# Methodology:

**Role of Student vs. Mentor**

Over the course of an accumulated few weeks, my mentor and I performed both my preliminary experiments as well as further testing to support my project. While my mentor managed the usage of scientific tools in the lab, including the centrifuge and the Rigaku XRD instrument, I was able to participate in the pipetting of liquids, sterilizing of food waste concentrates, forming, and analyzing of graphs, as well as the measuring of the wet samples of grown cellulose.

**Equipment and Materials**

Fruit peels and boiling water were used to extract the juice from the peels. This juice was then sterilized at the lab using a centrifuge to separate the pulp and Therma Scientific Nalgene Filtration devices. 6-well plates were used to set up each of the culture mediums and these plates were set in the incubator to allow the bacteria to grow. After this, for wet mass samples, a typical scale was used, and for the XRD assessment, a Rigaku XRD instrument was used to observe the comparison between the crystallinity of the typically made cellulose and the food-waste grown cellulose. To set up the sample to be used in this device, Polyimide tape (Kapton) was used to keep the sample in place and Avicel Microcrystalline cellulose powder was pressed into the center of the sample holder in the Rigaku device to ensure the depth of the holder was filled properly. Smart Lab Studio software was used alongside the Rigaku to collect the data from the XRD analysis.

**Sterilizing Food Source**

Peels of a fruit were collected and washed thoroughly to remove any external dirt. These peels were then cut into smaller pieces and set to boil in water for 20 minutes. After this time, the liquid was strained through both a cheese cloth and fine strainer to obtain the pure liquid concentration of the

fruit peel. As there were various fruits being tested, any fruits that had remaining pulp would be put in the centrifuge for several minutes to separate the pulp from the juice. All the juices were put through a sterile filter into an autoclaved storage container before being used in the culture medium to grow the cellulose to eliminate the risk of contamination.

**Preparation of Cellulose Samples**

Six-well plates were set up for each culture medium, the food source mediums and the typically used HS medium. These well plates were set in the incubator for three days before undergoing a drying process. This step is critical to remove moisture without altering the cellulose's intrinsic structural properties. Uniform drying is ensured to maintain consistency in sample quality.

**Sample Mounting on Rigaku XRD**

The dried cellulose samples were carefully placed onto the sample holder of the Rigaku CRD instrument. Polyimide tape (Kapton), known for its X-ray transparent properties, was used to ensure the sample remains flat and evenly distributed. This minimizes any potential interference with the X-ray beam.

**X-Ray Irradiation and Detection**

The samples were then exposed to X-rays generated by the Rigaku system. For routine organic samples, an angle range of 3 to 40 degrees was employed, with a scanning speed of 5 degrees per minute. X-rays interact with the sample, leading to diffraction. This diffraction occurs when X-rays are scattered by the electrons in the cellulose, creating patterns that are detected by the instrument. These diffraction patterns reveal the crystal structure of the cellulose.