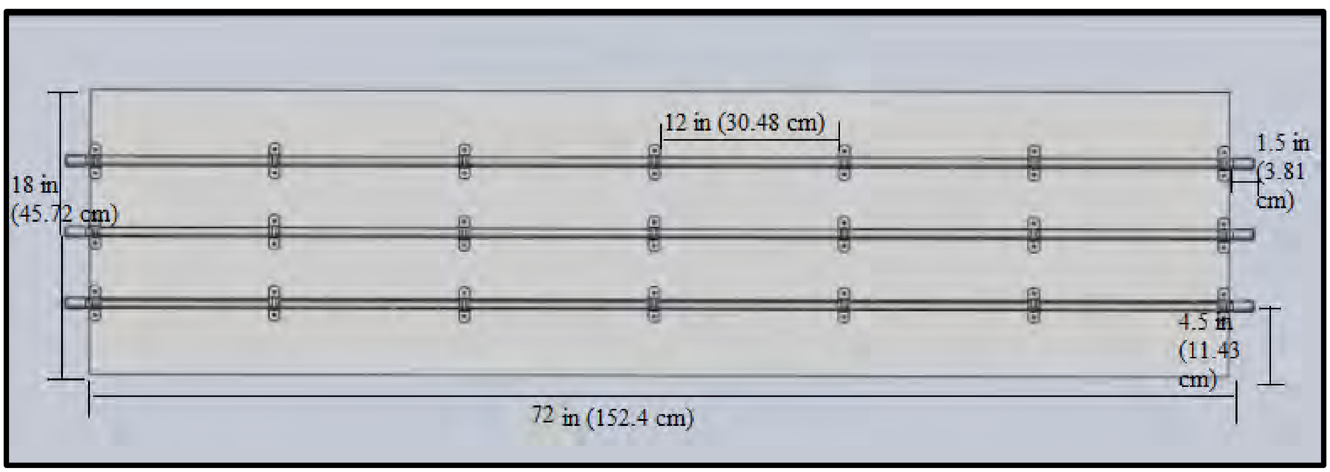

Figure 3.3 Design Concept 3

Table 3.2 Pros and Cons of the Three Design Concepts

Design Concept #	Pros	Cons
1	● Takes up less space; would fit in the original workstation ● Requires minimal building	● May cause flexibility issues with the loops in the tubes ● Is not the most feasible
2	● Fits SCOBY in the spooled configuration ● Takes up less space; would fit in the original workstation ● Requires minimal building	● Due to gravity, SCOBY may not grow consistently in thickness
3	● Allows SCOBY to grow uniformly ● Easy to maintain ● Requires minimal building	● Takes up more space

There were multiple opportunities to present creative solutions to the problem. For example, since the SCOBY takes the shape of its container, different geometric shapes were utilized in designing the structure to grow the thread. In Design Concept 1, the tubes are arranged in a snake formation, to allow for a smaller space of occupancy and controlled environment in a bin. For Design Concept 2, the tubes are wrapped around a large cylinder, modeled by a bucket. This also allows it to take up a small amount of space, but does not constrain the SCOBY to grow in sharp angles, as in Design Concept 1. Lastly, Design Concept 3 focused less on minimizing its size and more on simplicity of growth. Later in the process the sponsor provided more space to be utilized by the team, which lessened the constraint on the design. This concept allowed the SCOBY to grow uniformly in an extended tube.

Construction & Assembly

1. Obtain one 6' x 1.5' x 0.5" sheet of plywood and three silicone tubes of 6.25' length and 0.5" diameter.
2. Lay the 6' x 1.5' x 0.5" sheet of plywood on a flat, even surface.
3. Mark the middle point of the plywood which will be at 36in in length and 9in in width. This is where the middle clamp of the middle tube will go and will serve as a reference point.

4. From the clamp placed at 9in in length from the base the clamp to the left will be drilled at 4.5in in width and to the right will be at 13.5in in width. Ensuring that each tube is equally spaced by 4.5 in.
5. Position 1/2 in C clamps at intervals of 12inalong the length of the tubes and mark the spots to be screwed. The clamps will also be placed at both ends of the plywood (at 0 in and 72in).
6. Pre-drill pilot holes through the plywood at every marked spot until the screw can fit tightly.
7. Screw in a ½ in long screw into each hole, through the C clamps, fastening the tube in place.

Figure 3.4 Assembled Design

4. Feasibility Assessment (Proposed Solution)

What criteria did you use to choose the solution method that you did? Is this solution the most feasible based on technical, operational, schedule, and economic considerations? What impact might each possible solution have on society in a global and contemporary context?

Before the SCOBY Bio-Thread was put into production the following aspects were evaluated: the chemical reaction of fermentation, size reduction of SCOBY, spool size as well as a cost and risk assessment were all taken into consideration. Within the reaction the fermentation time as well as whether an open or closed system is being used can affect the overall product of the SCOBY. Pilot tests were conducted on small batches were used to evaluate the shrinkage of the SCOBY. This analysis was used to aid in the determination of tube mold dimensions.

Fermentation Chemical Reaction

The overall fermentation chemical reaction is described as:

$$C_6H_{12}O_6\ (glucose) \rightarrow 2C_2H_5OH\ (ethanol) + 2CO_2\ (carbon\ dioxide)$$

More specifically to Kombucha fermentation, below is the biochemical pathway of obtaining Kombucha from the inputs introduced in the procedures:

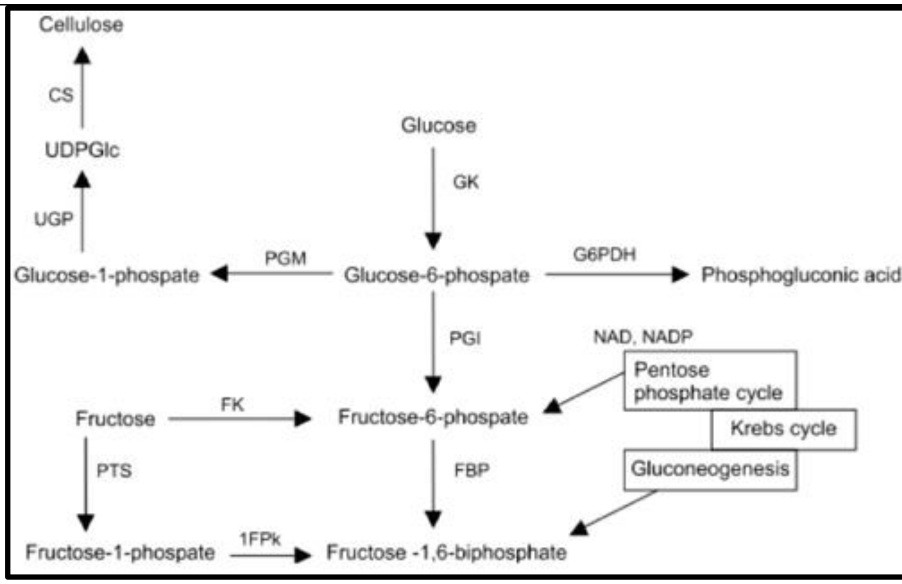

Figure 4.1 Biochemical Pathway for Kombucha Fermentation[10]

Variables

Once the tea is made, it will be left to ferment under three conditions:

<u>Condition #1: Control Variable</u>

- Tube Dimensions: 6.25 ft with an ID of 0.5 in
- Open/Closed Tube: Closed
- Fermentation Time: 2 weeks

<u>Condition #2: Time Variable</u>

- Tube Dimensions: 6.25 ft with an ID of 0.5 in
- Open/Closed Tube: Closed
- Fermentation Time: 3 weeks

<u>Condition #3: Open System Variable</u>

- Tube Dimensions: 6.25 ft with an ID of 0.5 in
- Open/Closed Tube: Open
- Fermentation Time: 2 weeks

The decision of having several trials isolates the variables being tested, which are how time and oxygen permeability leads to stronger mechanical properties and faster production, respectively.

SCOBY Size Reduction

The tables and calculations below demonstrate the measurements and calculations obtained to determine the length of the tubes. The SCOBY initially grows in a wet environment, therefore it is hydrated. One of the characteristics of the SCOBY is its high water retention, which influences the size of the SCOBY prior to drying. When it is left to dry, the SCOBY experiences a drastic change in height. Table 4.2 demonstrates the measurements (length, width, & height) taken when initially removed from the medium and when it is completely dried. Similarly, when the SCOBY will later be twisted into the thread, there will be another decrease in width, which is proposed in Table 4.3.

Table 4.2 Comparison of Length, Width, and Height from When the SCOBY is Wet and When it is Dry

Trial #	Starting Length (Wet)	Starting Width (Wet)	Starting Height (Wet)	Final Length (Dry)	Final Width (Dry)	Final Height (Dry)	Length Decrease (%)	Width Decrease (%)	Height Decrease (%)
1	8.255 cm	9.2075 cm	0.6 mm	5.3975 cm	5.715 cm	0.3 mm	34.74	37.93	50
2	7.9375 cm	7.9375 cm	0.2 mm	5.715 cm	5.715 cm	0.1 mm	28	76.378	50
3	7.62 cm	11.43 cm	0.7 mm	7.3025cm	11.1125 cm	0.3mm	4.18	2.69	57.14
4	6.6675 cm	11.7475 cm	0.4 mm	5.715 cm	11.43 cm	0.1 mm	14.29	2.7	75

Note: Calculations are found in the appendix

Table 4.3 Comparison of Length, Width, and Height from When the SCOBY is Dry and When it is Twisted

Trial #	Starting Length (Dry)	Starting Width (Dry)	Starting Height (Dry)	Final Length (Twist)	Final Width (Twist)	Final Height (Twist)	Length Decrease (%)	Width Decrease (%)	Height Decrease (%)
1	5.3975 cm	5.715 cm	0.3 mm	1.905 cm	1 mm		64.70%	98.25%	
2	5.715 cm	5.715 cm	0.1 mm	1.905 cm	1.1 mm		66.67%	98.08%	
3	7.3025cm	11.1125 cm	0.3mm	1.905 cm	1 mm		73.91%	99.1%	
4	5.715 cm	11.43 cm	0.1 mm	1.905 cm	1 mm		66.67%	99.13%	

Note: Calculations are found in the appendix

Spool Size Determination

One of the requirements of the design project is that the final product should be able to spool. A variable to consider in satisfying this requirement is the size of the spool. Since the final thread will be continuous in length at a maximum of 5 feet, the spool is expected to be able to fit the entire thread, without extending from the edge of the base.

To determine the proper spool size for a desired thread length, measurements from the spool's design are used in calculating how long of a thread it can hold. The formula to determine the length of a thread the spool can hold is found below (Figure 4.4)[11]. Upon calculating the reel factors and length of cables for each of the spools found online, it was determined that the 2.5'' x 2'' spool would be best fit for the project. This spool holds approximately 7.54 ft of thread, which is slightly over the 5-feet target.

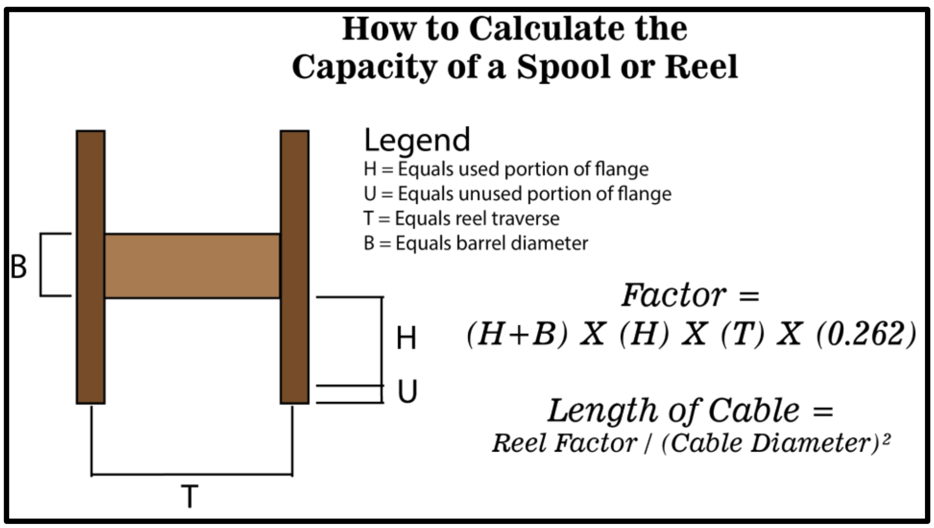

Figure 4.4 Formula for Calculating Spool Factor and Determining the Length of Cable for the Spool

Table 4.5 Sample Spools Researched to Determine Best Fit for 5-Foot Thread

Diameter	Length	H	U	T	B	Reel Factor	Length of Cable
2.5 in	2 in	15.3 mm	2.7 mm	53 mm	28 mm	9199.33614	7.54 ft
2.5 in	4 in	18.1356 mm	3.2004 mm	101.6 mm	20.6400 mm	18719.12112	15.33 ft
3.2 in	3.3 in	26.5625 mm	4.6874 mm	84 mm	19 mm	26635.36824	21.84 ft

As our project is creating the most efficient technique on the creation of a thread extracted from a fermentation process that involves the use of kombucha, a regular drink that is easily accessible at any stores and the use of sugar and tea. Once we create the thread, the future application would be wound dressing. Therefore, it would need to go through the Food and Drug Administration approval as well as some relevant standards that the device must account for. Some of the Standard Organization for Standardization and FDA Regulations that our device has to go through are listed below.

Standards

Table 4.6 Standards Used

Standard	Name
ISO 21710:2020	Biotechnology — Specification on data management and publication in microbial resource centers
ISO 15882:2008	Sterilization Of Health Care Products - Chemical Indicators - Guidance For Selection, Use And Interpretation Of Results
ISO 979:1974	Sodium Hydroxide For Industrial Use -- Method Of Assay
ISO 21527-2:2008	Microbiology Of Food And Animal Feeding Stuffs - Horizontal Method For The Enumeration Of Yeasts And Moulds - Part 2: Colony Count Technique In Products With Water Activity Less Than Or Equal To 0,95
ISO 20743:2021	Determination of antibacterial activity of textiles products
ISO 4045:2018	Chemical tests- Determination of pH and difference figure.

Cost Assessment

Within table 4.7 below the financial breakdown of the project is shown. A large portion of the cost for this project came from the materials that were needed. Around half of the materials went to the construction of the base and tube molds whereas the other half was for the production of the SCOBY Bio-Thread. Since the base and tube mold are reusable after the implementation of this process the overall cost should decrease and only require the materials needed to make the solution. That being the sugar, black tea, water, and Kombucha. These materials per unit will cost $12.94. It is expected that as the process is replicated that the overall price of the thread will decrease from what was calculated. The completion of the project remained within the budget of $500. The Project Cost was $289.32 and the product cost was $256.33. The price of the project was $424.32 with a unit price of $386.72.

Table 4.7 Cost Assessment

Section	Parts	Price	Per	Quantity	Total	Cash Outlay
Materials						
Final Product						
	Plywood	$66.67	Unit	1	$66.67	$66.67
	Silicone Tubes	$12.99	Unit	3	$38.97	$38.97
	Rubber Caps	$5.99	10 Units	6/10	$3.40	$3.40
	Screws	$1.28	14 Units	42	$3.84	$3.84
	Steel Clamps	$6.38	25 Unit	21/25	$5.36	$5.36
	Sugar	$1.54	1 lb (454g)	50g	$0.17	$0.17
	Black Tea	$4.65	100 Unit	2/100	$0.09	$0.09
	Kombucha	$3.65	474 ml	118ml	$0.90	$0.90
	Water	$0.98	3.78 L	750ml	$0.19	$0.19
	Spool	$22.79	20 Unit	1/20	$1.14	$1.14
	NaOH	$25.05	1 L	50ml	$1.25	$1.25
	Dremel	$23.38	Unit	1	$23.38	$23.38
Testing & Verification						
	Solids pH Meter	$97.99	Unit	1	$97.99	$97.99
	Caliper	$9.99	Unit	1	$9.99	$9.99
	Tape Measurer	$5.99	2 Unit	1	$2.99	$2.99
	Stress/Strain Apparatus	$799	1 Unit	1	$799	$0
	PASPORT Rotary Motion Sensor	$185	1 Unit	1	$185	$0.00
	PASPORT Force Sensor	$139	1 Unit	1	$139	$0.00
	Capstone	$695	1 Unit	1	$695	$0.00

	Software					
Software	Solidworks	$3,995	Unit	1	$3,995	$0.00
	Microsoft Project Plan	$1,129.99	Unit	1	$1,129.99	$0.00
	Slack	$0	Unit	1	$0.00	$0.00
Materials Total	$7,232.31			Materials	$7,232.31	$289.32
Unit Materials Total	$256.33			Unit Materials	$7,199.32	$256.33
Labor		$15	Hour	4.5	$67.50	$0.00
Product Cost					$323.83	$256.33
Total Project Cost					$356.82	$289.32

Overhead	40% Labor + 14% Material Cost
Total	$67.50
Unit	$62.89
Total Project Price	$424.32
Total Unit Price	$386.72

Risk Assessment

In order to evaluate the risk assessment, the major highlight was possible skin irritation or an allergic reaction since the final product is made from yeast and bacteria, the removal of these two was implemented by subjecting the final SCOBY thread into a purification process which involves the use of NaOH in which at the end the only present bioactive compound would be cellulose which is our initial focus.

Table 4.8 Potential Hazards

Potential Hazard	Generic Cause	Specific Cause	Probability	Severity	Control Mode	Control Method
Allergic Reaction	Interaction between the thread and skin	Allergy to Cellulose	Remote	Critical	List of Materials	Include the caution of a cellulose allergy
Choking Hazard	The thread is small and could have pillings over time	Small dimensions of product	Remote	Critical	Listed	Recommend secure placement on the user

5. Project Management: Organization/Work Breakdown Structure

What activities were done to accomplish the project? Who performed what tasks? What was the time frame for the completion of each task, and then the entire project? What project management software was utilized? Did the team work together in a professional and ethical manner?

The following activities were completed in order to complete this project design specifications, instructions for assembly, manufacturing details, verification testing as well as quality assurance procedures. The following team members were given their collaborated on certain aspects of the project,; Catalina Zambrano focused on Design and Engineering Analysis, Nathaniel Alexander and Rene Elvir Verification and Construction, Sydney Zamorano process and manufacturing of the thread, and Megan Boge assisted in the design process as well as within the manufacturing. Any major decision within each individual's responsibility was consulted with at least one other team member before a final decision was made. The time frame for each task was around 4-7 days depending on the size of the task. Deadlines were given as soon as possible but had to be reset or extended based on advice given from advisors. The turnaround time for anything that had to be readdressed was around 2-3 days to try and maintain as close to schedule as possible. All work was done within a shared google drive to allow for collaboration amongst all team members. Outside software such as solidworks, Miro (Online visual software) as well as Microsoft Project Plan were used. The Slack communication platform was also used as the main form of communication with the sponsor.

Table 5.1 Project Timeline

Component	Responsibility	Due Date
Clinical Research	Catalina Zambrano & Sydney	12/6/2021

	Zamorano	
Current Modalities	Catalina Zambrano	12/6/2021
Market Requirements	ALL	2/11/2022
Design Inputs	ALL	2/11/2022
Quality Function Deployment (QFD Analysis)	Catalina Zambrano	2/15/2022
Design Concept 1	Catalina Zambrano	2/28/2022
Design Concept 2	Catalina Zambrano	2/28/2022
Design Concept 3	Catalina Zambrano	2/28/2022
Process Design	Sydney Zamorano	2/20/2022
Technology Assessments	Catalina Zambrano & Sydney Zamorano	2/28/2022
Risk Assessments	Rene Elvir & Megan Boge	2/28/2022
Cost Assessment	Sydney Zamorano	2/28/2022
Regulatory Assessment	Rene Elvir & Megan Boge	2/28/2022
Patent Search Results	Catalina Zambrano	2/28/2022
Solidworks Design	Catalina Zambrano	3/6/2022
Verification Test Procedures	Nathaniel Alexander	3/6/2022
Verification Testing	Nathaniel Alexander & Rene Elivir	4/8/2022
Power Point	ALL	4/17/2022
Poster	ALL	4/17/2022
DHF	ALL	4/17/2022
DMR	ALL	4/17/2022

6. Engineering Analysis and Decision-Making

What kind of engineering analysis was required to make the design decisions? How was the analysis performed, and what was the result? What application of mathematical, physical, and life sciences was used in the engineering analysis? What modern engineering tools (including, for example, modeling software) were used for the analysis? On what basis were design decisions made? For example, what criteria were used for materials choices? If experiments were performed, how was data measured, analyzed, and interpreted to assist in design decisions?

1. SolidWorks Design

SolidWorks was utilized to depict the design for the three proposed design concepts. For Design Concept 1, three parts were created: the curved tube, the straight tube, and the bin. Firstly, the bin was formed by creating the rectangular shape and following the dimensions from a plastic bin it was based on. Using the length from inside the bin, the dimensions of the curved tube was formulated. This part was duplicated and connected with the other curved tube via a straight tube component. With this assembly, it was then added to the bin part, making another assembly, and the final design, as seen in Figure 6.1.

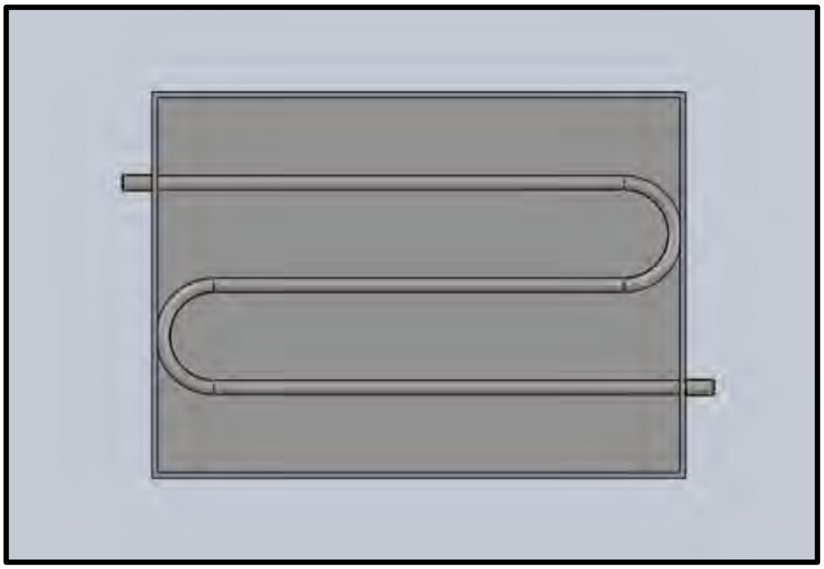

Figure 6.1 Design Concept 1

Design Concept 2 did not consist of an assembly, but rather of a singular part. The cylindrical structure was modeled after a 5-gallon bucket, and its dimensions were used to create the component. Next, using the Helix and Spiral option in Solidworks, the tube was formed in the wrapped configuration around the structure, finalizing the second design concept, shown in Figure 6.2.

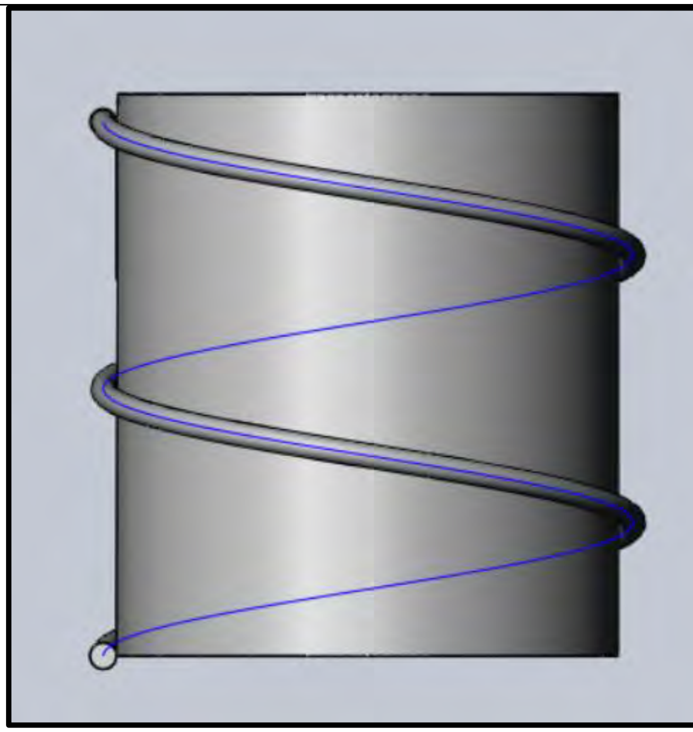

Figure 6.2 Design Concept 2

Lastly, Design Concept 3 was initiated by the design of the plywood base. Its dimensions were determined from the length of the tubes, allowing for a section of them from extending off of the edge. Next, the enclosed tube was made along with the rubber end caps, along with the opened tube, which served with the same design process only differing in cutting the cross-sectional area through the center. The clamp and screw was created based on previously formed templates. Lastly, all components were placed on an assembly and mated to remain secured on a structure, as it would in process (Figure 6.3)

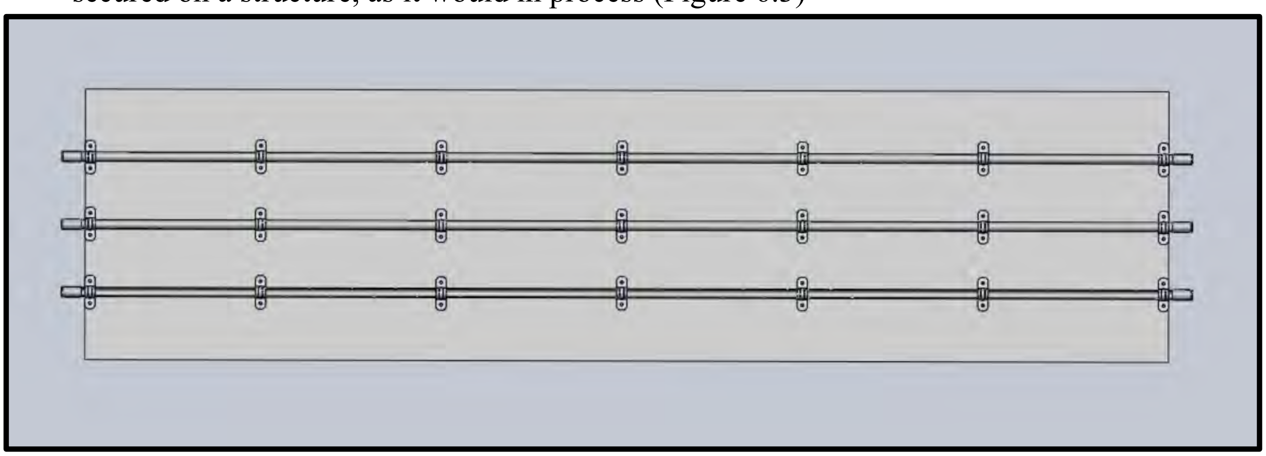

Figure 6.3 Design Concept 3

2. **Heat Transfer Simulation**

The Surface-to-Ambient Radiation analysis in Comsol describes the radiation of defined boundaries to the ambient[15]. The equation used to perform the analysis involves the net inward heat flux, as shown below:

$$-n \times q = \varepsilon\sigma(T_{amb}^4 - T^4)$$

In this equation, ε represents the surface emissivity, which is selected from the material specified. This value ranges from 0 to 1, where 0 means that no radiation is emitted from the surface and 1 means that the surface is a perfect emitter, or a blackbody. The σ variable represents the predefined Stefan-Boltzmann constant, which is $5.6704 * 10^{-8}$ W/m^2*K. Lastly, T_{amb} is the ambient temperature in Kelvin, which is manually inputted by the user.

This simulation was performed on the project to determine how long it would take the tea within the tubes to return to room temperature. The temperature of the tea (T) was inputted as 30 °C, and the ambient temperature (T_{amb}) was 25.5 °C. The results demonstrated that it would take approximately 8 minutes for the temperature to return to the modified room temperature.

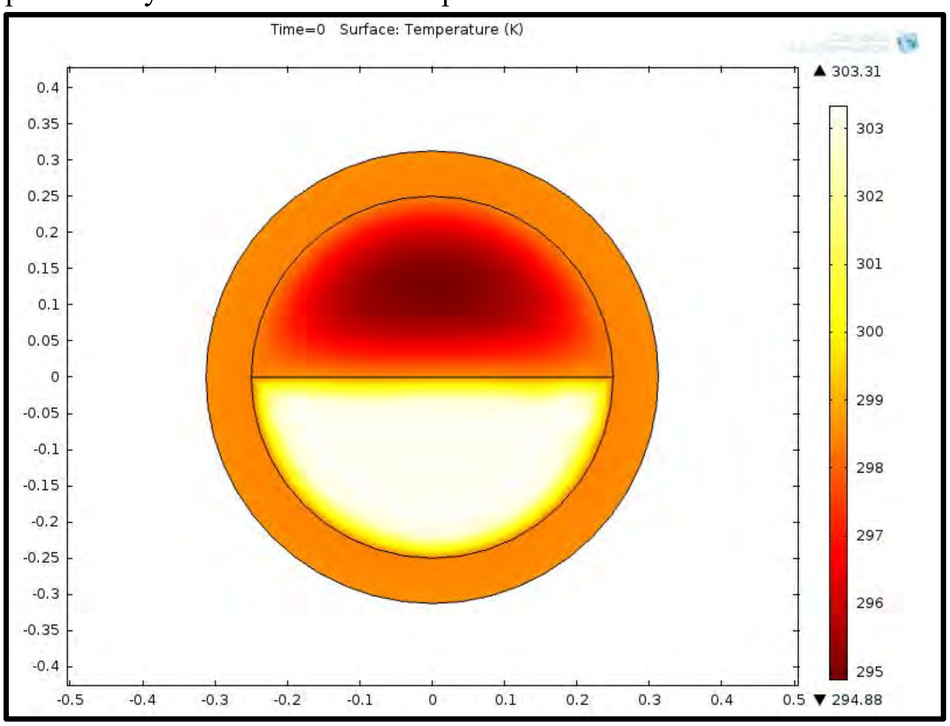

Figure 6.4 Temperature Distribution at Time=0 Seconds

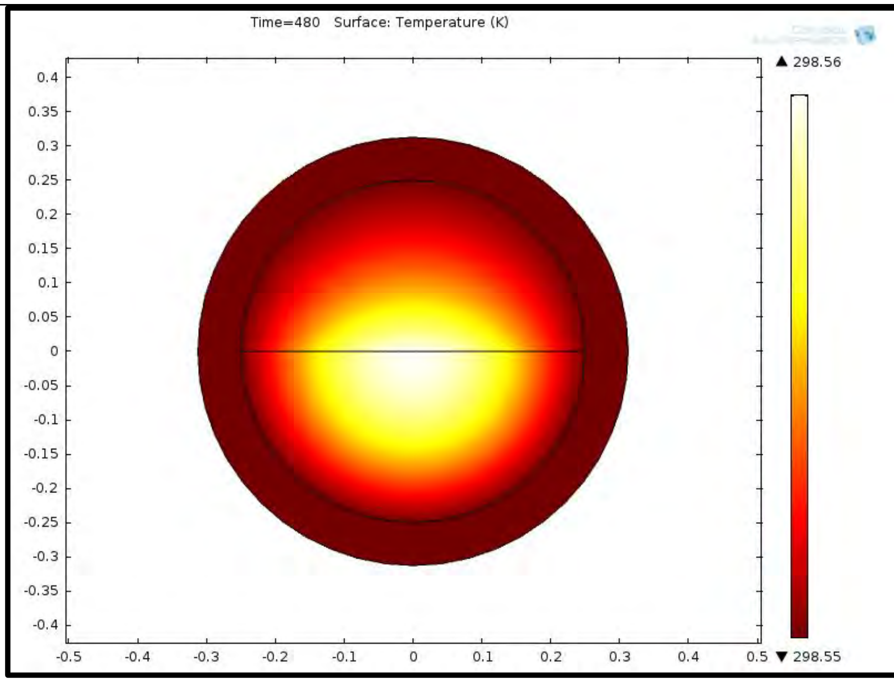

Figure 6.5 Temperature Distribution at Time=480 Seconds

3. NaOH Solution Preparation and Reaction Kinetics

Calculation of NaOH Solution (Prior to Preparation of Solution)

Used in Industry: Chlor-Alkali Process: **Electrolysis Process** of Sodium Chloride Solutions to Manufacture Chlorine, Molecular Hydrogen and Sodium Hydroxide

Half Reactions

$ClO^- + H_2O + 2e^- \leftrightarrow Cl^- + 2OH^-$ Reduction

$H_2O + 2e^- + 2Na^+ \leftrightarrow H_2 + 2NaOH$ Oxidation

Whole Reaction **Exothermic Reaction**

$$2Na^+Cl^-(s) + H_2O\,(l) \rightarrow 2Na^+OH^-(aq) + H_2(g)\uparrow + Cl^-(g)\uparrow$$

n = number of moles

Molarity $\boldsymbol{\mathcal{M}} = \frac{number\ of\ moles}{Liters}$

Molecular Weight (M) = $\frac{grams}{number\ of\ moles}$

Knowns:

$M_{Na} = 23.00\ [\frac{g}{mol}]$

$M_O = 16.00\ [\frac{g}{mol}]$

$M_H = 1.01\ [\frac{g}{mol}]$

Therefore,

$M_{NaOH} = 40.00\ [\frac{g}{mol}]$

Density, $\rho = \frac{mass}{volume}$

$\rho_{H2O} = 1.00\ [\frac{g}{mL}]$

Desired: $\boldsymbol{\mathcal{M}}_{NaOH} = 1\ [mol/L]$

Volume of Solvent (Water) = 100 mL

Therefore: n_{NaOH} = 1 [mol/L] NaOH * 100 [mL] * 10^{-3} [L/mL] = 1 [mol] NaOH

g_{NaOH} = 1 [mol] * $40.00\ [\frac{g}{mol}] = 4\ [g]$

Preparation of NaOH Solution

Pre-prep: Calculate solution measurements then put on protective eyewear and gloves. NaOH pellets and NaOH solution will burn the skin if in immediate contact. High Density Polyethylene, HDPE, containers are highly recommended as over time the sodium hydroxide can react with glass to form sodium silicate, that may result in etched or shattered glass. Be sure to use a container that is twice the size of the total desired volume collected in preparation of any unforeseen accidents.

- Using a scale with a resolution of at least tenths to the gram precision: In **separate** containers weigh out each component, Water and Sodium Hydroxide, with their respective amount previously calculated.
- Slowly, add the **NaOH** to the **WATER**. Continuously, mix the solution until all NaOH flakes have been added to the solution and dissolved completely. Container will be hot. Do not cover the container until the solution has cooled to room temperature.

4. Thread Dimensions Calculations

The table and calculations below demonstrate the measurements and calculations obtained to determine the SCOBY Bio-Thread diameter, standard deviation and standard error of the mean. The Bio-Thread is a result of the twisted dehydrated SCOBY, influencing the consistency of the diameter across the entire length. To investigate this, Table 6.1 demonstrates the measurements (diameter,standard deviation (SD), & standard error of the mean (SEM)) taken at every 2.5 cm along the length of the Bio-Thread.

Table 6.1 Results from the Dimensional Verification Test

Trial	Position/cm	Two Dimensional Diameter Average/ mm	Overall Average Diameter/ mm	SD Between Successive Points/ mm	SD Across Entire Specimen/ mm	SEM Across Entire Specimen
1	0	1.2	1.073	0.071	0.088	0.019
2	2.5	1.1		0.000		
3	5	1.1		0.071		
4	7.5	1.2		0.000		
5	10	1.2		0.071		
6	12.5	1.1		0.000		
7	15	1.1		0.071		
8	17.5	1.2		0.071		
9	20	1.1		0.000		
10	22.5	1.1		0.000		
11	25	1.1		0.071		
12	27.5	1		0.000		
13	30	1		0.000		
14	32.5	1		0.071		
15	35	1.1		0.141		
16	37.5	0.9		0.000		
17	40	0.9		0.071		
18	42.5	1		0.071		
19	45	1.1		0.071		
20	47.5	1		0.000		

21	50	1		0.071		
22	51.5	1.1		N/A		

Equations:

$$Standard\ deviation\ (SD)\ =\sqrt{\frac{\Sigma(x-\underline{x})^2}{n}}$$

$$Standard\ Error\ of\ the\ Mean\ (SEM)\ =\frac{SD}{\sqrt{n}}$$

5. **Thread pH Calculations**

The table and calculations below demonstrate the measurements and calculations obtained to determine the SCOBY Bio-Thread pH, standard deviation and standard error of the mean. The Bio-Thread is a result of the twisted dehydrated SCOBY, from a fermentation process. The fermentation consistency across the entire length of the tube influences the consistency of the pH across the entire length. To investigate this, and to ensure the Bio-Thread is not acidic, Table 6.2 demonstrates the measurements (pH, standard deviation (SD), & standard error of the mean) taken at every 2.5 cm along the length of the Bio-Thread.

Table 6.2 Results from the pH Verification Test

Trial	Position/ cm	pH	Average pH	SD Between Successive Points	SD Across Entire Specimen	SEM Across Entire Specimen
1	0	7.31	7.311	0.007	0.037	0.008
2	2.5	7.3		0.035		
3	5	7.35		0.042		
4	7.5	7.29		0.007		
5	10	7.3		0.000		
6	12.5	7.3		0.042		
7	15	7.24		0.042		
8	17.5	7.3		0.007		

9	20	7.29		0.071		
10	22.5	7.39		0.007		
11	25	7.38		0.000		
12	27.5	7.38		0.049		
13	30	7.31		0.000		
14	32.5	7.31		0.000		
15	35	7.31		0.007		
16	37.5	7.32		0.035		
17	40	7.27		0.014		
18	42.5	7.29		0.014		
19	45	7.27		0.035		
20	47.5	7.32		0.028		
21	50	7.28		0.035		
22	51.5	7.33		N/A		

Equations:

$$Standard\ deviation\ (SD)\ =\sqrt{\frac{\Sigma(x-\underline{x})^2}{n}}$$

$$Standard\ Error\ of\ the\ Mean\ (SEM)\ =\frac{SD}{\sqrt{n}}$$

6. Thread Stress Measurements

The table below shows the measurements obtained to determine the SCOBY bio-thread Ultimate Tensile Stress. The Bio-Thread is a result of the twisted dehydrated SCOBY, from a fermentation process. The fermentation consistency across the entire length of the tube influences the strength of the Bio-Thread across the entire length. Table 6.3 demonstrates the maximum force applied in tension to the Bio-Thread, the average cross-sectional area of the Bio-Thread and the Ultimate Tensile Stress of the Bio-Thread.

Table 6.3 Results from the Tensile Stress Verification Test

Maximum Force (N)	Cross-sectional Area (m^2)	Ultimate Tensile Stress (MPa)
5.8	9.04E-07	6.41

7. Detail Design

Should include all production specifications, such as machine drawings, assembly drawings, material and process specification, work instructions, etc.

Engineering Drawings

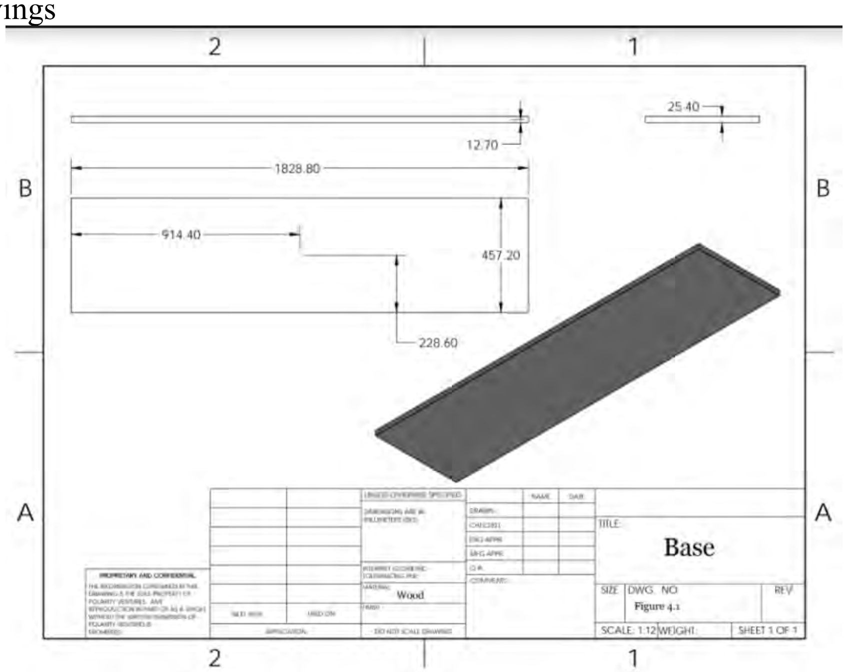

Figure 7.1 Base Design

Design drawing of the base that holds the silicone tubes. The length of the base is 6 feet and the width is 1.5 feet, with a height of 1 inch.

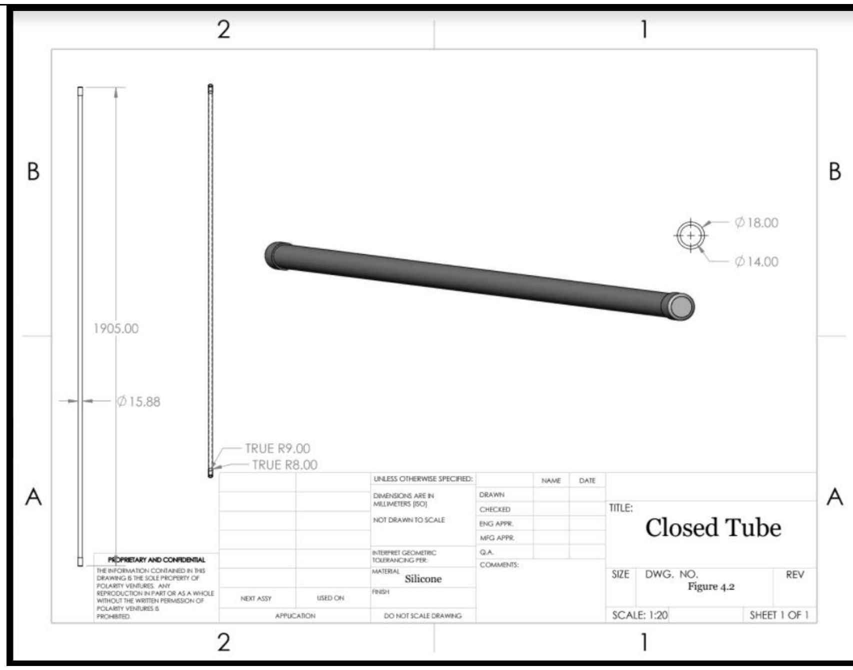

Figure 7.2 Closed Tube Design

Design drawing of closed silicone tube. The length of the tube is 6.25 feet with an ID of 12.7 mm and an OD of 15.875 mm. The cap placed on the tube has an ID of 16 mm and an OD of 18 mm.

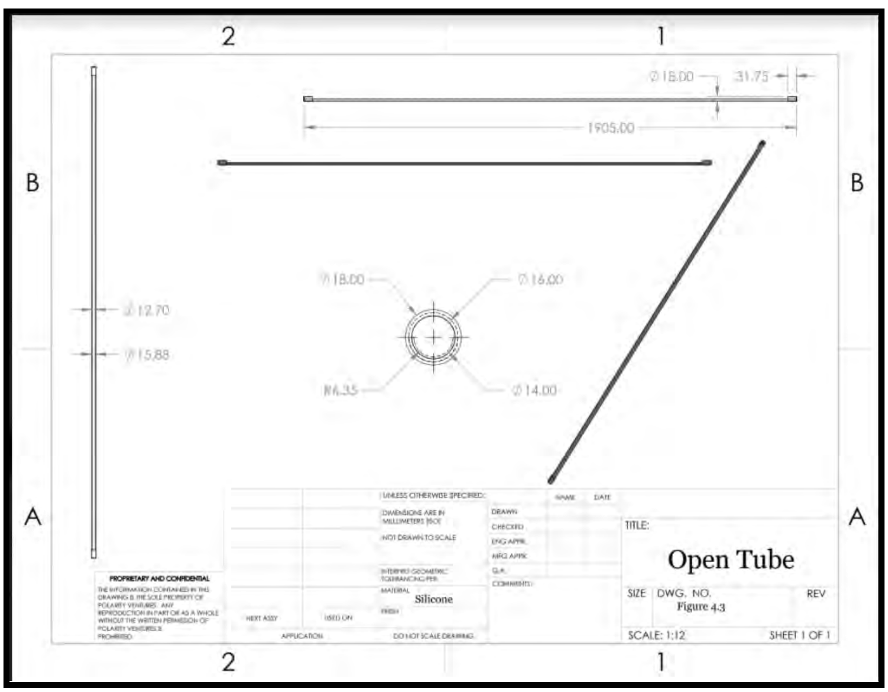

Figure 7.3 Open Tube Design

Design drawing of open silicone tube. The open tube is cut through the middle of the cross-section. The length of the tube is 6.25 feet with an ID of 12.7 mm and an OD of 15.875 mm. The cap placed on the tube has an ID of 16 mm and an OD of 18 mm.

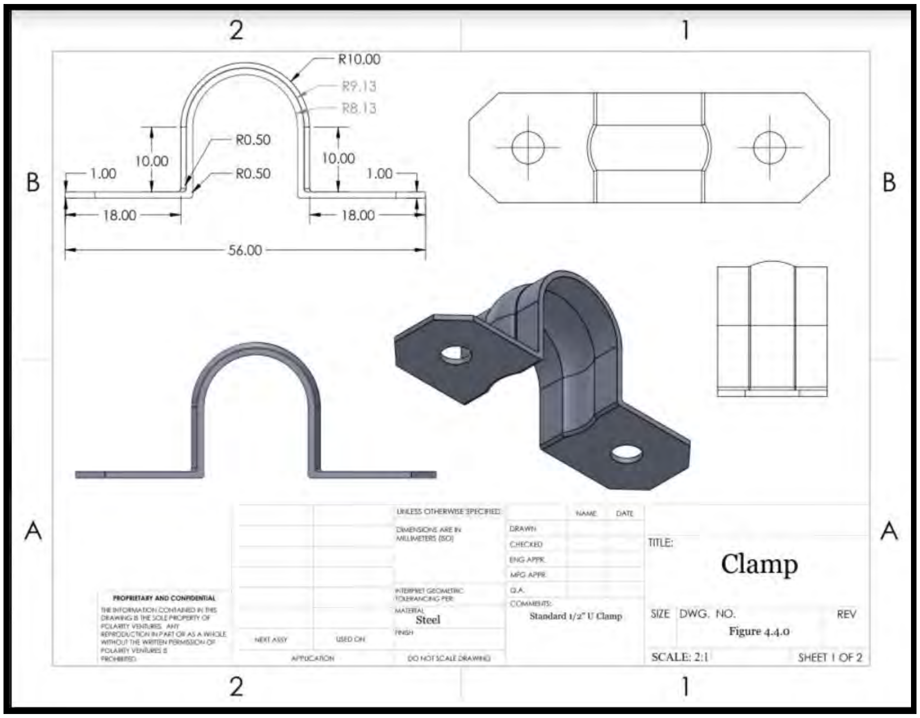

Figure 7.41 Clamp Design

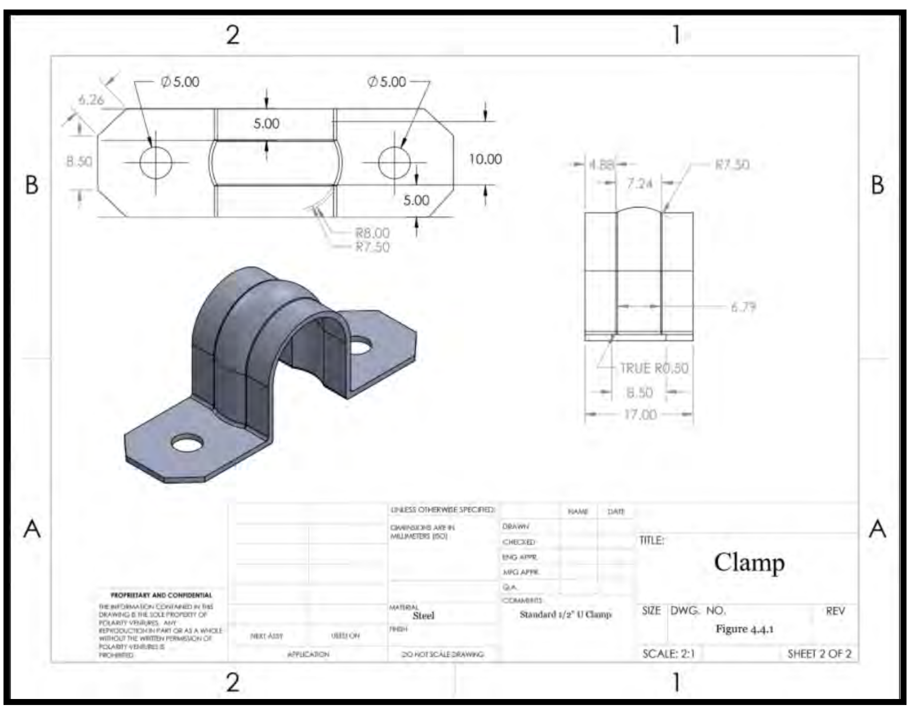

Figure 7.42 Clamp Design

Design drawing of the clamp. The length of the clamp is 56 mm, and the width is 17 mm. The diameter of each hole is 5 mm.

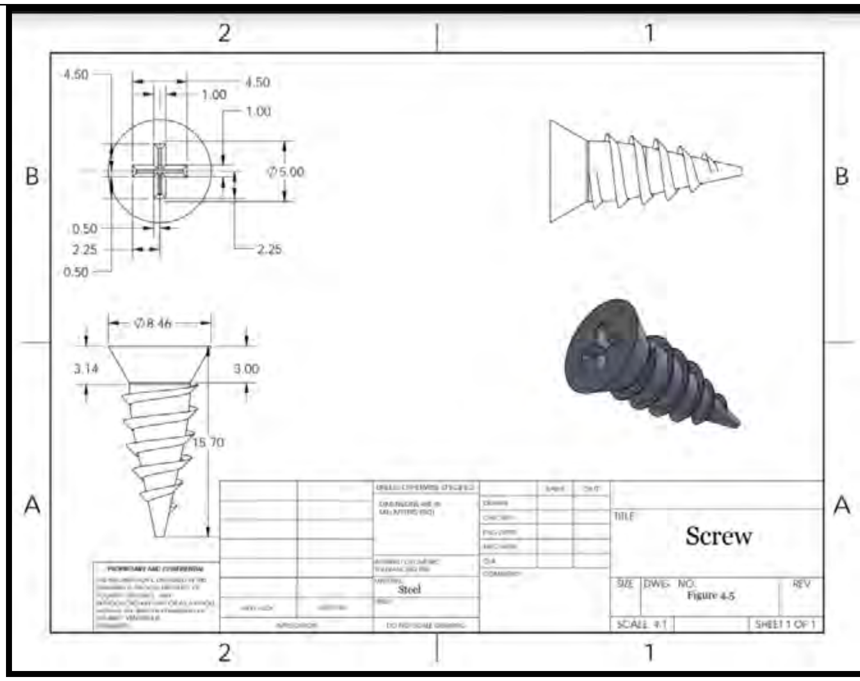

Figure 7.5 Screw Design

Design drawing of the screw. The head has a diameter of 8.26 mm and the tail has a length of 15.70 mm.

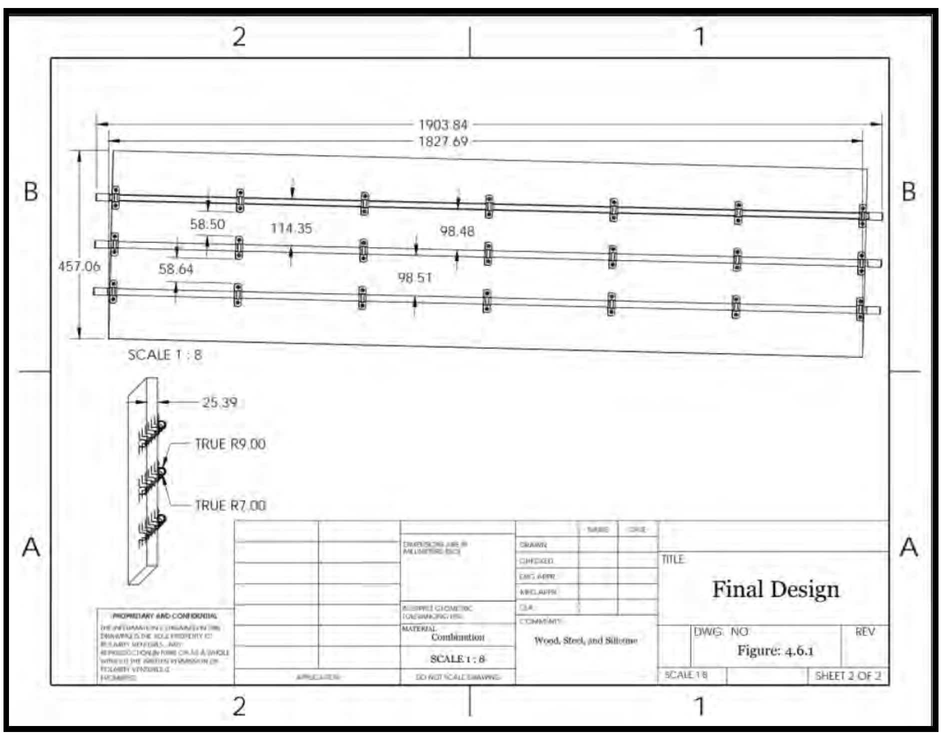

Figure 7.61 Completed Structure Design

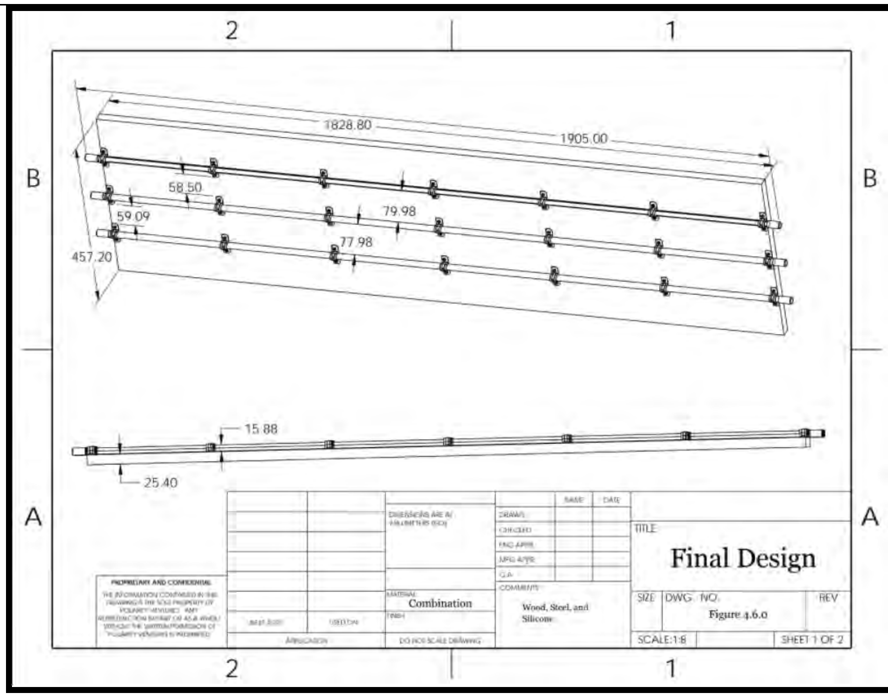

Figure 7.62 Completed Structure Design
Design drawing of the entire structure including two closed silicone tubes and one open silicone tube on a base. The distance between the middle of each tube is 4.5 inches.

Table 7.1 Material Specifications

Material	Specifications
Silicone Tubes	1/2" ID x 5/8" OD; Thickness: 1.2 mm; Length: 6.25 feet
Rubber End Caps	ID 16 mm
Plywood Base	Length: 6 ft; Width: 1.5 ft, Height: 1 in
Galvanized Steel Pipe Clamps	¾ in, 2-holes
Screws	3/16 in x 1 in
Spring Water	750 mL
White Sugar	100 g
Black Tea	4 bags
Kombucha	235 mL
Pot	10.63’’ x 10.63’’ x 5.12’’. Hold 4 quarts

Mixing Spoon	13.5’’ x 2.75’’ x 1.2’’
Hot Plate	9.4’’ x 3.1’’ x 8.5’’; 1000 watts
Dremel	5 speed 3.7V
Isopropyl Alcohol	50 mL; 90%
NaOH	100 mL; 1M

Procedures

Brewing
1. In a pot, 375ml of water will be brought to a boil (100 °C)
2. 50g of sugar will be added and then dissolved
3. 2 standard tea bags will be steeped for 15 minutes and then removed. *Note each tea bag contains around 3g of tea
4. 375ml of room temperature water (around 26°C) will then be added to help decrease the cooling time.
5. Once the mixture cools to 30°C, 118ml of unflavored, unpasteurized Kombucha can be added to the tea and then stirred

Fermentation

6. Fill the tubes with 100ml solution
    0. For the open tube, ensure that both rubber caps are tightly secured and then with a measuring cup and funnel the solution will then be poured into the mold filling it to just below the edge of the wall.
    1. For the closed tubes, secure one end of the tube with the rubber cap and then using the funnel the solution will be poured into the open end. Then secure that end with a rubber cap.
7. The solution will be left to ferment for 2 weeks (open and control tube) 0r 3 weeks.

Purifying

8. After fermentation, the remaining solution will need to be drained.
9. The thread will be rinsed with around 20ml of 1 M NaOH and then rinsed with distilled water.
10. The SCOBY will then be left to dry for 1 week.

Spooling

11. The thread will be removed from the mold after 1 week of drying.
12. The thread will be twisted upon itself after it has been dried. A dremel will aid in the twisting. One end of the thread will be secured to a hook with the other end attached to the dremel. When the dremel is turned on and in use it will twist the cord in about 5 seconds.
13. After twisting the thread will be wrapped around a spool and the ends will be fastened so that it does not untangle.

The above procedure will be slightly altered depending on which of the three variables. This can be best observed within the following flow chart. All changes will occur in the fermentation stage which can be seen within the diagram below.

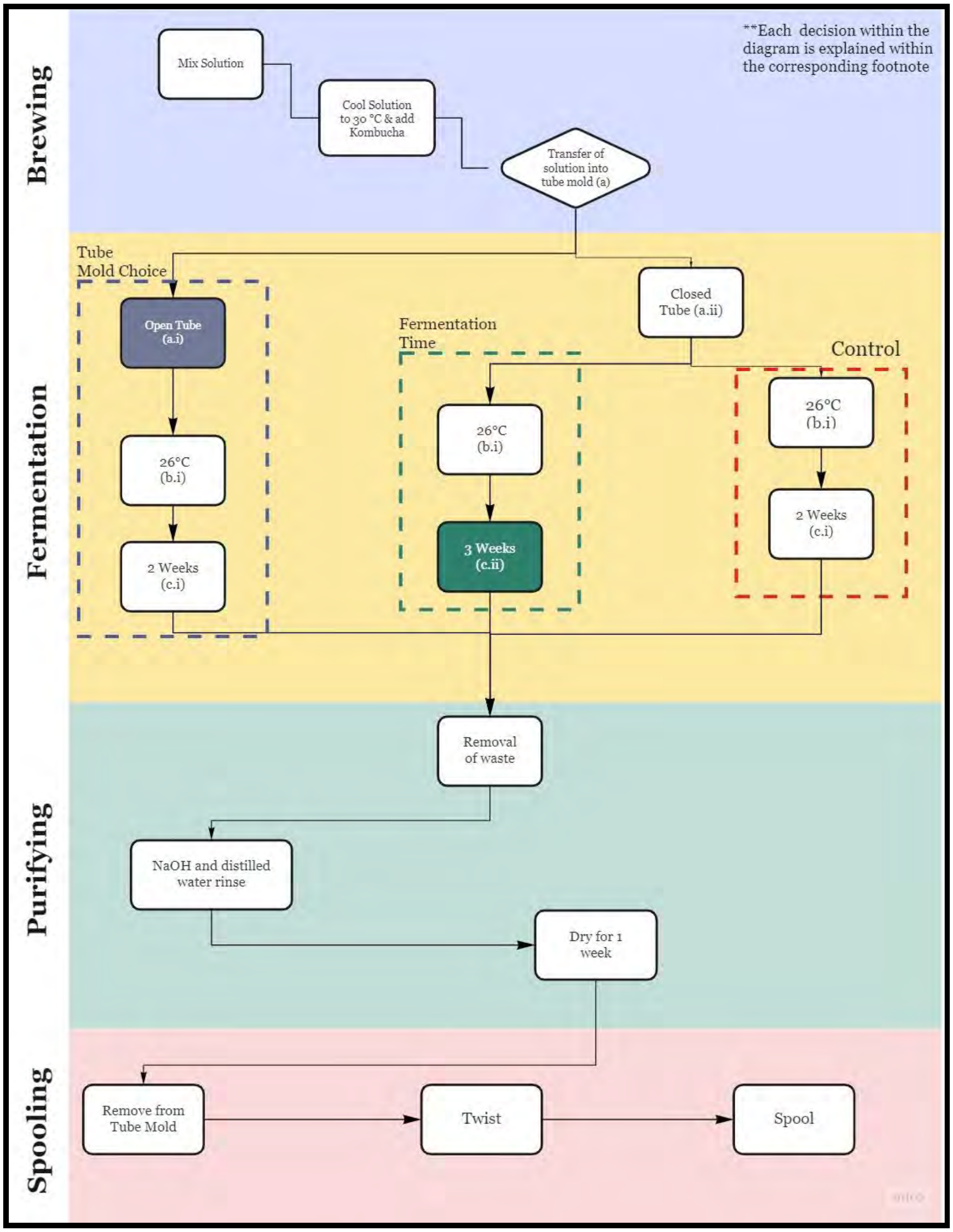

Figure 7.7 Flowchart of Procedures Followed in the Design Project

a. Tube Mold Choice
    i. Open Tube

The tube mold will be one made from silicone due to its high capacity of oxygen permeability. Since the fermentation process of SCOBY requires the presence of oxygen, having the tube be open in a horizontal cross section will allow for a greater presence of oxygen. Allowing for a decrease in the overall production of a SCOBY thread.

    ii. Closed Tube (control)

The closed tube mold still allows for the oxygen permeability however, it will be entering the fermentation system at a much lower rate when compared to the open tube.

b. Temperature
    i. 26 Degrees Celsius (control)

Kombucha tea is traditionally fermented at room temperature which is why this will serve as the control.

c. Fermentation Time
    i. 2 Weeks (control)

This is considered the general fermentation time for SCOBY regardless of what its final intentions are. It will serve as the control fermentation time when testing the effectiveness of altering the remaining variables.

    ii. 3 Weeks

The longer the SCOBY is left to ferment the thicker it will become. By setting the fermentation time for an extra week this will allow for the thread to be more uniform in its consistency and thickness.

8. Construction

How was it constructed? By whom? Using what manufacturing techniques, tools and processes? What problems were overcome?

Construction of the Base and Tube Mold:

The physical design will be constructed out of the following materials: 6.5 ft by 1.5 ft piece of plywood, 6.25 ft silicone tubes with an inner diameter of ½ inch, ½ in diameter clamps and ½ inch screws. A drill was the main form of equipment since the plywood was pre-cut by the distributor to its desired dimensions. It was constructed by the team, no outside sources were used. While constructing the base it was necessary to keep in mind how the assembly would be translated into a large scale operation. With that in mind the steps of assembly were kept as simple as possible. With the physical design the main thing was to make sure that each tube was evenly spaced along the width of the board. The middle tube was first placed on the board and then the 1st and 3rd tube were placed on either side 4.5 inches away from the middle tube and the end of the board. Clamps were used to secure the tube down to the base and also keep the tube straight. These clamps were placed every 12 inches along the length of the base, this includes the beginning and end of the base. This process can be increased to a larger scale by increasing the length of the tube or number of tubes. The spacing should remain consistent regardless of the tube length. The ability to increase to a larger production scale will allow this constructed base to be suitable for batch process manufacturing. The only problem that occurred within this construction was difficulty ensuring that the tubes and clamps were evenly spaced and straight. This can be avoided in large scale production by having a base with pre-made ruler markings or by automating the drilling of the clamps.

Figure 8.1 Clamps Spaced 4.5 Inches Apart

Figure 8.2 Set of Clamps Placed 12 Inches Apart

Figure 8.3 Screwing of the Clamps Securely

9. Testing

How was verification testing designed and conducted? How was validation testing designed and conducted? What application of mathematical (including statistics), physical, and life sciences was used in the analysis? What modern engineering tools (including, for example, sophisticated testing and analytical equipment) were used for the testing and analysis? What were the results?

Research was performed on verification tests for bioactive compounds and which compounds may be present, tests for determining total length and width, standard error and standard deviation, tests for fatigue, shape memory, and pH.

Table 9.1 Verification Test 1: Test for bioactive compounds

Market Requirement	Design Input	Verification Testing
The Bio-thread will contain bioactive compounds.	The final product will have cellulose.	Schulze's Reagent. A purple color change occurs in the presence of cellulose
	Rationale: Cellulose is a biocompatible material that has the capability of promoting wound healing.	

To test for bioactive compounds, more specifically cellulose, the SCOBY Bio-thread was placed at the base of an open container and using a measuring cylinder, 50 mL of Schulze's reagent was measured. Using a syringe, Schultze's reagent was applied along the length of the Bio-thread. All color changes present and any other observations which may be present were recorded and if the Bio-thread changed to a purple color, cellulose was present, and the verification test was passed.

Table 9.2 Verification Test 2: Test for Bio-thread dimensions

Market Requirement	Design Input	Verification Testing
An uninterrupted Bio-thread with dimensions that will facilitate large scale manufacturing	The Bio-thread must present repeatability with a length of 152.4 +/- 15.24 cm and a diameter of 2 +/- 0.2 mm to allow for future large scale production.	Measure the final length of the Bio-thread using a tape, measure the width two dimensionally across each foot of the Bio-thread using calipers, determine the standard deviation of the width and compare it to the standard deviation across the entire length.
	Rationale: Future large scale production	

To test for an uninterrupted Bio-thread with a continuous length of 152.4 cm and a diameter of less than 2 +/- 0.2 mm to allow for future large-scale production, the SCOBY Bio-thread was first placed along a long, flat surface and using a tape measure, the total length of the Bio-thread was measured. Keeping the Bio-thread in the same position, the width was measured two dimensionally across every 2.5 centimeters of the Bio-thread using calipers and all results were tabulated. The standard deviation of the width across every 2.5 centimeters of the Bio-thread was measured and compared to the standard deviation across the entire length. The standard error of the mean was determined by dividing the standard deviation by the square root of the sample size. Once the Bio-thread had a continuous, uninterrupted length of 152.4 +/- 15.24 cm and a diameter of 2 +/- 0.2 mm, the verification test was passed.

Table 9.3 Verification Test 3: Test for strength and flexibility

Market Requirement	Design Input	Verification Testing
The Bio-thread must be strong and flexible for future use in wound dressings.	The Bio-thread must endure a tensile stress of 32 MPa[16] and fit around a spool with a diameter of 1.0 cm	Tensile Strength testing: The Bio-thread must endure a minimum tensile stress of 32 MPa cycles to ensure strength. Flexibility testing: The bio-thread must spool around a spool with 1.0 cm diameter without fracturing.
	Rationale: To allow for future use in wound dressings	

To test for strength and flexibility, a 40 cm specimen of the SCOBY Bio-thread was spooled on a spool with 1.0 cm diameter. Next, an 11.5 cm specimen of the SCOBY Bio-thread was loaded into a tensile stress test machine. The bio-thread was pulled at a constant rate of extension specified in the standard and the test was stopped once the bio-thread fractured. Once the biothread endures a tensile stress of 32 MPa and is successfully spooled on a spool with 1.0 cm diameter without fracturing, the verification test is passed.

Table 9.4 Verification Test 4: Test for biothread pH

Market Requirement	Design Input	Verification Testing
The SCOBY must not be acidic	The pH of the SCOBY must not be below 4.7	pH meter for soft solids. Measure the pH at every 2.5 cm and calculate the standard deviation.
	Rationale: The average pH of natural skin is 4.7. Since the SCOBY is in contact with various acids, it may adopt an acidic concentration, which will need to be measured to ensure it will not harm the skin.	

To test for acidity, the SCOBY Bio-thread was placed along a long, flat surface and the pH was measured at intervals of 2.5 cm. The standard deviation of the pH across every 2.5 cm of the Bio-thread was determined and compared to the standard deviation across the entire length. The standard error of the mean was determined by dividing the standard deviation by the square root of the sample size. Once the Bio-thread had a pH of greater than 4.7, the verification test was passed.

For this project, the validation and verification testing overlapped and were therefore conducted together. The final product was required to be a non-acidic, 152.4 cm long, less than 2 mm wide, uninterrupted, flexible bio-thread containing bioactive compounds, and this was validated through the following verification tests.

Testing for bioactive compounds, more specifically cellulose, the SCOBY Bio-thread was placed at the base of an open container and using a measuring cylinder, 50 mL of Schulze's reagent was measured. Using a syringe, Schultze's reagent was applied along the length of the Bio-thread. All color changes present and any other observations which may be present were recorded and if the Bio-thread changed to a purple color, cellulose was present, and the product was validated.

Testing for an uninterrupted Bio-thread with a continuous length of 152.4 cm and a diameter of less than 2 +/- 0.2 mm to allow for future large-scale production, the SCOBY Bio-thread was first placed along a long, flat surface and using a tape measure, the total length of the Bio-thread was measured. Keeping the Bio-thread in the same position, the width was measured two dimensionally across every 2.5 centimeters of the Bio-thread using calipers and all results were tabulated. The standard deviation of the width across every 2.5 centimeters of the Bio-thread was measured and compared to the standard deviation across the entire length. The standard error of the mean was determined by dividing the standard deviation by the square root of the sample size. Once the Bio-thread had a continuous, uninterrupted length of 152.4 +/- 15.24 cm and a diameter of 2 +/- 0.2 mm, the product was validated.

Testing for strength and flexibility, a 40 cm specimen of the SCOBY Bio-thread was spooled on a spool with 1.0 cm diameter. Next, an 11.5 cm specimen of the SCOBY Bio-thread was loaded into a tensile stress test machine. The bio-thread was pulled at a constant rate of extension specified in the standard and the test was stopped once the bio-thread fractured. Once the biothread endures a tensile stress of 32 MPa and is successfully spooled on a spool with 1.0 cm diameter without fracturing, the product was validated.

Testing for acidity, the SCOBY Bio-thread was placed along a long, flat surface and the pH was measured at intervals of 2.5 cm. The standard deviation of the pH across every 2.5 cm of the Bio-thread was determined and compared to the standard deviation across the entire length. The standard error of the mean was determined by dividing the standard deviation by the square root of the sample size. Once the Bio-thread had a pH of greater than 4.7, the product was validated.

Microsoft Excel and PASCO Capstone Software were used to determine the tensile stress, standard deviation and standard error of the mean for specimen length, diameter, and pH. The obtained results were tabulated and reported in the Evaluation section below.

A PASCO Capstone tensile stress tester shown in figure 9.1 below was used to determine the ultimate tensile stress of the SCOBY Bio-thread. The thread was clamped on both ends and slowly drawn apart. Simultaneously, the PASCO Capstone software was active in the background, recording the tensile stress exerted on the SCOBY Bio-thread.

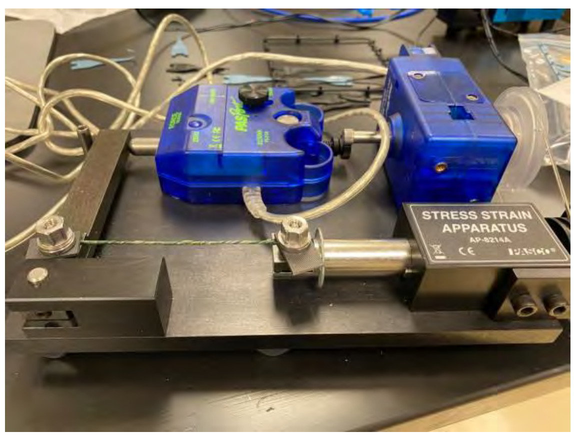

Figure 9.1 PASCO Capstone Tensile Stress Test

10. Evaluation

What application of mathematical (including statistics), physical, and life sciences was used to analyze and interpret the results? Was a statistical software package, or other modern engineering tool, used to assist in data interpretation? What were the specific evaluation criteria? Were they met?

To evaluate the final results, test protocols were designed to confirm all design inputs. The final SCOBY Bio-thread was required to contain cellulose, have a continuous length of 152.4 +/- 15.42 cm with a diameter of 2 +/- 0.2 mm, endure a tensile stress of 32 MPa, be spooled on a 1.0 cm diameter spool, and have a pH of greater than 4.7. The PASCO Capstone hardware and software were used to perform the tensile stress verification test, an electronic caliper was used to measure the width of the Bio-thread along its entire length, a solid pH meter was used to determine the pH of the Bio-thread along its entire length and Microsoft Excel was used to determine any averages, standard deviations and standard errors of the means.

Verification Test Results

Table 10.1 Verification Test Results

Parameter	Trials	Verification Test Result	Average Measurement	Standard Error of the Mean	Average Standard deviation across every specified unit	Standard deviation across all units
Cellulose presence	1	Passed	Passed	N/A	N/A	N/A
Continuous Length / cm	1	Failed	51.5	N/A	N/A	N/A
Diameter / mm	1	Passed	1.073	0.019	0.044	0.088
Tensile Stress Test / MPa	1	Failed	6.41	N/A	N/A	N/A
Flexibility test	1	Passed	N/A	N/A	N/A	N/A
pH	1	Passed	7.311	0.008	0.023	0.037

Verification Test for Cellulose:

The presence of cellulose within the SCOBY bio-thread was examined for one trial. Once the bio-thread was spooled, it was subjected to the Schultze's reagent, an oxidizing solution consisting of potassium chlorate $KClO3$ and nitric acid $HNO3$. If a blue color change were observed, cellulose would be deemed present.

Results:

Once there was contact between Schultze's reagent and the SCOBY Bio-thread, a color change was observed from dark green to blue, indicating the presence of cellulose.

Figure 10.1 Original Bio-thread

Figure 10.2 Bio-thread treated with Schultze's reagent.

Dimensional Verification Test:

In order to test the continuous length of the Bio-thread, a measuring tape positioned alongside the Bio-thread and results were recorded for one specimen. Initially, the dried SCOBY was 193 cm long but if twisted, fracturing would have occurred. As a result, the SCOBY was folded in thirds and twisted, providing the Bio-thread with much greater strength and stability. In order to test the diameter of the Bio-thread along its entire length, a caliper was used at every 2.5 cm. Further analysis was performed using Microsoft Excel to determine the average width, standard deviation and standard error of the mean.

Results:

The Bio-thread, analyzed for one specimen, was continuous in length for 51.5 cm, falling short of the desired length of 152.4 cm. However, the Bio-thread had an average width of 1.073 +/- 0.019 mm, satisfying the design input requiring the thread to be no greater than 2 +/- 0.2 mm in diameter, and passing the verification test.

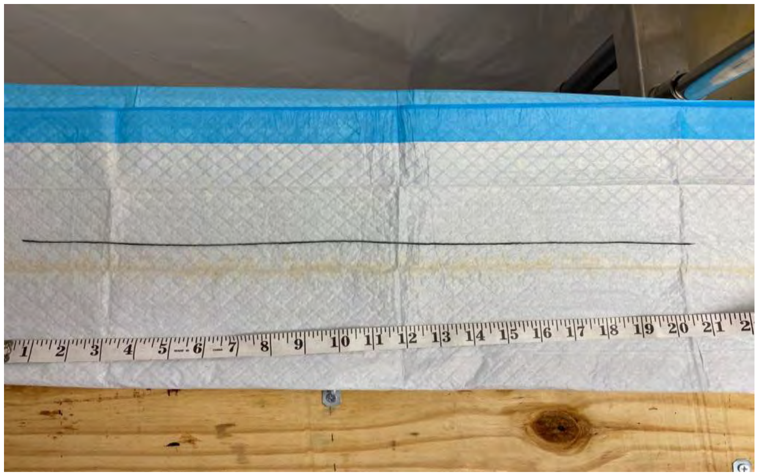

Figure 10.3 Final Bio-thread

Table 10.2 Results of the Diameter Verification Test

Trial	Position/cm	Diameter / mm	Average Diameter / mm	SD across specimen	SEM across Specimen
1	0.0	1.2	1.073	0.088	0.019
4	7.5	1.2			
7	15.0	1.1			
10	22.5	1.1			
13	30.0	1.0			
16	37.5	0.9			
19	45.0	1.1			
22	51.5	1.1			

Tensile Stress and Flexibility Verification Tests:

For the tensile stress test, an 11.5 cm sample of the SCOBY Bio-thread was secured at both ends of the PASCO Capstone tensile stress tester. This Bio-thread specimen was from a 2 week old SCOBY, with a diameter of 1.073 mm. The Bio-thread was slowly pulled apart until the point of fracture. The maximum endured force was recorded and the tensile stress was calculated. For the flexibility verification test, the 51.5 cm long Bio-thread was spooled on a spool with a diameter of 1 cm. If spooled without fracture, the Bio-thread would be deemed flexible.

Results:

The 1.073 diameter Bio-thread endured a force of 5.8 N, corresponding to a tensile stress of 6.41 MPa. This was much lower than the desired 32 MPa and therefore failed the tensile stress verification test. However, the Bio-thread was successfully spooled on a 1.0 cm diameter spool, deeming the Bio-thread flexible.

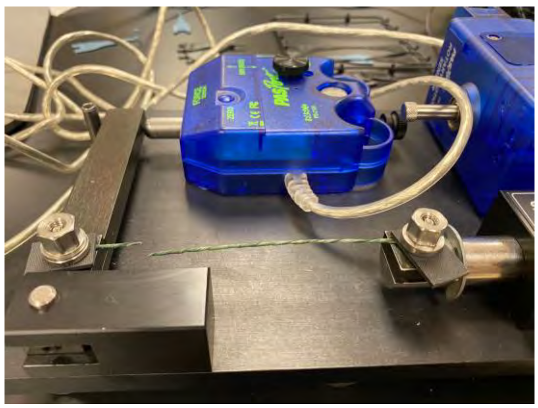

Figure 10.4 PASCO Capstone Tensile Stress Test

Table 10.3 Data Collected from the Tensile Stress Test

Maximum Force / N	Area / m^2	Ultimate Tensile Stress / MPa
5.8	9.04E-07	6.41

Figure 10.5 Showing the Spool Flexibility Test

pH Verification Test:

In order to test the pH of one Bio-thread specimen along its entire length, a solid pH meter was used at every 2.5 cm. Further analysis was performed using Microsoft Excel to determine the average pH, standard deviation and standard error of the mean.

Results:

The Bio-thread, analyzed for one specimen, had an average pH of 7.311 +/- 0.008, satisfying the design input requiring the thread pH to be no less than 4.7, and passing the verification test.

Table 10.4 Results of the pH Verification Test

Trial	Position/cm	pH	Average pH	SD across specimen	SEM across Specimen
1	0.0	7.31	7.311	0.037	0.008
4	7.5	7.29			
7	15.0	7.24			
10	22.5	7.39			
13	30.0	7.31			
16	37.5	7.32			
19	45.0	7.27			
22	51.5	7.33			

11. Conclusion

In conclusion, the process to create a SCOBY Bio-thread was successfully designed using a 4 step process, and the produced bio-thread was 51.5 cm long, 1.073 mm wide, had an average pH of 7.31, endured a tensile stress of 6.41 MPa and was flexible, successfully passing 3 out of 5 verification tests.

12. Relevance of Project to the BME Curriculum.

Briefly explain how you were able to satisfy each of the eight BME program Student Learning Outcomes by your project. Identify specific courses that directly related to the completion of your project and explain how?

Student Learning Outcomes

1. Ability to apply knowledge of mathematics (including differential equations and statistics), physical and life sciences, and engineering to carry out analysis and design to solve problems at the interface of engineering and biology;
2. Ability to design and conduct experiments, as well as to measure, analyze and interpret data from living systems;
3. Ability to design a system, component, or process to meet desired needs, including systems that involve the interaction between living and non-living materials, within realistic constraints such as economic, environmental, social, political, ethical, health and safety, manufacturability, and sustainability;
4. Ability to identify, formulate, and adapt engineering solutions to unmet biological needs,
5. Ability to use the techniques, skills, and modern engineering tools necessary for engineering practice, including the ability to model and analyze biological systems as engineering systems;
6. Ability to function on multi-disciplinary teams;
7. Ability to communicate effectively;
8. Awareness of the characteristics of responsible professional engineering practice, including ethical conduct, consideration of the impact of engineering solutions on society in a global and contemporary context, and the value of life-long learning.

Table 11.1 Learning Outcomes

Learning Outcome	How Outcome was Satisfied	Related Course
1, 2, 5	Verification testing for tensile strength and flexibility	BME 4050L, BME 4051L, BME 4100 Biomaterials
3,4	Selection of silicone as the tube mold material	BME 4100 Biomaterials
2, 5	Conducting a heat transfer analysis to determine consistency of temperature inside the tube molds	BME 3632 BME Transport, BME 2740 BME Modeling and Simulations

6,7,8	Decision making in regards to the final design	BME 4800C
6, 7, 8	Organization of team dynamics, individual responsibilities, and team commitments	BME 4800C

Appendix

Additional information and calculations.

Tea Volume Determination

Calculations:

$$Radius: 0.25\ in\ >\ 0.625\ cm$$
$$Height: 75\ in\ >\ 190.5\ cm$$

$\frac{1}{2} \times \pi \times (1.27)^2 \times 190.5 = 116.89\ cm^3$

SCOBY Size Reduction

Calculations:

Equation:

$$Percentage\ Decrease = \frac{(Starting\ Value - Final\ Value)}{[Starting\ Value]} \times 100$$

Trial 1:
Length:

$$\frac{(8.255 - 5.3875)}{[8.255]} \times 100 = 34.74\%$$

Width:

$$\frac{(9.2075 - 5.715)}{[9.2075]} \times 100 = 37.93\%$$

Height:

$$\frac{(0.6 - 0.3)}{[0.6]} \times 100 = 50\%$$

Trial 2:

Length:

$$\frac{(7.9375 - 5.715)}{[7.9375]} \times 100 = 28\%$$

Width:

$$\frac{(12.7 - 3)}{[12.7]} \times 100 = 76.378\%$$

Height:

$$\frac{(0.2 - 0.1)}{[0.2]} \times 100 = 50\%$$

Trial 3:
Length:

$$\frac{(7.62 - 7.30125)}{[7.62]} \times 100 = 4.18\%$$

Width:

$$\frac{(11.42 - 11.112)}{[11.43]} \times 100 = 2.69\%$$

Height:

$$\frac{(0.7 - 0.3)}{[0.7]} \times 100 = 57.14\%$$

Trial 4:
Length:

$$\frac{(6.6675 - 5.715)}{[6.6675]} \times 100 = 14.29\%$$

Width:

$$\frac{(11.7475 - 11.43)}{[11.7475]} \times 100 = 2.7\%$$

Height:

$$\frac{(0.4 - 0.1)}{[0.4]} \times 100 = 75\%$$

Calculations:

Equation:

$$Percentage\ Decrease = \frac{(Starting\ Value - Final\ Value)}{[Starting\ Value]} \times 100$$

Trial 1:
Length:

$$\frac{(5.3972 - 1.905)}{[5.3975]} \times 100 = 64.70\%$$

Width:

$$\frac{(5.715 - 0.1)}{[5.715]} \times 100 = 98.25\%$$

Trial 2:
Length:

$$\frac{(5.715 - 1.905)}{[5.715]} \times 100 = 66.67\%$$

Width:

$$\frac{(5.715 - 0.11)}{[5.715]} \times 100 = 98.08\%$$

Trial 3:
Length:

$$\frac{(7.3025 - 1.905)}{[7.3025]} \times 100 = 73.91\%$$

Width:

$$\frac{(11.1125 - 0.1)}{[11.1125]} \times 100 = 99.1\%$$

Trial 4:
Length:

$$\frac{(5.715 - 1.905)}{[5.715]} \times 100 = 66.67\%$$

Width:

$$\frac{(11.43-0.1)}{[11.43]} \times 100 = 99.13\%$$

Spool Size Determination

Calculations:

$$Diameter = 64\ mm$$

$Barrel = 28\ mm$

$\frac{64\ mm - 28mm}{2} = 18\ mm\ (flange)$

$18 \times 0.85 = 15.3\ mm\ (H\ Value)$

$18 - 15.3 = 2.7\ mm\ (U\ value)$

$$(15.3 + 28) \times (15.3) \times (53) \times (0.262) = 9199.33614\ (Reel\ Factor)$$

$\frac{9199.33614}{2^2} = 2299.83\ mm = 7.54\ ft\ (Length\ of\ Cable)$

$$Diameter = 2.5\ in$$

$Barrel = 0.8126\ in\ =\ 20.6400\ mm$

$\frac{2.5 - 0.8126}{2} = 0.8437\ in = 21.336\ mm\ (flange)$

$21.336 \times 0.85 = 18.1356\ mm\ (H\ Value)$

$21.336 - 18.1356 = 3.2004\ mm\ (U\ value)$

$$(18.1356 + 20.6400) \times (18.1356) \times (101.6) \times (0.262) = 18719.12112\ (Reel\ Factor)$$

$\frac{18719.12112}{2^2} = 4679.78\ mm = 15.33\ ft\ (Length\ of\ Cable)$

$$Diameter = 81.5\ mm$$

$Barrel = 19\ mm$

$\frac{81.5 - 19}{2} = 31.25\ mm\ (flange)$

$31.25 \times 0.85 = 26.5625\ mm\ (H\ Value)$

$21.336 - 25.5625 = 4.6874\ mm\ (U\ value)$

$$(26.5625 + 19) \times (26.5625) \times (84) \times (0.262) = 26635.36824\ (Reel\ Factor)$$

$\frac{26635.36824}{2^2} = 6658.84\ mm = 21.84\ ft\ (Length\ of\ Cable)$

References

What reference and sources were used to gather the background information to understand and complete your project? Multiple sources should have been used, including peer reviewed journal papers, trade journals, textbooks and government websites.

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