Examining Enhanced *Komagateaibacter hansenii* Production Via Unconventional Food Waste Sources

Grant Proposal

Anika Karre

Massachusetts Academy of Math and Science

Worcester, MA

Abstract

Bacterial cellulose is becoming increasingly popular as researchers discover its wide variety of applications. The various structural properties allow it to be a versatile and functionable material, making it useful for a plethora of worldly applications (Lahiri et al., 2021). Bacterial cellulose is considered a biopolymer that can be manipulated and used in many ways. This research aims to dive deeper into the production of bacterial cellulose from the Genus species, *Acetobacter xylinum*, focusing on altering the growth medium of the bacteria and observing for changes caused by these shifts in the medium to help identify a cost-efficient way to grow this commonly used material. This project hones in on alternate food waste sources as a replacement for typical culture mediums to determine whether food waste sources are a viable option for growing successful yields of bacterial cellulose. As a result of previous research emphasizing sterilized juices and their benefits in growing bacterial cellulose, a specific juice was first utilized, and two samples were formed. The control sample with the typical environment was set up, as well as the sample with the specified food source. The bacteria can then grow, and the overall yield can be measured and photographed to collect data to hopefully support the conclusion that specific foods will have a positive effect on the growth and yield of the bacterial cellulose.

Keywords: Bacterial Cellulose, food-waste alternative, production medium, carbon source, production yield

Examining Enhanced *Acetobacter xylinum* Production Via Unconventional Food Waste Sources

Over 400 billion dollars in food is wasted annually, equating to around 100 billion pounds of food in the United States alone (*Food Waste in America | Feeding America*, n.d.). Food waste has been, and continues to be, a major economic and environmental issue. However, even as such a prevalent issue in today's world, the impact of food waste continues to be overlooked, as society lets this food waste accumulate without finding better uses for it. One way to begin tackling this issue is by looking for alternate ways to use food waste in a financially and environmentally beneficial way. An example of an alternate path is using certain food waste to aid in growing bacteria that has industrial applications. This approach will be beneficial as bacteria already has an abundance of applications; however, being grown with natural food waste not only makes the bacteria more biocompatible but is also increasingly cost-efficient.

For this project, the bacteria being observed produces bacterial cellulose. Bacterial cellulose is becoming an increasingly popular resource mainly due to its appealing chemical and thermal properties (Choi et al., 2022). Overall, in comparison to plant cellulose, bacterial cellulose has stronger structural properties including a higher water-holding capacity, higher crystallinity, and higher tensile strength (Elsacker et al., 2021). These properties allow it to have various applications, especially in the medical field as it is such a versatile biomaterial. Some of the most common uses in the medical field include wound dressing, blood vessel regeneration (Lahiri et al., 2021), and even forming drug delivery systems.

To briefly hone in on its applications in drug delivery systems, it's important to understand the immense benefits of constructing efficient systems in the first place. Many patients are stuck in the loop of having to go back and forth from the hospital repeatedly, which can be mentally taxing and cause fatigue and exhaustion (*What Are the Advantages and Disadvantages of Chemotherapy?*, n.d.). As a result, treatment plans are not the most realistic or conventional route. This lack of convenience is a common trend with various diseases as the treatment itself may work fine, but the concept of having to go in repeatedly to get the drug administered is stressful and draining for the patient. A significant part of creating an effective drug delivery system that will aid in remedying this issue, is selecting a strong biopolymer that will improve efficiency as it serves as an effective encapsulation method for the controlled drug release of the system (Gheorghita et al., 2021). Bacterial cellulose is classified as a biopolymer, so through its production, helping form these drug delivery systems can be a strong example of where its specific medical applications could lie.

This project focuses on a specific cellulose-producing bacterial strain known as *Acetobacter xylinum*. It is considered a model microorganism when it comes to creating bacterial cellulose. What makes the cellulose produced by this strain a well perceived option is its excellent properties, ranging from strong biocompatibility and high purity to high crystallinity and polymerization (Yang et al., 2023). Additionally, this strain, in particular, was designed for the purpose of enhanced production of bacterial cellulose (Hur et al., 2020). Because of this, *Acetobacter xylinum* is a commonly used cellulose-producing strain by researchers in this field.

Previous Knowledge:

A commonly used production medium for growing this bacterial cellulose is Hestrin Schramm (Costa et al., 2017). While this medium is effective at producing yield, it can be costly, especially when scaled up to an industrial sized manufacturing. Researchers have been actively looking into what happens when the carbon source of the medium is altered. In other words, does changing the environment of where the bacterial cellulose is being grown affect how it ends up growing and producing cellulose? A study conducted by Kaczmarek et al. (2022) dived deeper into confirming the effects using this strain of bacteria would have on the production of cellulose. The authors set up thirteen strains, some with and some without ethanol, to check for the direct influence of the strain on the production of cellulose. While there was an outlier in strain 11, the rest of the data provided visual evidence of the efficacy of adding an additional carbon source in growing cellulose.

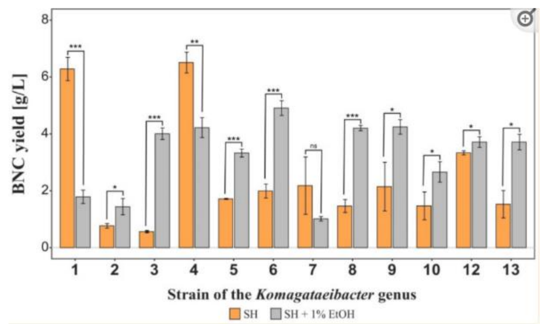

Figure 1: A graph depicting the influence of bacterial strain on cellulose production, Kaczmarek et al., 2022, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8950309/

Broad Researched Solution:

To target the concern of higher costs associated with the production of this bacterial cellulose, the use of food waste has been researched more and more. To explain why, it's important to recognize and understand what exactly makes a good culture medium for bacterial cellulose to grow in. Bacterial cellulose production is dependent on its production medium, meaning its environment. The amount and quality of the produced yield is specifically dependent on the carbon source used in that environment. Currently, components like glucose, fructose, and sucrose are used in the culture medium to improve efficiency and quantity. The Hestrin-Schramm medium uses various chemicals and glucose to grow the cellulose, while food sources come with the natural components necessary built-in. The connection between its production and the use of food waste can be made. Many foods are high in different types of sugars, especially things like juices, which can be derived from fruit waste.

Specified Researched Solution:

Juices are an excellent starting point for doing research on how food waste will affect the properties will affect the overall growth of bacterial cellulose. From there, it is much more reasonable to branch out and use that initial knowledge to test different common food wastes. For example, options that are typically more expensive, such as pure glucose and peptone, could be replaced by food source alternatives, including apple wastes and various tea mixtures (Amorim et al., 2023). As mentioned before, initializing this change will not only have the potential to produce a higher and more biocompatible cellulose, but it is much more cost efficient and can utilize food wastes. This method translates better for larger scale production sites where this change in cost could have a big influence on their ability to successfully produce large quantities.

Knowledge Gap:

The main knowledge gap in this area is surrounding what kinds of foods and what properties in those foods make them such a good option for bacterial cellulose growth. Seeing as factors like specific sugars impact how well the bacterial cellulose will grow is important to consider when making the decision on what foods are the best options of course, as some may aid in higher yields. To this day, research is still being done on how specific foods impact the production of the bacteria as there are a lot more factors to consider than just the sugar content. For example, with things like juices, even if a specific juice is high in a specific sugar, it may also be more acidic, which would then have a different effect on the growth.

Section II: Outline of Project Process

This proposal's objective is to examine how altering the growth medium of BC Production costs to utilize lower cost food wastes. various food sources will impact both the production yield and quality.

The central hypothesis of this proposal is to examine if there is a transition from commonly used substrates to food waste sources, then are there any significant effects on the quality and quantity of the bacterial cellulose. The rationale is that by researching specific aspects of bacterial cellulose production, this material can then be used for a multitude of applications in various fields, especially the medical field. With more research on this topic, it can add to the convincing argument of shifting to food waste sources for sustainable bacterial cellulose production. This method will not only be environmentally friendly, but also very cost-efficient, which is a current issue with its production today. The work proposed here will ultimately shed light on a more cost effective and environmentally friendly way to produce a specific strain of bacterial cellulose, which will have various applications in a variety of fields, especially the medical field.

The expected outcome of this work is to see a rise in production yield and production quality of the bacterial cellulose strain, *Acetobacter xylinum*, through the testing of different food sources as alternate culture mediums for the bacteria to grow. By comparing the yield of bacterial cellulose using the specific food source side-by-side with the yield of bacterial cellulose through the control, the Hestrin Schramm medium, a clear difference should be visible. This difference can then be measured and photographed to support the conclusion that certain food waste serves as a beneficial substitute for commonly used subtracted for the creation of bacterial cellulose. There will also be a medium that is 50% of the control and 50% of the sterilized food source as an initial backup if clear results aren't portrayed from the medium with just the food source. By starting with juices that are high in sugar, the juices must be sterilized and properly blended first to get a concentrated form that can effectively aid in the production of the strain of bacterial cellulose. From there, the baseline testing will continue to provide substantial evidence of an increase in yield size.

Section III: Project Goals and Methods/Actionable Items

Relevance/Significance:

The relevance of this project directly ties back to its numerous applications in areas such as water purification, cosmetics, and, most significantly, the medical field. The properties of bacterial cellulose make it an excellent choice in developing bio-based products. Its structure and capabilities also allow for it to be successful in accomplishing many things in the medical field, including suppressed microbial growth, the ability to aid in controlled release of certain drugs, and the ability to encourage efficient wound healing (Hur et al., 2020). If certain food waste proved to be an effective method of developing bacterial cellulose, would have the ability to engage in a transition of higher production costs to utilize lower cost food wastes. Knowing this is very important, mainly because it's a material that many researchers are looking more and more at as they discover its numerous beneficial properties. By being more cost efficient, this material will automatically become more accessible as larger scale production can take place without an economic toll on the company. Not only that, but this would be doing the environment a massive favor in reducing a portion of the food waste that continues accumulating worldwide, especially in the United States. Even if not as significant at first, the more this change in materials takes place, and more companies decide to partake in using food waste sources as a replacement for current substrates, the more beneficial it will be for them and the environment.

Specific Aims:

Specific Aim #1: The objective is to obtain a cost friendly approach to growing bacterial cellulose.

Justification and Feasibility. Shifting to food waste products in place of current high-cost substrates as a means of culturing bacteria will be a very cost-friendly shift. In comparison to many of the currently used essentials in the production medium, any kind of food waste is certainly the better economic option. Even if by a smaller margin, the more that needs to be produced, the more significant this price change will be. The success can be measured by comparing the prices of the food source used and how much it costs to use the typical HS medium.

Concise Analysis of Preliminary Data. After running baseline preliminary testing to identify the effects of replacing the culture medium with a food source alternative, the results clearly displayed the increase in bacterial cellulose yield when using a 100% concentration of pineapple juice as a substitute culture medium. As shown in the graph, as you move to the right, the yield decreases. This is displaying how as the concentration of pineapple juice reduces from 100%, to 50%, to 0%, the cellulose yield is also decreasing. This provides clear evidence towards the efficacy of using a food source in place of the typically used HS medium. One thing that was noted was how the cellulose produced by the pineapple juice medium was less transparent than the cellulose formulated by the HS medium. While this didn't play a factor in the testing, it was something to keep in mind for future testing as it was a property of the cellulose that was affected by the change in culture medium.

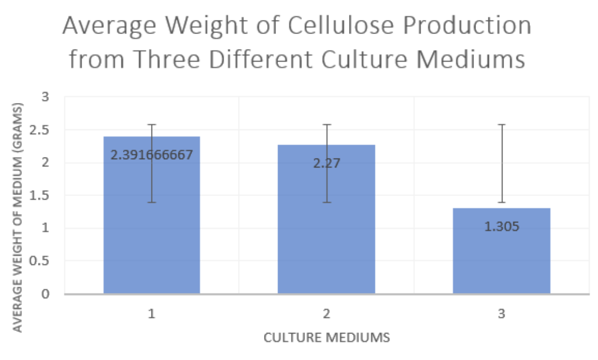

Figure 2: A graph depicting the averages of three groups of cellulose culture mediums and their respective yields. The bar on the left represents 100% pineapple juice as the medium, the bar in the middle is 50% pineapple juice, 50% HS medium, and the bar on the right represents the 100% HS medium.

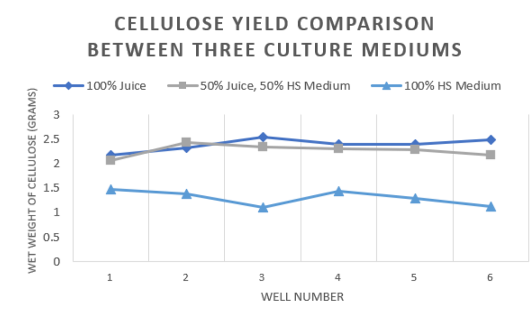

Figure 3: A line graph displaying the specific weights of cellulose produced from each six-well plate. The different lines represent the different culture mediums, as labeled in the graph. The different form of graph offers another look at how the culture mediums with a higher concentration of pineapple juice performed better in producing a higher yield of cellulose.

Expected Outcomes. The overall outcome of this aim is to determine which inexpensive foods produce the highest yield of bacterial cellulose. This knowledge will be used for initiating the transition between current substrates for growing the bacteria and selecting food waste alternatives.

Potential Pitfalls and Alternative Strategies. Besides already published literature, it may be a challenge to uncover which foods work best for growing bacterial cellulose efficiently. Therefore, it is possible that this process will require multiple trials to discover the most effective food source. While time consuming, this method is evidently the strongest strategy in determining accurate results of various food sources.

Future Explorations. Seeing as this could prove to be a time-consuming approach, future explorations would continue to dive into this idea and test a variety of food waste sources. With the base knowledge in mind, this approach would be a good opportunity to observe whether there are specific patterns in the food's properties that make it a better option or not.

Specific Aim #2: The aim is to solidify a sustainable and environmentally friendly approach to growing bacterial cellulose. This can be achieved by selecting various food sources and testing them as culture mediums to observe which food sources work better in growing cellulose.

Justification and Feasibility. As mentioned earlier, growing cellulose with a food waste culture medium would be more beneficial to our environment as food waste is a big issue today. Any way the massive amount of food waste in our society can be repurposed for better uses is a major financial and environmental success.

Conclusion:

This project is focused on highlighting the effects of using food waste as a substitute for growing bacterial cellulose to determine whether certain food wastes will aid in improving the yield and quality of cellulose produced. From preliminary testing using pineapple juice, the results displayed an evident increase in cellulose yield when the culture medium was comprised of purely pineapple juice. This gave a clear example of the effectiveness of using a food source as the culture medium for growing this bacterial cellulose. Direct implications of this knowledge are having a cost friendly way of growing this material, which translates well when companies look to utilize this material to save money and work effectively. Not only that, but this is a sustainable way of growing bacterial cellulose, as it reduces food waste by putting it to better use. Future work lies in testing the cellulose for its specific properties, including properties like crystallinity and biocompatibility to compare with traditionally grown bacterial cellulose. Doing this will help show how effective food waste sources are at growing a higher yield of cellulose that also retains or improves on it's mechanical properties.

Section III: Resources/Equipment

Most of the necessary materials would be located in a biomedical lab, including materials like the control medium (Hestrin-Schramm), the actual bacteria that will be grown, and then the specific sterilized food waste to test as a separate medium. Common lab tools such as an autoclave to sterilize the medium, well plates, a refrigerator or freezer, and a couple others are all required to proceed with the testing and can all be found inside the lab.

Section V: Ethical Considerations/Safety of the Project

As this project does not require any kind of testing on humans, there is no safety concern in that aspect. The bacteria are grown in a lab where professionals have enough background knowledge to keep the experiment safe. Besides the Hestrin-Schramm medium, the other culture medium will just be a food source which is very ethical.

Section VI : Timeline

Month	Work Accomplished
September	- Initial brainstorming regarding potential project ideas - Checking feasibility of ideas - Using mind maps and fishbone diagrams to hone in on specific topics and projects based off those topics
October	- Began to zoom in on a specific topic and narrow down on ideas for a project that fit the time constraints and were reasonable for a high school student - Read through various pieces of scientific research, documenting the progress and any notes in my project notes to get familiarized with the subject area
November	- Started researching for labs that were doing similar research that aligned with my project idea - Sending out professional emails to those labs - Continued to do more research on different testing strategies for my project and how I could implement them with the resources I had
December	- Got in contact with a lab that was working on the topic of my project - Began preliminary research at the lab for my project, worked with PhD student to grow bacteria and take the wet mass samples to graph, visually representing the increase in growth of cellulose when changing the culture medium - Presented all the work done at December Fair

January	- Continued working in the lab to conduct more experiments and plan out future testing strategies for my project - Began working on stem thesis and finalizing grant proposal (professional documents about my project)
February	- Finalizing all testing done for my project, preparing poster board for February Fair - Practicing pitches and going through all the work done towards project - Present at February fair

Section VII: Appendix

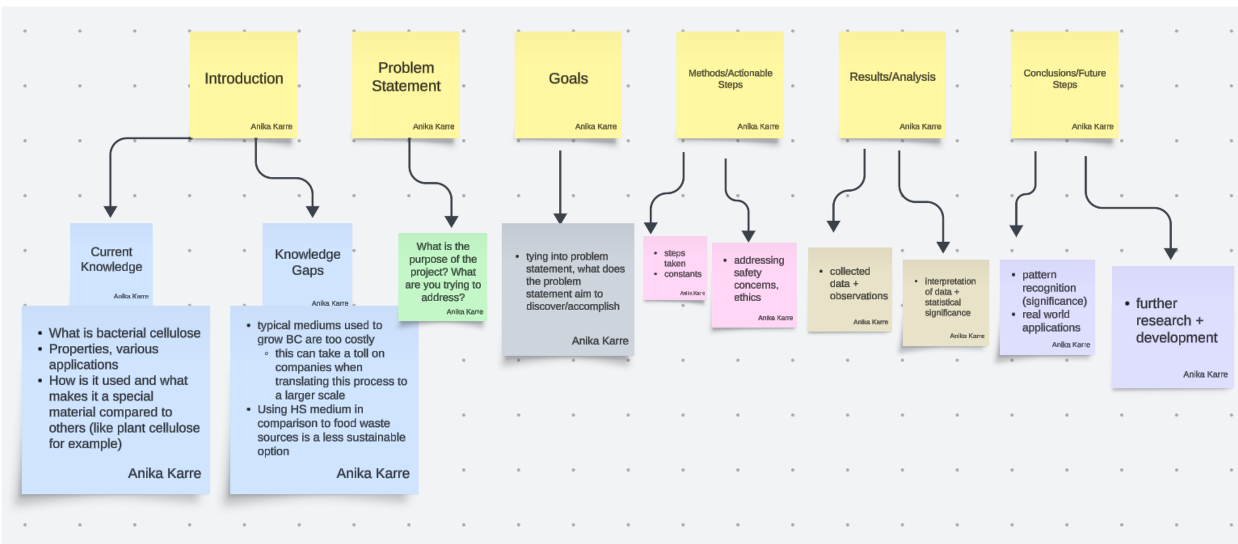

Figure One: Mind Map outlining the different components of the grant proposal document.

Section VIII: References

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