AUTOMATED SHEARING DEVICE FOR THE CONTROL OF AGGREGATION IN *TAXUS CHINENSIS* SUSPENSION CULTURES

A Report for Major Qualifying Project Requirement

Submitted to the Faculty of the

Chemical Engineering Department
Worcester Polytechnic Institute
Worcester, MA 01609

April 25th, 2024

Jada Smith

Deah Zajmi

This report represents the work of one or more WPI undergraduate students submitted to the faculty as evidence of completion of a degree requirement. WPI routinely publishes these reports on the web without editorial or peer review.

Abstract

Paclitaxel, a chemotherapeutic drug derived from the *Taxus* species, combats various forms of cancer, including ovarian and lung cancer. Traditional methods of harvesting paclitaxel from Pacific Yew trees are impractical due to slow growth rates and low yield concentrations. Plant cell culture (PCC) offers a viable alternative, which enables the controlled production of paclitaxel. Challenges like cellular aggregation hinder paclitaxel's production. This MQP investigates the efficacy of an automated shearing device designed to decrease cellular aggregation. Manual and automated shearing techniques were compared in which the mean aggregate sizes and viability of *Taxus* suspension cultures were taken. It was found that manual shearing had steeper rates of disaggregation than automated shearing. This was because the automated shearing device was set to a speed of 7, whereas speed 10 would have been a more accurate representation of the speed of manual shearing. The viability of the suspension cultures remained relatively unchanged between both shearing methods. Various speeds on the shearing device were compared, and speed 10 exhibited the greatest decrease in mean aggregate size, therefore establishing the correlation that a higher flow rate corresponds to a larger decrease in mean aggregate size. Continuous shearing with the automated device was also tested. After said continuous shearing, mean aggregate sizes were able to recover their original sizes, and the viability of suspension cultures was left relatively unchanged.

Acknowledgments

For this MQP and all of the immense work that we have put into it, we would like to greatly thank our friends and families who have supported us as we have conducted research after long hours and days in the lab. We would like to thank Caroline Rauber, our graduate student advisor who oversaw our work and made sure we took the most appropriate steps in our research to properly conduct our MQP. Lastly, we would love to thank our advisor, Professor Susan Roberts, for inspiring us as we moved forward with this project, and for guiding us to the end.

Table of Contents

Abstract 1
Acknowledgments 2
Table of Contents 3
Table of Figures 4
Chapter 1: Introduction **5**
1.1. The Importance of Researching Paclitaxel Production 5
1.1.1. Paclitaxel's Success as a Chemotherapeutic Drug 5
1.2. Techniques that Increase Paclitaxel Production in Plant Cell Cultures 6
1.2.1. Plant Cell Culture 6
1.2.2. Elicitation with Methyl Jasmonate 6
1.2.3. Aggregation and Disaggregation of Paclitaxel Cells 7
1.2.4. Automated Shearing Device 8
1.3 Research Aims 8
Chapter 2: Methodology **10**
2.1. General Experiment Design 10
2.1.1. Manual vs Automatic Shearing Experiment 10
2.1.2. Continuous Shearing Experiments 11
2.1.2.2. Continuous Shearing for Three Hours 11
2.2. Maintaining Taxus Suspension Cultures 13
2.3. Shearing Taxus Suspension Cultures Manually 13
2.4. Shearing Taxus Suspension Cultures Automatically 13
2.5. Resazurin Metabolic Activity and Viability Assay 13
2.6. Measuring Mean Aggregate Sizes of Taxus Suspension Cultures 14
Chapter 3: Results and Discussion **15**
3.1. The Viability of Taxus Suspension Cultures Was Unaffected After Being Sheared Manually or Automatically 15
3.2. Manual Shearing Disaggregated Better Than Automated Shearing at a Flow Rate of 2.8 mL/s 16
3.3. A Flow Rate of 8.6 mL/s is an Accurate Representation of the Flow Rate Associated with Manual Shearing. 17
3.4. Aggregate Size Can be Recovered After Continuous Shearing 18
3.5. Higher Flow Rates on the Automated Shearing Device are More Successful at Producing Smaller Aggregate Diameters 19
3.6. The Viability of Taxus Suspension Cultures Was Unaffected After Being Sheared Continuously 19
Chapter 4: Conclusion and Recommendations **21**
4.1. Conclusions in Conjunction with Presented Research Aims 21
4.2. Future Recommendations to Further Test the Automated Shearing Device 21
Chapter 5: References **23**
Chapter 6: Appendices **24**
Appendix A: Calculations for Automated Device Speed That Best Represents Manual Shearing 24

Table of Figures

Figure 1. The chemical structure, formula, molecular weight, average mass, and systemic name of paclitaxel (Kampam, 2015). 5

Figure 2. Diagram displaying the signal transduction cascade that takes place in plants (Brzycki, 2021). 7

Figure 3. A labeled photograph of the automated shearing device. 8

Figure 4. Key for methodology schematics in Figures 5, 6, and 7. 10

Figure 5. A schematic for the experiment testing manual shearing against automated shearing. 11

Figure 6. A schematic for the experiment testing continuous shearing for three hours. 12

Figure 7. A schematic for the experiment testing continuous shearing for one hour three times. 13

Figure 8. Percent Viability of Taxus Suspension Cultures Manually and Automatically Sheared. Four biological replicates were made for each group (unsheared, manually sheared, and automatically sheared). The open data points represent data taken before shearing on a particular day, while closed data points represent data taken after shearing on a particular day. The error bars are standard error of four measurements. 15

Figure 9. Mean Aggregate Diameter of Taxus Suspension Cultures Manually and Automatically Sheared. Four biological replicates were made for each group (unsheared, manually sheared, and automatically sheared). The open data points represent data taken before shearing on a particular day, while closed data points represent data taken after shearing on a particular day. The rate of disaggregation (m) is given under each trendline. The error bars are standard error of four measurements. 16

Figure 10. Mean Aggregate Diameter Before and After Shearing Manually or Shearing Automatically at a High Flow Rate. The automatically sheared samples were sheared at speed 10 (8.6 mL/s) with the shearing device. Three biological replicates were taken. The error bars are standard error of three measurements. 17

Figure 11. Mean Aggregate Diameter of *Taxus* Suspension Cultures Sheared Continuously for Three Hours at a High Flow Rate. The automatically sheared samples were sheared on speed 10 (8.6 mL/s) with the shearing device, for a continuous three hours. Three biological replicates were taken. The error bars are standard error for three measurements. 18

Figure 12. Mean Aggregate Diameter of *Taxus* Suspension Cultures Automatically Sheared at Various Speeds for One Hour Three Times. Three biological replicates were taken. The error bars relate to the standard error from three measurements. 19

Figure 13. Percent Viability of *Taxus* Suspension Cultures Sheared Continuously for Three Hours at a High Flow Rate. The automatically sheared samples were sheared on speed 10 (8.6 mL/s) with the shearing device, for a continuous three hours. Three biological replicates were taken. The error bars relate to the standard error from three measurements. 20

Chapter 1: Introduction

1.1. The Importance of Researching Paclitaxel Production

1.1.1. Paclitaxel's Success as a Chemotherapeutic Drug

Cancer is a devastating disease that impacts people all over the world without bias. Ovarian and lung cancer are especially grueling diseases that affect millions. In 2023 alone, there were 19,710 new cases of ovarian cancer diagnosed and 13,270 deaths in the United States (National Cancer Institute 1). As for lung cancer, in 2023 there were 238,340 new cases diagnosed and 127,070 deaths in the United States (National Cancer Institute 2). There is a treatment that can battle these two, among other types of cancer, and it is called Paclitaxel.

Paclitaxel, more commonly known as Taxol, is a chemotherapeutic drug. The chemical structure of Taxol, which is derived from the Pacific Yew Tree or *Taxus brevifolia*, can be seen in Figure 1. In 1964, crude extracts of *Taxus brevifolia* were obtained by the National Cancer Institute (NCI) in collaboration with the United States Department of Agriculture (USDA), and found to be cytotoxic, or toxic to living cells. By 1992, Taxol had become FDA-approved to treat ovarian cancer. In the following years, it would also come to be approved for breast cancer, lung cancer, and Kaposi's sarcoma. Doctors also recommend it be used off-label to treat gastroesophageal, endometrial, cervical, prostate, and head-and-neck cancers, as well as sarcoma, lymphoma, and leukemia (Weaver, 2014). Paclitaxel treats the aforementioned diseases by targeting microtubules, specifically by inhibiting their depolymerization (Kampam, 2015).

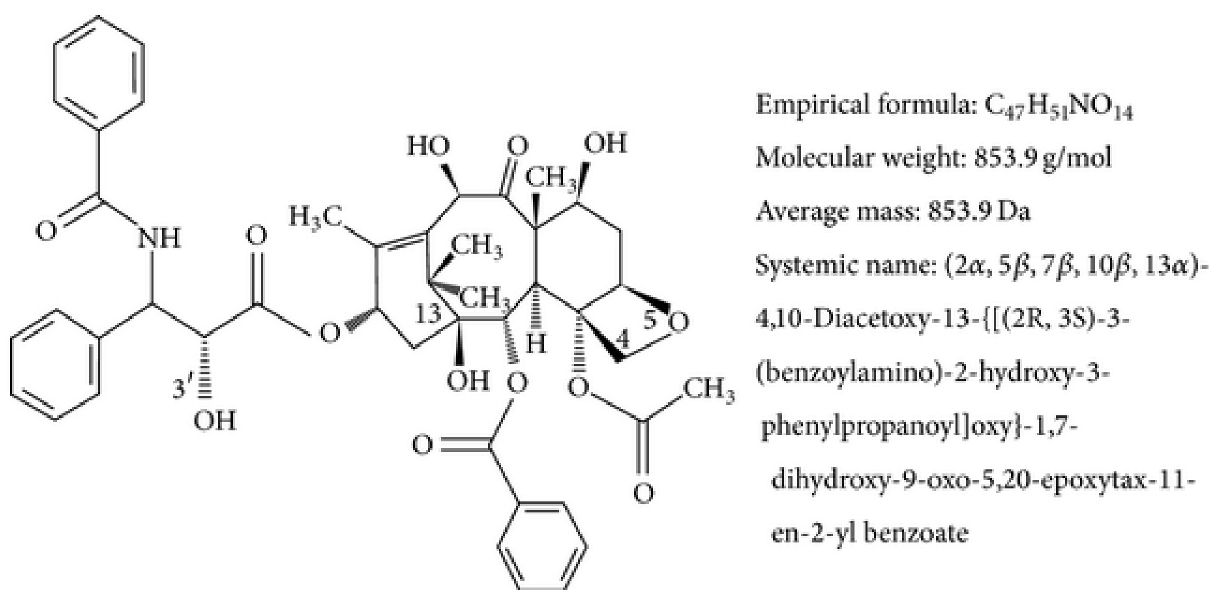

Figure 1. *The chemical structure, formula, molecular weight, average mass, and systemic name of paclitaxel (Kampam, 2015).*

1.2. Techniques that Increase Paclitaxel Production in Plant Cell Cultures

1.2.1. Plant Cell Culture

In nature, paclitaxel is found in very low concentrations within the Pacific Yew Tree, and it grows incredibly slowly. In order to accumulate 2.5 grams of paclitaxel (the amount needed for one treatment), harvesting from eight mature yew trees would be necessary (Howat, 2014). Because of this, harvesting naturally from the trees is not a viable option. Alternative options to natural harvesting have been researched, including total synthesis, semi-synthesis, and heterologous expression, but each of these methods brings about its own set of disadvantages. These disadvantages include the fact that total synthesis is too complex while producing a low yield, semi-synthesis heavily relies on environmental factors, and heterologous expression is limited by epigenetic factors (Howat, 2014).

Plant cell culture (PCC) involves inducting callus cultures with the abundant *Taxus brevifolia* tissue (usually in the needles or branches), treating the culture with hormones to obtain dedifferentiated plant tissue, and then transferring said callus into a liquid medium that supports cell growth in suspension (Tabata, 2004). PCC is seen as the most viable option for obtaining the paclitaxel product because it achieves what the methods stated above cannot (it is not too complex, and it is not dependent on environmental or epigenetic factors). It has additional benefits, such as the fact that it provides uniform quality in its paclitaxel product and it is environmentally friendly because it does not rely on the limited natural resource that is the Pacific Yew Tree. PCC is not without its disadvantages though. PCC could provide low growth rates, variable yields, and high susceptibility to shear stresses (Howat, 2014). Elicitors, such as methyl jasmonate, can help increase paclitaxel yield in these cases though, which is discussed further in the section below.

1.2.2. Elicitation with Methyl Jasmonate

In the context of *Taxus brevifolia*, paclitaxel is a secondary metabolite and methyl jasmonate (MeJa) is an elicitor. Plants undergo stress like attacking insects, herbivores, or extreme weather among other things. Many plants, including *Taxus brevifolia*, have mechanisms called secondary metabolites. Unlike primary metabolites, secondary metabolites are not necessary for vital plant processes like respiration and photosynthesis; instead, secondary metabolites act by stimulating defense responses against the aforementioned stressors the plant may encounter. Elicitors are agents that induce or stimulate a defense response in plant cells by producing secondary metabolites. When a plant is exposed to a specific elicitor, signals are sent throughout the plant to produce a specific secondary metabolite (Narayani, 2017).

Eliciting MeJa in *Taxus* plant cell cultures has shown an increase in the production of paclitaxel. This happens because when MeJa is exposed to taxus cells, a signal transduction cascade initiates in which signaling molecules are sent to bind with receptors in the taxus cells, ultimately sending signals to upregulate the secondary metabolite known as paclitaxel (Brzycki, 2021). Figure 2 displays the transduction mechanism in more detail. Furthermore, MeJa elicitation can be incredibly helpful in increasing paclitaxel yield in a commercial setting, as the yield can otherwise be low when utilizing the method of PCC to artificially harvest paclitaxel.

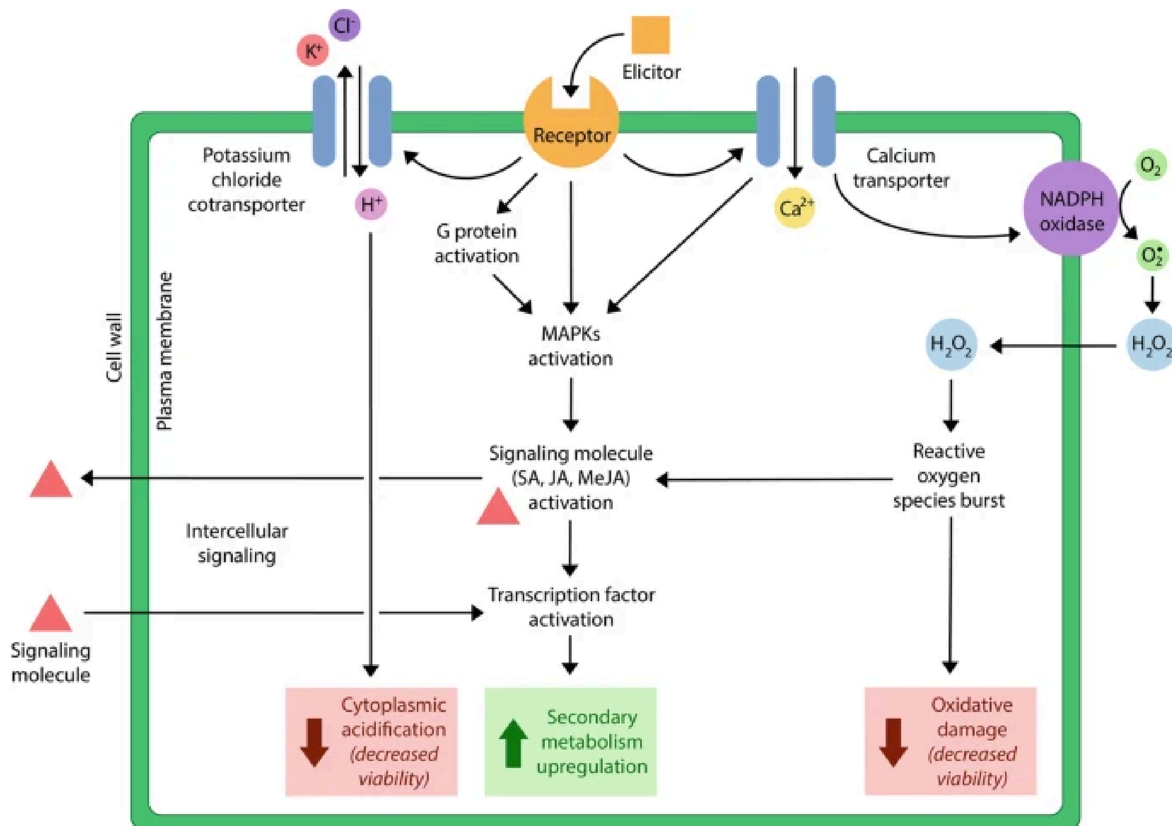

Figure 2. *Diagram displaying the signal transduction cascade that takes place in plants (Brzycki, 2021).*

1.2.3. Aggregation and Disaggregation of Paclitaxel Cells

A downside of plant cell culture is cellular aggregation. This is because even after the plant cells divide, the cell walls encapsulating them stay connected (Kolewe, 2011). Different aggregates, which can range from 100 to 2000 micrometers, all within the same sample can have varying environmental conditions. This leads to differences in each aggregate's exposure to shearing, differences in how cells within the aggregate signal each other, and therefore differences in the amounts of secondary metabolite being produced (Wilson, 2020). This means that cellular aggregation is an incredibly important parameter when it comes to the production of paclitaxel via PCC. Past researchers have taken note of this and ultimately found that *Taxus* suspension cultures with smaller aggregates produced more paclitaxel than cultures with larger aggregates (Kolewe, 2011).

Disaggregation is the reduction of cellular aggregates. Disaggregation can occur via enzymatic or mechanical methods. For instance, enzymes such as pectolyase or cellulase can degrade the cell wall, thus separating cell aggregates. Another method of enzymatic disaggregation could include inhibiting the proteins contributing to aggregation (Wilson, 2020). Alternatively, methods of mechanical aggregation, such as shearing cultures with a pipette, can be pursued. Mean aggregate sizes of *Taxus* suspension cultures have been decreased substantially using this method of manually shearing with a pipette.

1.2.4. Automated Shearing Device

An automatic shearing device was created in Doctor Susan Robert's lab at Worcester Polytechnic Institute in 2023 and is presented in Figure 3 (Rauber, 2023). As opposed to pipetting the cultures by hand, the device pipetted the cultures on its own under a variety of different parameters. These include the test type, pump speed, test duration, cycles per set, and the time between sets.

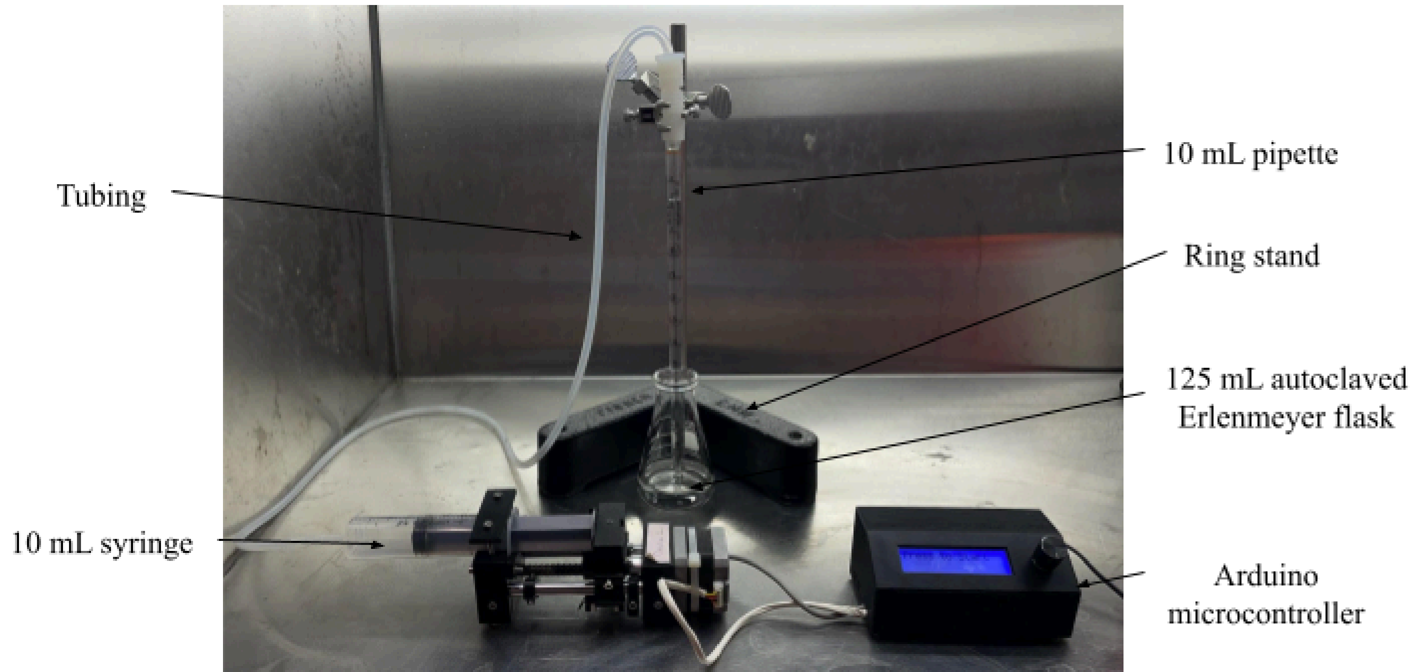

Figure 3. *A labeled photograph of the automated shearing device.*

There are two kinds of test types, continuous shearing or intermittent shearing. The continuous setting allows for shearing to take place, non-stop, for a certain amount of time, anywhere from 5 minutes to 6 hours. The intermittent setting allows for a certain number of cycles of shearing to take place and then resting before moving on to the next cycle set. There are 10 different pump speeds, ranging from speed 1 to speed 10. The flow rates of each speed setting are as follows: 1.0 mL/s for speed 1, 1.1 mL/s for speed 2, 1.2 mL/s for speed 3, 1.4 mL/s for speed 4, 1.7 mL/s for speed 5, 2.2 mL/s for speed 6, 2.8 mL/s for speed 7, 4.2 mL/s for speed 8, 5.2 mL/s for speed 9, and 8.6 mL/s for speed 10. The test duration, meaning the amount of time spent shearing, can reach a maximum of 360 minutes, or 6 hours. Cycles per set and time between sets go hand in hand, where cycles per set dictate how many times the pipette will shear up and down the suspension, while the time between sets dictates how long the break between said cycles will be. All of these settings can be changed on the Arduino microcontroller. As there is a method now for a more convenient and useful disaggregation method, it was imperative to further test its limit and specific properties while working with *Taxus* cells, which leads to the following objective and aims for conducting this research.

1.3 Research Aims

The automated shearing device has been previously tested under various shearing parameters, including shearing at shearing at speed 10 during the lag phase, exponential growth phase, and the stationary phase, shearing at speed 10 for 25, 50, 75, and 100 cycles, and shearing at speed 10 for 1

hour, 2 hours, and 3 hours. The primary objective of this research project is to further test and examine the uses and impact of the automated shearing device on *Taxus* plant cells under conditions that have not already been tested. To complete this objective, three primary aims were established. Those aims are listed as follows:

Aim 1: Compare mean aggregate sizes and viability of *Taxus* suspension cultures that are manually sheared with suspension cultures that are automatically sheared.

To compare both methods of shearing, an experiment was devised in which suspension cultures were sheared throughout the course of a week, either manually or automatically. There was also a control group that was not sheared. The mean aggregate sizes of the suspension cultures were taken before and after they were sheared, as well as viability assays. If shown to be successful, the automatic shearing device could prove useful in an industrial setting where large amounts of paclitaxel need to be sheared with minimal manpower. For viability, resazurin assays were taken in order to find how viable cultures are when handled by the two shearing methods. Shearing automatically is expected to yield smaller mean aggregate sizes for *Taxus* suspension cultures.

Aim 2: Determine if there is a correlation between the flow rate of the automated shearing device and the decrease in size of the *Taxus* aggregates.

To make such a correlation, *Taxus* suspension cultures will be sheared using the automated shearing device either continuously for 3 hours at various speeds or intermittently for one hour at a time at various speeds. Each speed on the device shears at a unique flow rate. If a correlation between the flow rate of the automated shearing device and the decrease in size of *Taxus* aggregates, then the optimal parameters for shearing *Taxus* suspension cultures can be determined, which can once again be useful in an industry setting where large amounts of paclitaxel need to be yielded. It is expected that higher flow rates will correlate with larger decreases in *Taxus* aggregate sizes.

Aim 3: Observe the viability of *Taxus* suspension culture cells after they are continuously sheared.

This will be examined by taking resazurin assays of *Taxus* suspension cultures after they are automatically sheared at varying speeds. This assay will allow for observation of the metabolism of the *Taxus* cells, and their recovery after continuous shearing. Understanding how *Taxus* cells respond to continuous shearing at various speeds is essential for optimizing shearing parameters for small aggregate sizes while also minimizing damage to cells' metabolism. It is anticipated that shearing continuously at higher speeds will compromise the viability of *Taxus* suspension cultures.

Chapter 2: Methodology

2.1. General Experiment Design

2.1.1. Manual vs Automatic Shearing Experiment

A total of twelve *Taxus* suspension cultures were prepared using the paclitaxel-producing cell line 48.82A.3S, which derives from a variety of the yew tree, *Taxus chinensis*. The suspension cultures were prepared in 125 mL flasks and contained 50 mL of cells and media. Four flasks were to be sheared manually, four were to be sheared automatically at speed 7 with the shearing device, and four were not to be sheared at all. Speed 7 on the shearing device was chosen because it was thought to best represent the speed of manual shearing, as seen in prior research done on the automated shearing device. The day the cultures were prepared was considered day 0 of the suspension culture cycle.

On the fourth day of the suspension culture cycle, a resazurin assay was performed to assess the viability of each sample, and the mean aggregate sizes were taken. One mL was removed from the suspension cultures for the resazurin cell viability, and two mL were removed from the suspension cultures for the measurement of mean aggregate sizes. The flasks were then sheared 50 times with a 10 mL pipette, either manually, automatically at speed 7, or not at all according to their designations. After the shearing (or lack of shearing) took place, the resazurin assay and the measurement of mean aggregate sizes were taken once more.

On day 6 of the suspension culture cycle, resazurin assays were performed on all twelve flasks. The flasks were then sheared (or not sheared) according to their designation, and another resazurin assay was taken. The measurement of mean aggregate sizes was taken as well. This process was repeated on days 8 and 10. A visualization of this experiment can be seen in Figure 5.

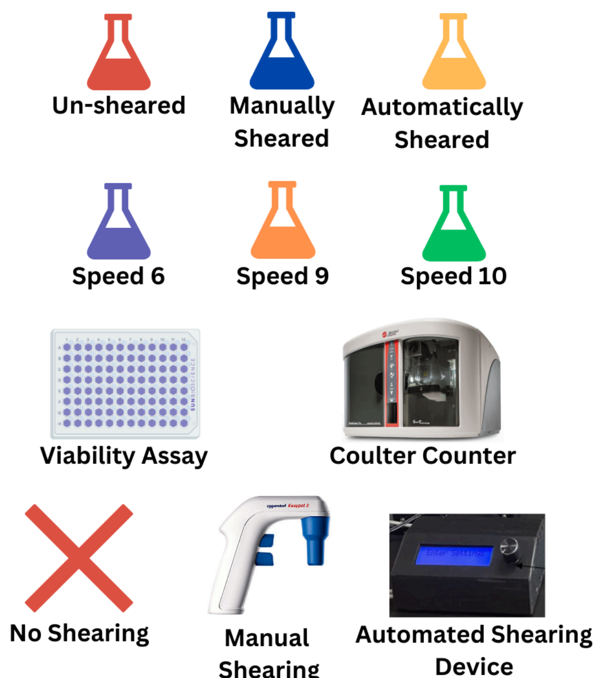

Figure 4. *Key for methodology schematics in Figures 5, 6, and 7.*

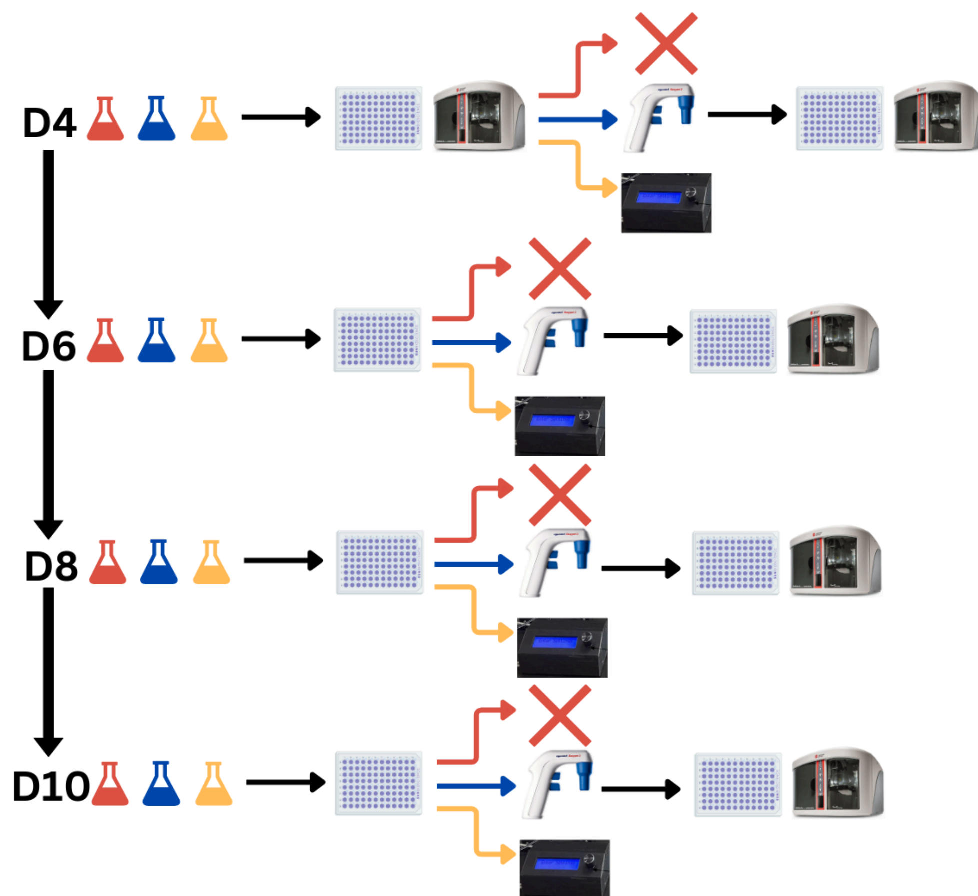

Figure 5. *A schematic for the experiment testing manual shearing against automated shearing.*

2.1.2. Continuous Shearing Experiments

2.1.2.2. Continuous Shearing for Three Hours

A total of nine *Taxus* suspension cultures were prepared using the paclitaxel-producing cell line 48.82A.3S. The suspension cultures were prepared in 125 mL flasks and contained 50 mL of cells and media. Three suspension cultures were designated to be sheared at speed 6 (2.2 mL/s), three were designated to be sheared at speed 9 (5.2 mL/s), and three were designated to be sheared at speed 10 (8.6 mL/s). The day the cultures were prepared was considered day 0 of the suspension culture cycle.

On day 4 of the suspension culture cycle, resazurin assays and measurements of mean aggregate sizes were taken. One mL was removed from the suspension cultures for the resazurin assay, and two mL were removed from the suspension cultures for the measurement of mean aggregate sizes. Then each sample was sheared continuously for 3 hours at its designated speed. This had to be done in shifts as there were only three shearing devices. After the 3 hours of continuous shearing, resazurin assays and mean aggregate sizes were taken once again. On days 6, 8, 10, and 14, resazurin assays were taken from each sample. A visualization of this experiment can be seen in Figure 6.

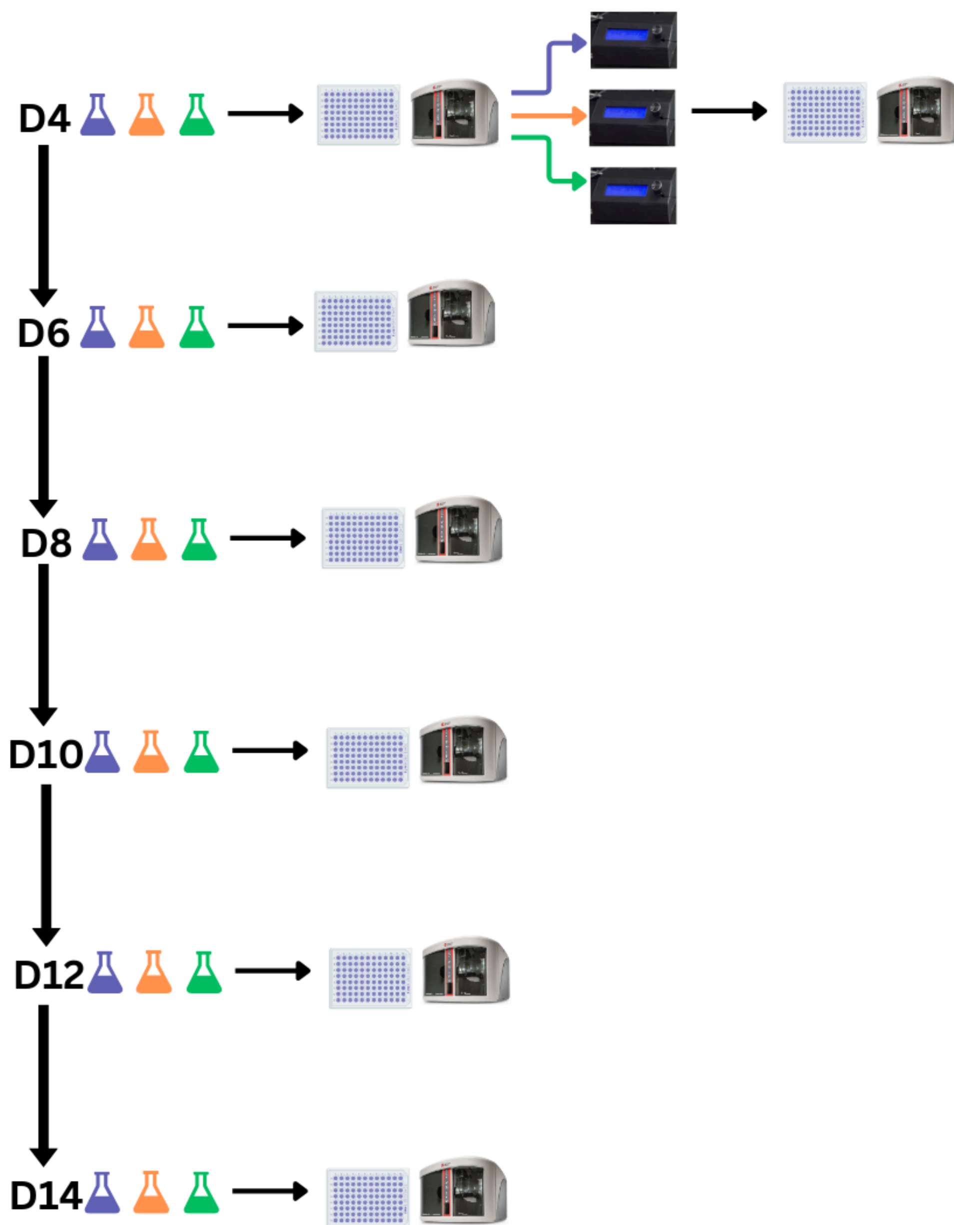

Figure 6. *A schematic for the experiment testing continuous shearing for three hours.*

2.1.2.1. Continuous Shearing Three Times for One Hour Each

A total of nine *Taxus* suspension cultures were prepared using the paclitaxel-producing cell line 48.82A.3S. The suspension cultures were prepared in 50 mL flasks and contained 15 mL of cells and media. Three suspension cultures were designated to be sheared at speed 6 (2.2 mL/s), three were designated to be sheared at speed 9 (5.2 mL/s), and three were designated to be sheared at speed 10 (8.6 mL/s). The day they were prepared was considered day 0.

On day 4 of the suspension culture cycle, resazurin assays and measurements of mean aggregate sizes were taken. One mL was removed from the suspension cultures for the resazurin assay, and two mL were removed from the suspension cultures for the measurement of mean aggregate sizes. Three suspension cultures were then sheared using the automatic shearing device at speed 8 (4.2 mL/s) for one hour, three were sheared at speed 9 (5.2 mL/s) for one hour, and three were sheared at speed 10 (8.6 mL/s) for one hour. Resazurin assays and measurements of mean aggregate sizes were taken after

shearing. This process was then repeated two more times so that each group of flasks was sheared for a total of three hours, with mean aggregate diameters being taken after each round of shearing. A visualization of this experiment can be seen in Figure 7.

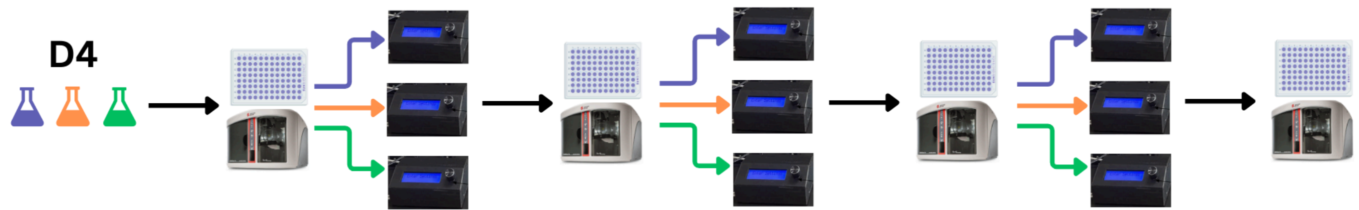

Figure 7. *A schematic for the experiment testing continuous shearing for one hour three times.*

2.2. Maintaining *Taxus* Suspension Cultures

To maintain *Taxus* suspension cultures, they were transferred every 14 days. To do this, a sterilized 125 mL Erlenmeyer flask was filled with 40 mL of pre-made liquid media and antioxidants using a 25 mL serological pipette (Kolewe, 2011). Then, 10 mL of suspension culture was added to the flask using a 10 mL serological pipette with a cut tip to allow for large aggregates to be transferred. The Erlenmeyer flask was then covered with a foam cap. All of this was done under a laminar flow hood. The flasks that had been transferred were then placed in an incubator set to 23˚C and 125 rpm in darkness.

2.3. Shearing *Taxus* Suspension Cultures Manually

Suspension cultures were sheared manually using a 10 mL serological pipette and an Eppendorf pipette controller under a sterile laminar flow hood. The serological pipette was placed inside the Erlenmeyer flask containing the *Taxus* suspension culture and held upright, while someone sheared via pushing the up and down buttons on the Eppendorf pipette controller.

2.4. Shearing *Taxus* Suspension Cultures Automatically

Suspension cultures were sheared automatically using the automated shearing device, a 10 mL serological pipette, and a ring stand under a sterile laminar flow hood. The ringstand held up the 10 mL pipette and the tubing it was connected to upright so that the tip of the pipette was inside the *Taxus* suspension culture. The settings on the automated shearing device's controller were then set (these settings included whether the test was continuous or intermittent, the pump speed, the test duration, the cycles per set, and the time between sets), and the automated shearing device began shearing.

2.5. Resazurin Metabolic Activity and Viability Assay

One mL of *Taxus* suspension culture was collected under a laminar flow hood and transferred into a snap cap tube for each sample on hand. Each tube was labeled with a number. The snap cap tubes were then taken to a lab bench, and the supernatant was removed from each of them. Next, 900 µL of PBS and 100 µL of resazurin were pipetted into each tube containing cells, as well as one extra

empty tube, which would be a blank. The tubes were left to incubate for an hour under aluminum foil to prevent light exposure. After one hour, each of the tubes was placed in a microcentrifuge for 1 minute at 2100 rpm. They were then pipetted into a 96-well plate in triplicates and read using a Victor Nivo PerkinElmer microplate reader. The plate reader was set to read at 570 nm.

The resazurin assay works by assessing the absorption wavelength of cells, starting at 570 nm (Lavogina, 2022). This means that by observing the color absorbance shift within a plant cell that is in its exponential growth phase, the resazurin assay can penetrate and illuminate the corresponding wavelength from within the cell membrane (Rampersted, 2012). By creating this illumination, the microplate reader can quantitatively measure the oxidized to reduced state of the sample and indicate the viability of the sample.

2.6. Measuring Mean Aggregate Sizes of *Taxus* Suspension Cultures

The Multisizer 4e Beckman Coulter Counter was used to measure the mean size of the cells. Upon filling and flushing the inside of the machine with 400 mL of Isoton II Diluent, a blank run of the 400 mL of Diluent was taken for 30 seconds. Once this run was complete, 2 mL of the *Taxus* suspension culture was pipetted into about a half-filled flask of Isoton II Diluent. More Isoton II Diluent was then added until the 400 mL line was reached to avoid overflow. The flask containing the *Taxus* suspension culture sample and Isoton II Diluent was placed inside the Beckman Coulter Counter. The system ran for 60 seconds at the highest stirrer speed and the Multisizer 4e software was set to calculate the mean diameter with the blank run loaded in the graph.

Chapter 3: Results and Discussion

3.1. The Viability of *Taxus* Suspension Cultures Was Unaffected After Being Sheared Manually or Automatically

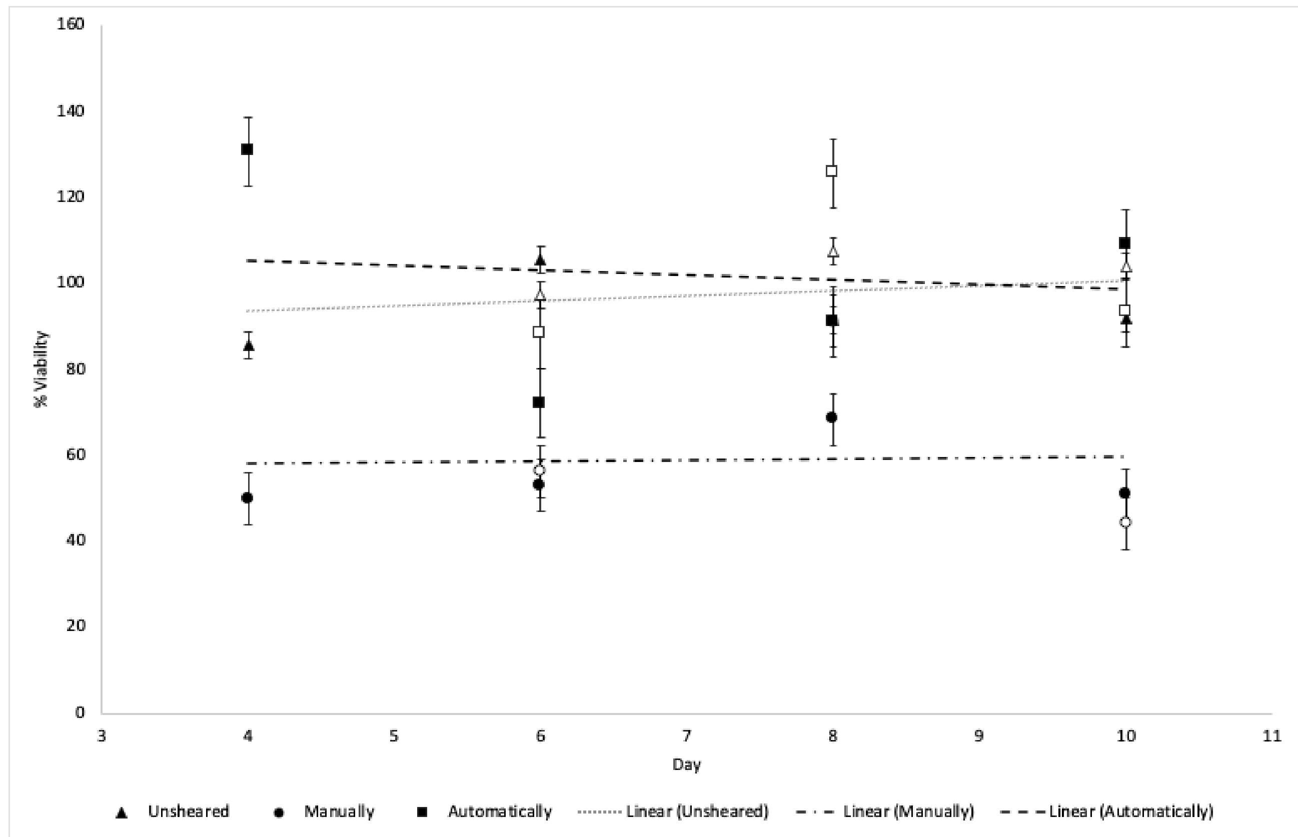

Figure 8. *Percent Viability of* Taxus *Suspension Cultures Manually and Automatically Sheared. Four biological replicates were made for each group (unsheared, manually sheared, and automatically sheared). The open data points represent data taken before shearing on a particular day, while closed data points represent data taken after shearing on a particular day. The error bars are standard error of four measurements.*

When referring to Figure 8 above, there were different levels of shearing being conducted on the flasks, with the only noticeable change in the viability coming from flasks that were unsheared. Since the rates of disaggregation for each shearing technique are mostly horizontal, the conclusion that can be reached is that the viabilities of the *Taxus* suspension cultures were mostly unaffected and that there was a relatively low metabolic shift in each of the shearing methods. This means that in a given situation, both methods of shearing, manually or automatically, will still generate viable cultures, with manual shearing having cultures with lower viability.

3.2. Manual Shearing Disaggregated Better Than Automated Shearing at a Flow Rate of 2.8 mL/s

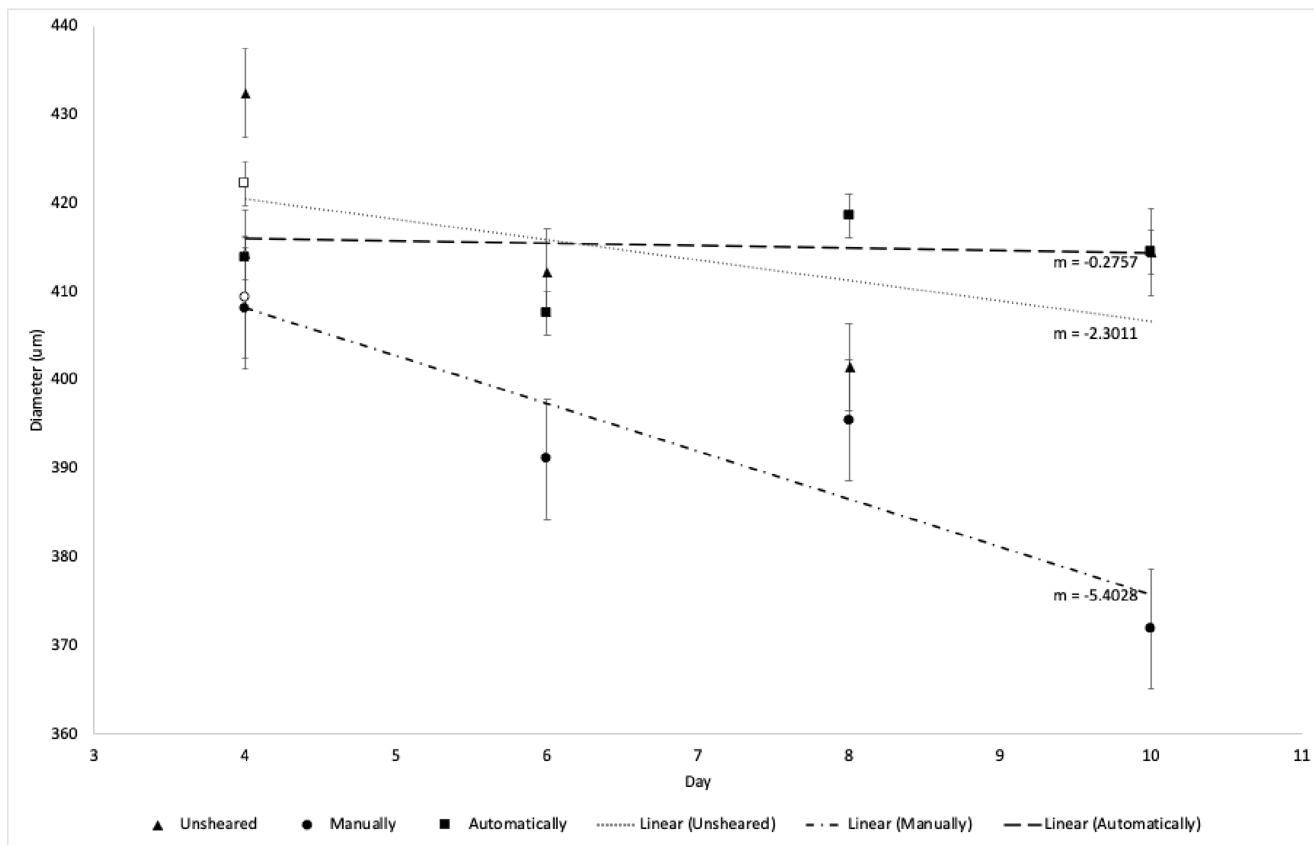

Figure 9. *Mean Aggregate Diameter of Taxus Suspension Cultures Manually and Automatically Sheared. Four biological replicates were made for each group (unsheared, manually sheared, and automatically sheared). The open data points represent data taken before shearing on a particular day, while closed data points represent data taken after shearing on a particular day. The rate of disaggregation (m) is given under each trendline. The error bars are standard errors of four measurements.*

When comparing manual shearing to automated shearing at speed 7, manual shearing displayed a greater decrease in aggregate diameter, which was contrary to the expectations set in the research aims. As seen in Figure 9, the rate of disaggregation for manual shearing was -5.4028 µm/day, while the rate of disaggregation for automated shearing was -0.2757 µm/day. This outcome suggests that speed 7, which has a flow rate of 2.8 mL/s, may not accurately represent the speed of manual shearing as was initially assumed. To keep the method of shearing (manual, automated, or none) as the independent variable, all other variables need to be controlled, including the flow rate of shearing. A t-test determined that the decrease in diameter for suspension cultures sheared manually was statistically different from suspension cultures sheared automatically

3.3. A Flow Rate of 8.6 mL/s is an Accurate Representation of the Flow Rate Associated with Manual Shearing.

Speed 7 was originally thought to be an accurate representation of the average speed of manual shearing, but after the aforementioned experiment was conducted, that was found not to be the case. When the automated shearing device is in use, there is a slight pause after it draws up the suspension culture and before it releases it back into the flask. This pause does not occur during manual shearing; the shearing is instead uninterrupted, ultimately meaning that manual shearing is going at a faster rate than automated shearing at speed 7.

An additional experiment was conducted to find a flow rate on the automated shearing device that better represents the actual flow rate that occurs with manual shearing. First, a suspension culture was sheared with the device at speed 7 for a total of 25 shears. The duration of this shearing was timed and found to be 197 seconds. Then, a suspension culture was sheared manually for that same amount of time. Manual shearing for 197 seconds equated to 65 shears. Calculations, which can be found in Appendix A, found that it would take 76 seconds to shear manually 25 times. As this is a much smaller time frame, the two highest speeds on the automated shearing device were tested, speeds 9 (5.2mL/s) and 10 (8.6 mL/s), were tested. Shearing 25 times at speed 9 took 120 seconds while shearing 25 times at speed 10 took 80 seconds. The time it took to shear 25 times manually (76 seconds) and the time it took to shear automatically (80 seconds) are incredibly close, and since there is no speed higher than speed 10 on the automated shearing device, it can be said that speed 10, or a flow rate of 8.6 mL/s, is an accurate representation of the speed of manual shearing. The full calculations for this experiment can be found in Appendix A.

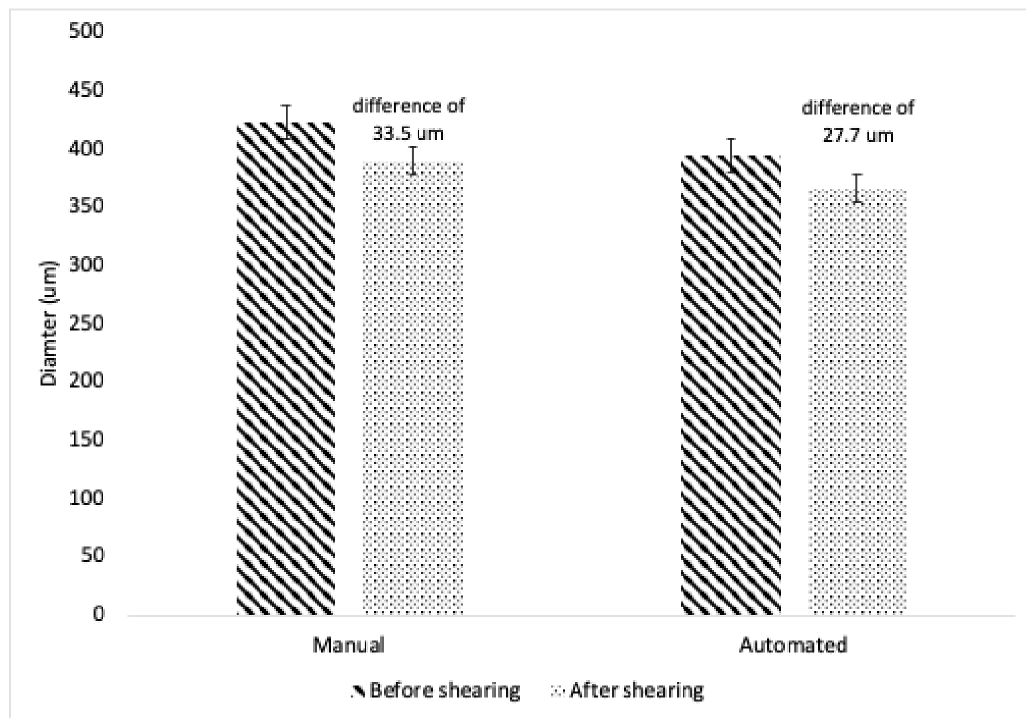

Figure 10. *Mean Aggregate Diameter Before and After Shearing Manually or Shearing Automatically at a High Flow Rate. The automatically sheared samples were sheared at speed 10 (8.6 mL/s) with the shearing device. Three biological replicates were taken. The error bars are standard error of three measurements.*

This small experiment explains why manual shearing led to larger decreases in aggregate diameter in Figure 9. It was because the speed 7 was far too slow when compared to manual shearing whereas speed 10 is almost just as fast as manual shearing. To put this new finding to the test, suspension culture samples were sheared either manually or automatically at speed 10 for 15 minutes each, with each group (manual or automated) having three biological replicates. The aggregate diameters of the samples were taken before and after shearing, the result of which can be seen in Figure 10. Shearing manual led to a difference of diameter of 33.5 µm while shearing automatically at speed 10 led to a difference of diameter of 27.7 µm. The fact that these two values are incredibly close further proves that a flow rate of 8.6 mL/s is an accurate representation of manual shearing. On days 6, 8, 10, and 14, resazurin assays were taken from each sample.

3.4. Aggregate Size Can be Recovered After Continuous Shearing

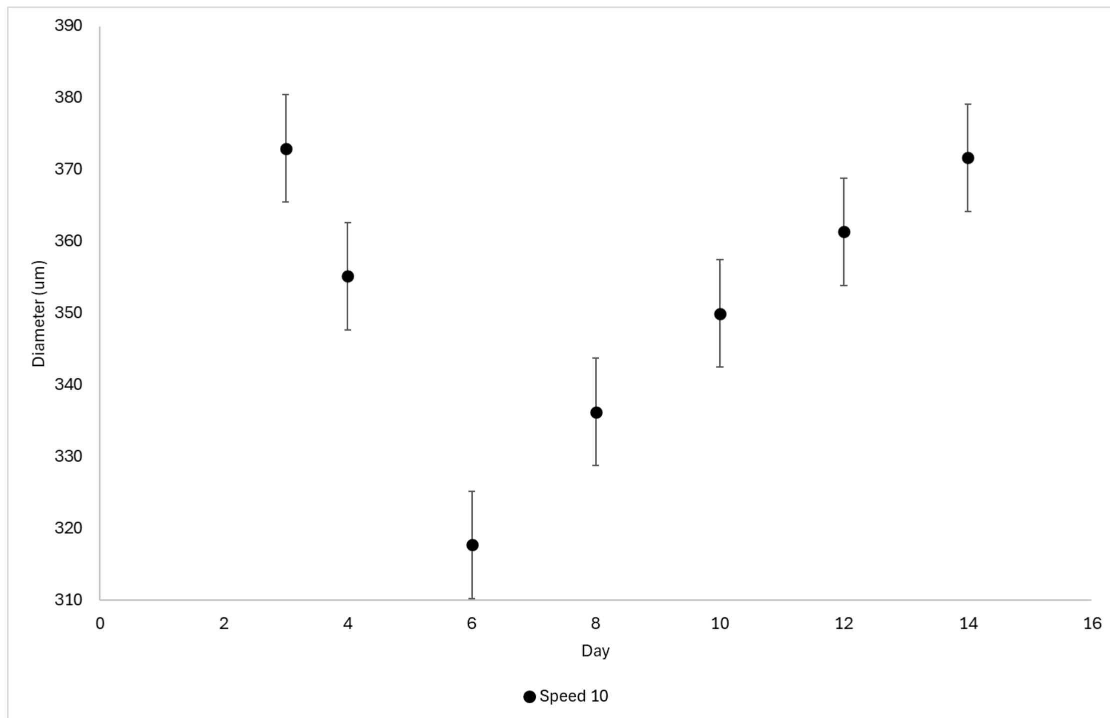

Figure 11. *Mean Aggregate Diameter of* Taxus *Suspension Cultures Sheared Continuously for Three Hours at a High Flow Rate. The automatically sheared samples were sheared on speed 10 (8.6 mL/s) with the shearing device, for a continuous three hours. Three biological replicates were taken. The error bars are standard error for three measurements.*

Figure 11 illustrates a significant reduction in *Taxus* aggregate size between days 4 and 6 after continuous shearing for 3 hours on day 4 of the suspension culture cycle, followed by a substantial increase in aggregate size for the remaining 8 days. This pattern suggests that speed 10 on the automated shearing device effectively reduces *Taxus* suspension culture size, but the subsequent increase in aggregate size implies that if cultures are not subjected to further shearing, then the original aggregate diameter size can be entirely recovered over time. The cultures were also in the middle of an exponential growth phase, which allowed for their diameter and biomass to increase as well. The mean

aggregate sizes while shearing for 3 hours continuously were only taken from the suspension cultures sheared at speed 10 because the cultures that were sheared at speeds lower than 10 did not have a noticeable aggregate decrease.

3.5. Higher Flow Rates on the Automated Shearing Device are More Successful at Producing Smaller Aggregate Diameters

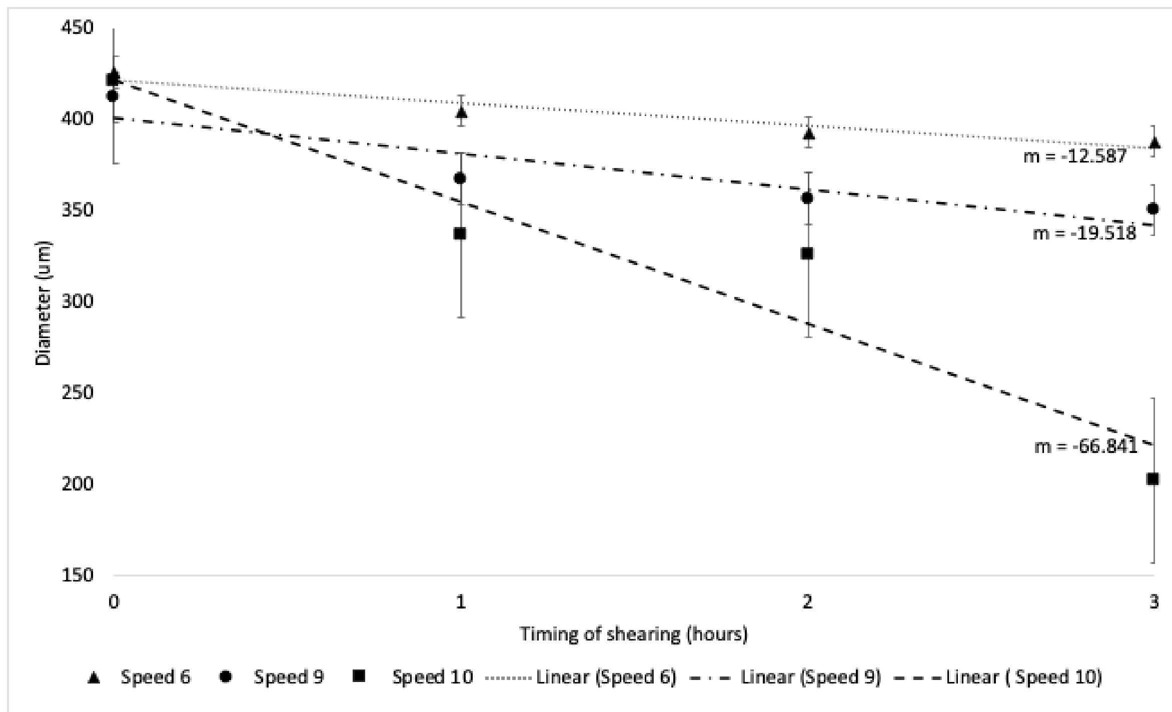

Figure 12. *Mean Aggregate Diameter of Taxus Suspension Cultures Automatically Sheared at Various Speeds for One Hour Three Times. Three biological replicates were taken. The error bars relate to the standard error from three measurements.*

In the experiment regarding the comparison of shearing speeds, the *Taxus* cultures were sheared at different speeds for one hour at a time over the course of three hours. Higher shearing flow rates correspond with greater reductions in particle diameter, as depicted in Figure 12. Speed 10 had a rate of disaggregation of -66.841 µm/hour, speed 9 had a rate of disaggregation of -19.518 µm/hour, and speed 6 had a rate of disaggregation of -12.587 µm/hour. According to these rates, speed 10, which has a flow rate of 8.6 mL/s, exhibited the largest drop in aggregate size over a total of three hours of shearing, while speed 6, which had a flow rate of 2.2 mL/s, exhibited the smallest drop in aggregate size over a total of three hours of shearing. This is believed to have occurred because higher flow rates put more friction on the *Taxus* suspension cultures, therefore breaking aggregates down more efficiently than lower flow rates.

3.6. The Viability of *Taxus* Suspension Cultures Was Unaffected After Being Sheared Continuously

Figure 13 showed a relatively good recovery after day 10, but shearing only took place on day 4 and was left alone for 10 days afterward. Overall, for all of the speeds as it relates to the viability,

none of the viability dropped to below 50%, meaning that all of the continuous cultures were still viable and useful when changing the timing of the shearing. Although none of them dropped below 50%, speed 10, however, did have the lowest viability percentage, which could be represented by the fact that speed 10 also had the highest aggregation out of the 3 speeds with the greatest boost in recovery. Subsequently, speed 10 had the highest viability towards the end of the experiment due to a higher metabolic shift and a possible error in the resazurin assay analysis in the final two days of the experiment.

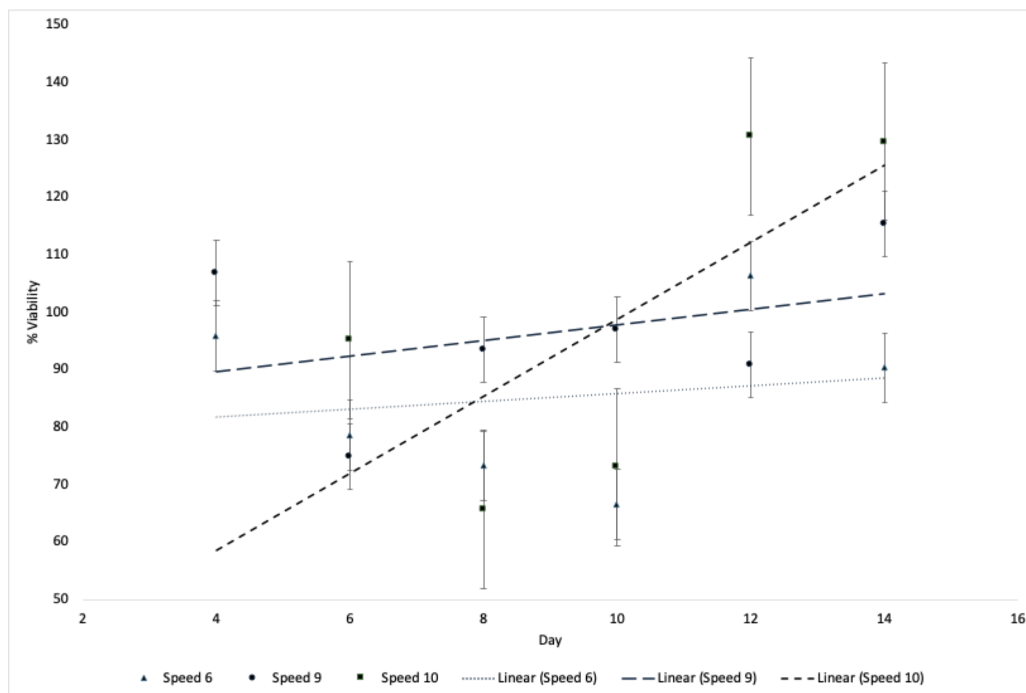

Figure 13. *Percent Viability of* Taxus *Suspension Cultures Sheared Continuously for Three Hours at a High Flow Rate. The automatically sheared samples were sheared on speed 10 (8.6 mL/s) with the shearing device, for a continuous three hours. Three biological replicates were taken. The error bars relate to the standard error from three measurements.*

Chapter 4: Conclusion and Recommendations

4.1. Conclusions in Conjunction with Presented Research Aims

The goal of this MQP was to further test the automated shearing device created in Professor Susan Roberts' lab at Worcester Polytechnic Institute on *Taxus chinensis* suspension cultures. Three aims were established to achieve this goal. The first aim was to compare mean aggregate sizes and viability of *Taxus* suspension cultures that are manually sheared with suspension cultures that are automatically sheared. The first aim was completed successfully but with unexpected results from the mean aggregate size data. When comparing automated and manual shearing, at speed 7 on the automated shearing device, which has a flow rate of 2.8 mL/s, manual shearing worked more effectively than automated shearing. This indicated that speed 7 was not representative of the speed of manual shearing. Our research found that speed 10 was more accurate instead. As for the viability of *Taxus* suspension cultures, it was found that samples sustained percent viabilities that were above 50% after they were sheared either manually or automatically at speed 7. Ultimately, the *Taxus* suspension cultures remained viable.

The second aim of this MQP was to determine if there was a correlation between the flow rate of the automated shearing device and the decrease in size of the *Taxus* aggregates. The results were as expected; higher flow rates on the automated shearing device correlated to larger decreases in *Taxus* aggregates. More specifically, the highest speed provided on the automated shearing device, which has a flow rate of 8.6 mL/s, exhibited the steepest rate of disaggregation. This allows for more friction and more space for disaggregated *Taxus* cells to breathe in under these conditions. Should industrial companies have or want a device of their own, they would most favor the higher flow rate as you get more Taxus cells for more treatment in a quicker amount of time.

The third aim of the MQP was to observe the viability of *Taxus* suspension culture cells after they were continuously sheared. The viability assays revealed that *Taxus* suspension cultures maintained viability levels above 50% across all shearing methods and parameters tested. Manual shearing exhibited lower viability percentages than automated shearing at speed 7, but both methods were viable for cell disaggregation. Continuous shearing at speed 10 left *Taxus* suspension cultures viable and showed subsequent recovery over a ten-day period of no shearing.

4.2. Future Recommendations to Further Test the Automated Shearing Device

If research on the automated shearing device were to continue in the future, there are some recommendations to further test the automated device's capabilities on *Taxus* cell cultures. The first of these recommendations would be to compare manual shearing against automated shearing at speed 10. As was previously mentioned, speed 10 is an accurate representation of the speed of manual shearing; therefore, future experiments comparing the rate of disaggregation of the viability of manual and automated shearing should utilize this speed, as opposed to speed 7.

The next recommendation would be to test the automated shearing device on *Taxus* suspension cultures of different cell densities. Variations in cell density may have led to discrepancies in mean aggregate size results, impacting the accuracy of the data collected. Conducting experiments with various shearing parameters on *Taxus* suspension cultures would allow for more precise comparisons.

The final recommendation would be to ascertain the limit of manual shearing. In other words, the point at which automated shearing begins to disaggregate better than manual shearing. It is already known that manual shearing has steeper rates of disaggregation than automated shearing at low flow rates, and it is assumed that automated shearing at higher flow rates would work better than manual shearing. By subjecting *Taxus* suspension cultures to different speeds on the automated shearing device, the speed at which automated shearing becomes more efficient than manual shearing can be identified.

In conclusion, these recommendations for future experiments hold the potential to advance the understanding of automated shearing techniques and optimize the automated shearing device for improved bioprocessing efficiency and productivity.

Chapter 5: References

Brzycki, C.M., Young, E.M., Roberts, S.C. (2021) “Secondary Metabolite Production in Plant Cell Culture: A New Epigenetic Frontier” https://doi.org/10.1007/978-3-030-58271-5_1

Howat S., et al., (2014) “Paclitaxel: biosynthesis, production, and future prospects” https://doi.org/10.1016/j.nbt.2014.02.010

Kampam M., et al., (2015) “Paclitaxel and its evolving role in the management of ovarian cancer” https://doi.org/10.1155/2015/413076

Kolewe M., et al., (2011) "Analysis of aggregate size as a process variable affecting paclitaxel accumulation in *Taxus* suspension cultures" https://doi.org/10.1002/btpr.655

Lavogina D., et al., (2022). Revisiting the Resazurin-Based Sensing of Cellular Viability: Widening the Application Horizon. Biosensors (Basel). https://www.ncbi.nlm.nih.gokv/pmc/articles/PMC9032648/

Narayani M. & Srivastava S. (2017) “Elicitation: a stimulation of stress in in vitro plant cell/tissue cultures for enhancement of secondary metabolite production” https://doi.org/10.1007/s11101-017-9534-0

National Cancer Institute. Cancer of the Lung and Bronchus - Cancer Stat Facts. National Cancer Institute. https://seer.cancer.gov/statfacts/html/lungb.html.

National Cancer Institute. Cancer of the Ovary - Cancer Stat Facts. SEER. https://seer.cancer.gov/statfacts/html/ovary.html.

Rampersad S. N. (2012). Multiple applications of Alamar Blue as an indicator of metabolic function and cellular health in cell viability bioassays. Sensors (Basel). 12(9):12347-60. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3478843/

Rauber C. (2023) Master’s thesis: Effect of Continuous Shear Stress and Enzymatic Disaggregation on Cell Health and Secondary Metabolite Production in *Taxus* Plant Cell Culture

Tabata H. (2004) “Paclitaxel production by plant-cell-culture technology” .https://doi.org/10.1007/b13538

Weaver, B. A. (2014) “How Taxol/paclitaxel kills cancer cells” Molecular Biology of the Cell, 25 (18), 2677–2681. https://doi.org/10.1091/mbc.e14-04-0916

Wilson S., et al. (2020) "A population balance model to modulate shear for the control of aggregation in Taxus suspension cultures" https://doi.org/10.1002/btpr.2932

Chapter 6: Appendices

Appendix A: Calculations for Automated Device Speed That Best Represents Manual Shearing

- Shear with device at speed 7, 25 times and time it
  - t1=197s (3min 17s)
  - x1=25 (times shearing)
- Shear manually for the same amount of times and count how many times sheared
  - t1=197s (3min 17s)
  - x2=65 (times shearing)
- Cross multiplication
  - 197*25=t2*65
  - t2=76s (1min 16s)
- Shear with device at speed 9, 25 times, and time it
  - t3=120s (2 min)
- Shear with device at speed 10, 25 times and time it
  - t4=80s (1min 20s)
- t4 is closest to t2, meaning speed 10 (8.6 mL/s) is the speed on the device that best replicates the rate of manual shearing