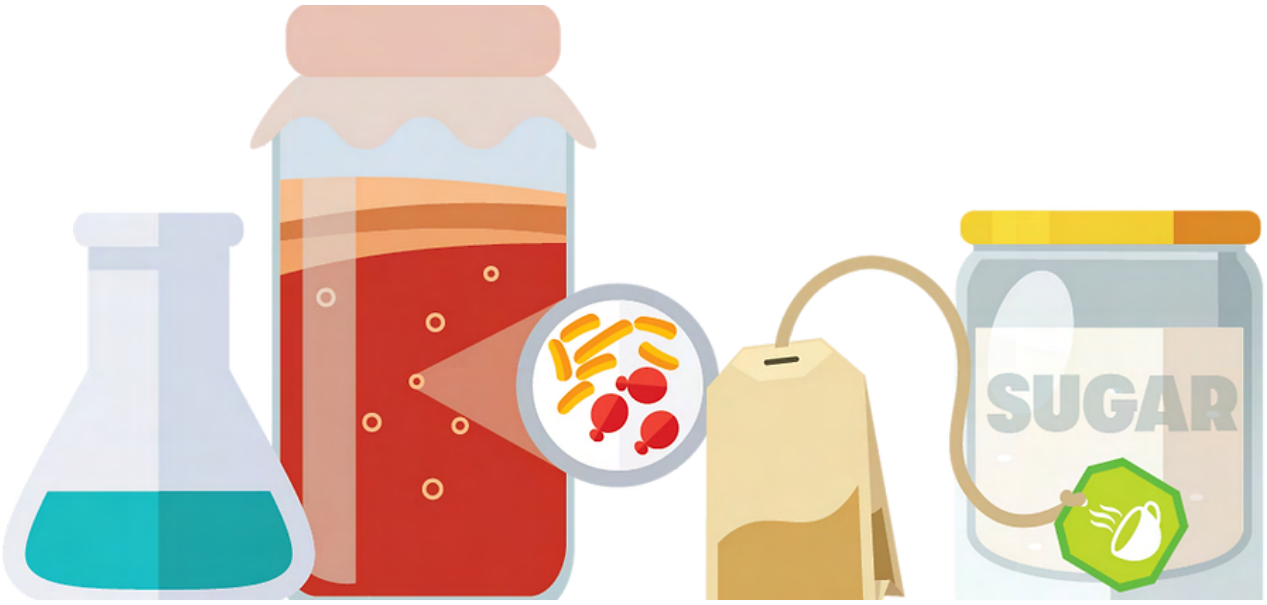
The Tech Interactive

Biotinkering Programs for Science Centers

Making with Microbes



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Making with Microbes Overview

Science Center Experience

Kombucha is a fermented tea that contains living yeast and bacteria that produce a layer of cellulose at the surface of the tea. In this activity, visitors collaborate with this community of tiny living organisms to design and grow a custom biomaterial. The microbes do the hard work of manufacturing the biomaterial, but visitors decide the final look and feel of it by choosing what ingredients to feed the microbes as the culture gets started. Materials can vary in strength, thickness, color, and even texture. While the microbes are growing, visitors can embed a pattern into other fresh biomaterial pieces and then create something artistic or useful with a fully dried piece that they can take home.

Subject
Biodesign

Ages
10+

Duration
30-40 min

Key Concepts
Microbes, bacteria, yeast, kombucha, biomaterials

Activity Goals

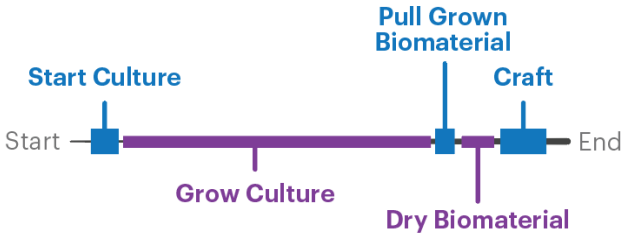
- Enable visitors to collaborate with tiny living microbes to design, mix, and grow a custom biomaterial.
- Support personalized design with microbiology by providing opportunities for visitor-driven choices that affect living microbes and the final product.
- Inspire visitors with experiments that use familiar materials and that they can continue iterating on in their own homes.

Operational Considerations

Base Biology	Format Complexity	Lab Requirements	Cycle Length	Cost
Kombucha Tea (yeast and bacteria)	High: Living Organism	Sink	1-3 weeks	$$$

Cycle Details

A full *Making with Microbes* cycle takes a minimum of several weeks due to mandatory wait times for organism growth and biomaterial drying (shown in purple). Using an assembly line approach, however, the visitor-facing activity can be consolidated into a single ~30 min experience (shown in blue).



Background Information

Kombucha Cultures

Microbes are living things that are so tiny we need a microscope to see them. These diverse and numerous organisms inhabit almost every environment on earth. They thrive in water, air, soil, our homes, and even on and inside our bodies.

The microbes featured in this activity are derived from kombucha, a popular beverage that is slightly sweet and tangy. Kombucha contains a mixture of living yeast and bacteria that work together to produce a fermented tea. Under an electron microscope, this looks like billions of tiny rod-shaped bacteria coexisting next to large, round yeast.

Like a sourdough starter, each kombucha is its own unique community of microbes, so the yeast and bacteria species can vary somewhat from culture to culture. There are many types of yeast that can break down sugar, but one type frequently found in kombucha is *Zygosaccharomyces*, which can tolerate the high acid and high sugar conditions. The bacteria needed to produce the biomaterial layer on top of the kombucha cultures all belong to the genus *Komagataeibacter*. At least some species from this genus are required, but many others can also be present in these complex communities.

Kombucha cultures have been fostered by humans for thousands of years and need just two basic ingredients to grow: sugar and tea. Sugar is the generic name for sweet-tasting carbohydrates; there are many types derived from a variety of natural sources which can be used. Any type of tea that is made from the true tea plant *Camellia sinensis* can work, including black, white, oolong, and green teas. Variation between these flavors is due to differences in production methods, such as leaf oxidation time, but they all have all the full set of nutrients needed to keep the microbes alive. Combining *Camellia sinensis* leaves with sugar to make sweet tea creates a complete food source for kombucha, making it a very easy culture to grow.

The Fermentation Process

Fermentation is a metabolic process used by many types of microbes to turn sugar into energy. During fermentation, sugar is broken down and converted into acids and/or alcohols. Over time, these byproducts accumulate and help preserve foods without refrigeration. People all over the world have been using fermentation for thousands of years to preserve foods and create a wide variety of yummy things like beer, wine, bread, cheese, yogurt, kimchi, sourdough, sauerkraut, soy sauce, chocolate, fish sauce, ketchup, miso, and some types of pickles.

The microbes that live in kombucha cultures work together to ferment the sweet tea. Yeast are responsible for getting the whole process rolling. They use the sugar molecules for energy and produce alcohols as a byproduct. This step also generates CO_2 gas, causing many bubbles to appear in actively fermenting cultures. Now the bacteria can get involved. They rely on the alcohols produced by the yeast for their energy. Once they use them up, what is left are acids. This is what makes the kombucha tea smell a bit like vinegar. As fermentation progresses, the tea gets more and more acidic, which makes it hard for other microbes to invade the fermenting culture. The yeast and bacteria in kombucha depend on each other for survival and have a dynamic relationship that allows them both to thrive.

Microbial Cellulose Biomaterial

The bacteria living at the surface of the kombucha culture make and release strands of cellulose into their environment. These long strands are composed of many individual glucose molecules strung together into a polymer. As millions of strands are made, they tangle together into a dense network of microscopic fibers. This microbial cellulose is what creates the visible mat of biomaterial on the surface of a growing kombucha culture that we can dry out into a leather-like biomaterial. It is thought that this layer could help keep contaminants out of the community, but its exact biological function is unknown.

Microbial cellulose is very similar to the cellulose plants make in order to stand upright. However, these two cellulose types have different characteristics. While both types of polymer are composed of glucose molecules, the glucose subunits are not connected in the same way. As a result, the fibers that make up microbial cellulose are finer and have a more intricate structure than plant cellulose. They are also longer, more absorbent, and stronger. Additionally, microbial cellulose can grow in almost any shape you want (plants are not so accommodating). The unique characteristics of microbial cellulose, including its chemical stability, molecular structure, and mechanical strength, have attracted scientists, designers, and others to explore possible uses for this grown material. Also, the biodegradability and renewable nature of microbial cellulose make it very environmentally-friendly!

What is a SCOBY?

SOCBY is an acronym that stands for Symbiotic Culture Of Bacteria and Yeast. It can refer to any microbial culture made of these two organisms including, but not limited to, kombucha. Technically, it describes the whole culture, but many people use it to refer to just the thick cellulose layer that grows on top. Kombucha brewers also use the term “mother” for this same layer because it can be used to help start a new culture. All of these terms are just different language for the same biomaterial product that is at the core of this activity.

Real-World Connections

Medicine

Microbial cellulose has been used as a backbone to grow new tissues and as a wound dressing. The cellulose network produced by a pure culture of *A. xylinum* has been used for experimental treatment of skin burns and other dermal injuries in Brazil.

Leather and Textiles

Designers of all kinds are becoming very interested in biofabrication and the new materials and textiles that can be produced with biology. One leader in this field is Suzanne Lee, a fashion designer based in New York. She was one of the first people to explore using microbial cellulose grown with kombucha cultures to create speculative clothing and shoes. These were one-off couture items made to highlight the possibility of growing clothes using microbiology, so the display items were not ever worn or produced at any larger scale. But it is still inspiring to imagine what might be possible!

Plastic Alternatives

Some designers are exploring the possibility of using microbial cellulose produced from kombucha as a food packaging material that is more sustainable than plastic. It could provide a sustainable and biodegradable material for single-use food service.

Other Commercial Products

In the late 1980s, Sony made the first headphones with a biocellulose diaphragm to amplify the sounds in the headphones. While the exact process for creating the diaphragm material is patented, it was grown using bacteria that produce cellulose just like we are doing in the activity! Biocellulose is thought to provide a clear acoustic profile that is unique among other diaphragm materials commonly found in headphones. This material is often used by headphone makers who are focused on sustainability, but biocellulose can now be found in many different types of headphones targeted to the audiophile market.

Useful Vocabulary

Term	Definition
Acid	A substance with a pH of less than 7.
Bacteria	A large group of single-celled organisms that have cell walls but no membrane-bound organelles.
Biomaterial	A material derived from, or produced by, biological organisms.
Carbohydrate	A compound made of carbon, hydrogen, and oxygen. Some examples include sugars, starch, and cellulose.
Cell	The smallest functional unit of life. Complex organisms are made of many cells.
Cellulose	A long molecule made of thousands of repeated subunits of glucose all strung together to form a chain. It is a core structural component in plant cells.
Culture	A nutrient source in which microorganisms are grown.
Fermentation	A metabolic process that converts sugar to acids and gasses or alcohol.
Glucose	A type of simple sugar molecule.

Kombucha	A drink produced by fermenting sweet tea with a culture of yeast and bacteria.
Microbe	A very small living thing that can only be seen with a microscope.
Polymer	A large molecule composed of many repeating subunits.
SCOBY	An acronym that stands for Symbiotic Culture Of Bacteria and Yeast, often used to describe the biomaterial layer on top of kombucha.
Yeast	A single-celled fungus that reproduces by budding or division.

Visitor Experience

Operational Summary

Context

Making with Microbes uses a visitor-supported assembly line approach to consolidate a 1-3 week biological process into a single 30-40 minute biotinkering experience for visitors. This activity was originally designed to run on a daily basis. While daily operations are not required, this schedule does allow for supply chain efficiency, as the materials made by past visitors can be used to support future visitors in a reliable and consistent fashion.

This activity was created as a semi-facilitated experience organized around four hands-on engagement stations: food mixing, growing, biomaterial drying, and crafting. Each station is designed to be largely self-guided, with the facilitator providing initial onboarding and support as needed to answer questions and encourage creativity and confidence. A facilitator-led introduction and individual supply distribution can happen at any time and location between when visitors enter and receive their challenge at the first station, depending on what works best for a given space and staffing model. The remainder of the experience can be fairly self-paced, with visitors progressing between stations when ready.

Activity Outline

1. Visitor Prep and Introduction
    - Visitors put on gloves.
    - Facilitator gives an overview of the activity.
    - Facilitator briefly introduces kombucha, microbes, and biomaterial.
2. Microbe Food Mixing Station
    - Facilitator explains the challenge: design food to help microbes grow a biomaterial.
    - Visitors get individual supplies: mixing containers.
    - Visitors explore the available shared ingredients and effects they have on biomaterial.
    - Visitors experiment with mixing different ingredients to create a unique microbe food.
3. Culture Growing Station
    - Visitors add microbes (active kombucha culture) to their food mixture.
    - Cultures are set aside to grow biomaterial over the next week.
4. Biomaterial Drying Station
    - Visitors harvest previously grown biomaterial from cultures.
    - Visitors choose textures and prepare biomaterial for drying.
    - *Optional:* Visitors may return to the Mixing Station to restart the experiment.
5. Crafting Station
    - Visitors select a piece of dried biomaterial and creatively explore.

Visitor Prep and Introduction

Overview

Provide a brief overview of the activity to orient visitors to the nature of the experience and have all participants put on gloves. The facilitator should introduce visitors to kombucha, microbes, yeast, bacteria, fermentation, and biomaterials. Tailor the focus and depth of the background information shared to the target audience and local community being served.

Essential Materials	Individual ● Gloves (all sizes) *Optional:* Physical examples to support the introduction (e.g., living kombucha culture, pieces of dried biomaterial, images of yeast and bacteria)
Example Setup	 

Engagement Strategies

Cultivate Confidence and Agency

- Successfully putting on gloves can be challenging and frustrating. Supports such as a hand measuring diagram can help young visitors navigate this step more independently.

Make Community-Relevant Connections

- Draw connections to fermented foods that are familiar to the audiences you serve.

Foster Scientific Curiosity

- Encourage visitors to use their observation skills to explore a kombucha culture for signs of living microbes (air bubbles, biomaterial layer, etc.).

Microbe Food Mixing Station

Overview

This station is the core challenge of the activity. Visitors design a custom sweet tea to help microbes grow a biomaterial with specific properties. They will need a variety of ingredients, including different types of teas, sugars, and colors. To support intentional design and decision making for a biological process that takes time, have visitors first explore dried final products made from various ingredients. Provide a mixing container with a clearly-defined volume to constrain the final volume of microbe food to one that is appropriate for the amount of microbes being used. After learning the challenge, visitors can be turned loose to explore the ingredients and tools as they experiment and mix. Once visitors have a final microbe food mixture, they can move to the Culture Growing Station.

Essential Materials	<u>Individual</u> ● Mixing container with clearly defined volume ● Liquid waste container <u>Shared</u> ● Dried biomaterial samples ● Variety of teas (oolong, white, black, etc.) ● Variety of sugars (agave, molasses, syrup, etc.) ● Mixing tools with different functions such as: ○ Pouring (funnels, pitchers, etc.) ○ Measuring (beakers, test tubes, graduated cylinders, etc.) ○ Stirring (spoons, whisks, etc.) ○ Mixing (jars, cups, etc.)
Example Setup	   

Key Visitor Steps	1. Gather individual supplies. 2. Explore samples of dried biomaterial made from different ingredients. 3. Choose microbe food ingredients. 4. Use tools to explore and test out possible formulations. ○ If needed, dump mixture in a waste container and start again. 5. Mix a final food mixture at the appropriate volume.

Engagement Strategies

Cultivate Confidence and Agency

- Give encouragement and validate ideas to help visitors embrace the personally-motivated design process, as many are uncomfortable with doing “science” without a recipe or protocol.
- Providing an individual waste container lets visitors change their mind or start different experiments without feeling constrained by their initial decisions.
- Curating a diverse but not overwhelming set of tools can highlight for visitors that there is more than one successful approach.

Emphasize Science as a Creative Process

- Enable visitors to make informed decisions about their ingredient choices by exploring dried biomaterials with specific ingredients. A touch board or other method of “research” into the effects of the ingredients on hand can help visitors imagine what they might want to create with science and why, which can provide a sense of purpose and creative direction for their experimentation.

Encourage Experimentation and Open-Ended Exploration

- Use questions instead of direct suggestions to engage with visitors and encourage them to share about their individual biomaterial design goals, try new tools, or iterate on their mixture formulation tests.

Make Community-Relevant Connections

- Providing ingredient options, such as specific varieties of teas or sugar sources, that are familiar to the populations you serve can allow additional points of cultural connection to the activity.

Culture Growing Station

Overview

This station kicks off the visitor-supported assembly line section of the activity. Visitors add living microbes from an active kombucha culture to the food mixture they just designed and leave it behind in an individually-sized growing container to manufacture biomaterial for future visitors. The biomaterial growth process takes about a week, but once visitors have set up a complete culture, they can move to the Biomaterial Drying station.

Essential Materials	<u>Shared</u> ● Kombucha starter culture in a dispenser (see Backend Preparations for details) <u>Individual</u> ● Culture growing containers
Example Setup	 
Key Visitor Steps	1. Add living microbes (active kombucha culture) to the final food mixture. ○ A ratio of around 1:1 microbes to food supports good growth. 2. Pour culture into an individual growth container. 3. Leave culture behind to grow biomaterial for a future visitor.

Engagement Strategies

Foster Scientific Curiosity

- Having an example of a culture with a fully-grown piece of biomaterial for visitors to interact with can help them understand and imagine what will happen to their culture after they leave it.

Highlight Authentic Science Practices: Collaboration

- Point out that biology can often be a collaborative science in a unique way: it can involve working with other living organisms to reach our end goal. In this activity, we have to wait and give the microbes time to grow our custom biomaterials.
- Emphasize that visitors are creating a biomaterial that a future visitor will get to work with. Multiple people will touch the same experiment and collaboratively contribute to its trajectory.

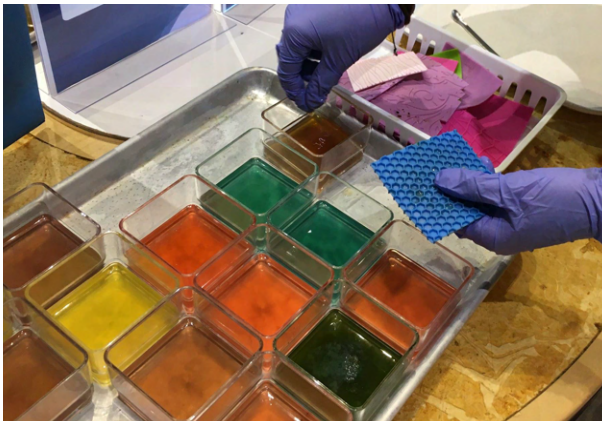
Cultivate Confidence and Agency

- Streamlining complex steps through user-friendly equipment like a bottle-top set-volume liquid dispenser can make a step that involves measuring a precise volume of living microbes easily accessible and empowering to all ages and experience levels. It also feels very “sciencey” to visitors, and successful interactions with it can build confidence in a lab setting.
- Instructional graphics can be used to remove the need for a facilitator at this step, allowing visitors to progress through this more procedural station in an efficient and self-guided fashion.

Biomaterial Drying Station

Overview

This station is part of the visitor-supported assembly line section of the activity. Visitors are tasked with helping to dry and customize an already-grown piece of biomaterial started by a previous visitor. This involves removing the floating biomaterial from the surface of a culture they choose. Visitors can then customize it further by selecting a textured surface pattern to dry the biomaterial on. It will pick up this texture as it dries, leading to a final product that has a design which was influenced by *two* separate visitors. Once the biomaterial has been fully prepped for drying, visitors have two options for their progression through the experience. They can choose to return to the Microbe Food Mixing Station to create another culture or they can move to the Crafting Station.

Essential Materials	<u>Individual</u> ● Previous visitor-made culture with a layer of grown biomaterial ● Flexible drying surfaces (textured silicone trivets, fondant molds, etc.) <u>Shared</u> ● Liquid waste containers ● Drying tray
Example Setup	 
Key Visitor Steps	1. Choose a culture made by a previous visitor. 2. Remove the layer of floating biomaterial from the surface of the liquid. 3. Lay the biomaterial out on a smooth or textured surface. 4. Leave the biomaterial on a rack to dry for a future visitor.

Engagement Strategies

Cultivate Confidence and Agency

- Tabletop instructional graphics can be used to remove the need for a facilitator at this station, allowing visitors to progress through this step independently.

Crafting Station

Overview

Visitors select a piece of fully dried biomaterial to craft with and take home. This station should be set up with a variety of crafting supplies for visitors to choose from that will allow them to decorate, reimagine, or alter their biomaterial.

Essential Materials	<u>Individual</u> ● Dried biomaterial <u>Shared</u> ● Crafting supplies with different functions: ○ Cutting (scissors, hole punches, etc.) ○ Coloring (markers, pens, etc.) ○ Attaching (string, yarn, keychains, etc.)
Example Setup	 

Engagement Strategies

Support Creativity

- Instructional graphics can be used to help visitors less familiar with paper-based crafts (e.g., origami, papel picado) get started on their own.
- Consider creating a location for visitors to leave behind their artwork to share with the community and serve as inspiration for future visitors.

Common Visitor Questions

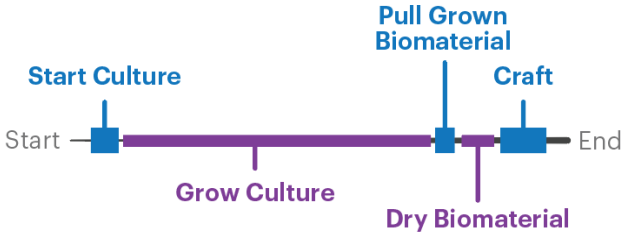
Visitors often ask unpredictable or incredibly specific questions about the content or process of an activity while they are participating in the experience. Every audience will have different interests or prior knowledge that they bring to the experience. Below are examples of the most common questions we hear from visitors and the types of answers we aim to provide.

Question	Information
Can I drink it?	*No! You should never drink anything in a lab.* Kombucha tea is a popular and entirely safe beverage, but it needs to be grown with care and caution if it is going to be consumed. We don't grow ours to be food-safe or to taste good because we are optimizing it to grow biomaterial.
Can I eat it?	*No! You should never eat anything in a lab.* Although a similar cellulose network floating on the surface of various fermented fruit juices is consumed in the Philippines as a delicacy, we don't grow ours under food-safe conditions or for taste.
What should I put in my microbe food?	*It's up to you to decide!* A kombucha culture does need at least some tea and some sugar to grow, but the rest is up to you! Choose what properties you think are important for your biomaterial and combine ingredients accordingly. Explore samples of dried biomaterial to get a sense of the effects of each ingredient on the final product.
Why is making purple so hard? How can I make it?	*Remember, the teas and sugars also add a bit of color to your mixture.* When adding dyes, think about how you might compensate for the bonus color coming from the tea and/or sugar. Then, test out your idea on a small volume first, using the provided tools. Adding more blue than red to the mixture often helps because it balances out the yellow undertones in many teas and sugars.
Can I take home the biomaterial from the food I designed?	*Unfortunately, no.* Because it takes about a week for the living microbes to grow a piece of biomaterial, it won't be ready in time. Instead, a future visitor will help finish the process and you can choose a piece of already dry biomaterial to take home that was started by a previous visitor.
Are there living microbes in the biomaterial?	*Not once it is dried out.* Bacteria and yeast live in all parts of an active kombucha culture, including the floating cellulose of the biomaterial. Once the biomaterial is removed from the liquid culture and dried out, however, those microbes can no longer survive.
Does kombucha tea have health benefits?	*There is no strong evidence of this.* Though it is claimed to be beneficial for several medical ailments, very little or no clinical evidence is available for specific outcomes from drinking kombucha.

Backend Preparations

Overview of Components

The biological base of *Making with Microbes* is a living culture of yeast and bacteria (kombucha tea), so some ongoing backend support is needed to operate this activity. A full activity cycle, from start to finish, takes a minimum of several weeks due to mandatory wait times (shown in purple) for organism growth and biomaterial drying.



A visitor-supported assembly line approach can be used to consolidate the visitor-facing activity into a single floor experience (shown in blue). To achieve this, there are some backend operational tasks that need to be done by staff to support the purple phases of the cycle. Keep in mind that some of the initial preparation steps take significant time and must be done prior to beginning activity operations. Each visitor-supported phase of the cycle should be maintained on a consistent and ongoing basis to ensure materials (kombucha starter culture, grown biomaterial, and dried biomaterial) are available at each step of the activity. To stabilize the assembly line approach against supply chain disruptions and visitor number variability, staff can process supplemental pieces of biomaterial when needed. This strategy can also be used to support operational formats other than daily programming, if desired.

Backend preparations for this activity include:

1. Establishing and Maintaining a Kombucha Starter Culture
2. Selecting and Preparing Microbe Food Ingredients
3. Selecting and Preparing Culture Growing Station Supplies
4. Growing Visitor-Made Cultures
5. Drying Grown Biomaterial

Institutional Note: Working with Fermenting Cultures

Growing kombucha at scale for large numbers of visitors is relatively easy, but since it involves fermentation there are associated odors that will need to be accommodated. Each institution will want to customize their setup and operations in the way that considers and best manages these inherent parts of the system for their particular space or audience.

Starting Up: Getting the Visitor Assembly Line Going

The first time this activity is run with visitors, staff will need to grow individual-sized kombucha cultures starting 1 week or more in advance to have supplies ready for each step of the activity. After that, the visitor-supported assembly line approach can be leveraged in an ongoing fashion to sustain supplies.

Establishing and Maintaining a Kombucha Starter Culture

Operating *Making with Microbes* requires having a staff-supported backend system for growing and maintaining an active kombucha starter culture. This must be done at a scale that is sufficient to support the number of visitors participating in the activity, as the backend starter culture is the source of living microbes for all visitor-made food mixtures. Getting a robust and thriving kombucha culture established can take several weeks, but is critical for successful biomaterial growth.

Materials

Reusable Equipment	
Item	**Notes**
Kettle	Any hot water source works
Mixing containers	Heat-resistant for brewing tea
Large culturing container(s)	Glass or plastic with a spigot is ideal
Culturing container cover(s)	Can use cracked-open lids or porous materials like coffee filters with rubber bands
Measuring cup and spoons	Optional as amounts don't need to be too precise

Consumable Supplies	
Item	**Notes**
Kombucha beverage or starter	Can be purchased from grocery stores or various online sources
Water	
Tea	Must be a "true tea," which means it is made from the *Camellia sinensis* plant
Sugar source	Granulated or syrup
70% alcohol	Rubbing alcohol

Procedures

1. Select a source of active kombucha to use for establishing a backend starter culture.
    - Option 1: Store-bought kombucha beverage (must contain living microbes)
        - Not all commercially available kombucha beverages will grow biomaterial. Some tips to increase the chances of obtaining an active culture when purchasing kombucha from a grocery store include:
            - Look for refrigerated bottles that say "live," "raw," "active," or "unpasteurized." These are the best bets for robust biomaterial growth.
            - Labels that caution against use by anyone who is pregnant or that list an alcoholic content (a result of fermentation by yeast) can indicate beverages that are more likely to contain live microbes.
            - "Jun"-style kombuchas seem to more reliably grow biomaterial, but the cultures can be extra smelly.

Technical Tip: Using Store-Bought Kombucha

If you are using store-bought kombucha, obtain several bottles and combine them, as a single bottle from an otherwise-reliable brand can sometimes be a dud.

- Option 2: Kombucha SCOBY (also called a kombucha “mother”)
    - These can be ordered from various sources as a slab of hydrated biomaterial.

2. Prepare sweet tea to feed the microbes in kombucha.
    - Brew tea using hot water.
        - Use any kind of tea derived from the plant *Camellia sinensis*. We’ve seen good long-term stability with black and oolong teas, but other types that meet specific preferences or needs can also be used (e.g., smell or base color considerations).
    - Add in a sugar source while the tea is hot and stir until dissolved.
        - Approximately 1 tbsp per 1 cup hot water works well, but this can be adjusted as needed to optimize growth of an individual culture in a specific environment.
        - Most types of real sugar work well. We’ve seen good long-term success with granulated white sugar.
    - Let the sweet tea cool to room temperature.
3. Prepare the large culturing container.
    - Wash the container thoroughly with soap and hot water.
    - Spray the inside with 70% alcohol and allow it to evaporate before use.
4. Start the kombucha culture.
    - If using a store-bought kombucha beverage, combine kombucha and sweet tea at approximately a 1:1 ratio to help microbes get established in a new environment.
    - If using a biomaterial kombucha starter (SCOBY), combine all contents of the package directly with the sweet tea.
5. Let the culture grow for a few weeks until it is robustly active.
    - Ensure that the culture is covered to keep bugs and debris out but that it is not airtight (the microbes need oxygen to survive).
    - Leave the culture at room temperature or slightly warmer for the best microbe growth.
    - Look for bubbles in the tea within four to five days and the appearance of biomaterial on top of the liquid within two weeks.
        - If these things are not observed, the culture is likely inactive, so start again.
    - Once the microbes are thriving, this kombucha can be sustained almost indefinitely.
6. Maintain this backend kombucha starter culture throughout activity operations.
    - We recommend using continuous brewing to create a perpetually-producing kombucha culture. This method involves removing a smaller amount from a primary culture and letting the same vessel repopulate with new microbes.
        - Transfer the entire starter culture to a large vessel with a spigot near the bottom.
        - Add sweet tea to fill the vessel and allow it to grow for a few weeks so that the microbes can fully activate the expanded culture volume.
        - As needed, remove no more than ⅓ of the total culture volume through the spigot and refill the lost culture volume of the vessel with fresh sweet tea.
            - Removed liquid can be used immediately in the activity or refrigerated for future use.
        - Allow several days for the culture to fully repopulate with new microbes.
        - Repeat this cycle of removing from the spigot and refilling the lost culture volume in a continuous fashion to support ongoing programming needs.
    - Several large backend starter cultures can be used on a rotating schedule to support higher volume or frequency operations.

Technical Tip: Noticing and Managing Starter Culture Contamination

Kombucha is remarkably resistant to outside contamination if grown in a reasonably clean environment, but like with any living culture, it is possible. The most common form of contamination is typically mold, like you see on food accidently left out in a kitchen. It may grow in the sweet tea before biomaterial begins to form, or on top of the floating biomaterial itself. If any unwanted microbial growth is observed, dispose of the culture, clean all containers well, and start again.

Selecting and Preparing Microbe Food Ingredients

These ingredients are used by visitors at the Microbe Food Mixing Station. The goal is to provide an array of options that allows visitors to make personalized choices about what properties, such as the texture, look, feel, and color they want the final dried biomaterial to have.

Materials

Reusable Equipment	
Item	**Notes**
Refrigerator	If storing ingredients long-term
Kettle	Any hot water source works
Mixing containers	Heat-resistant for brewing tea
Ingredient bottles	Condiment or squeeze bottles work well

Consumable Supplies	
Item	**Notes**
Teas	Provide several options - black, green, white, and oolong all work well
Sugar sources	Provide several options - granulated sugars and syrups work well
Water	
Dyes or colorants	Food-safe recommended
70% alcohol	Rubbing alcohol

Procedures

1. Select ingredients for the visitor-facing activity.
    - Choose several types of teas with different base colors and impacts on dried biomaterial that visitors can use in their microbe food mixture.
        - Teas can be loose-leaf or bagged, but must be a “true tea” (e.g., black, green, white, or oolong teas).
        - Avoid herbal teas, rooibos, or flavored teas with oil (e.g., Earl Grey) as these lack important nutrients or contain ingredients that can disrupt biomaterial formation.
    - Choose several sugar options with different base colors and impacts on dried biomaterial that visitors can use in their microbe food mixture.

        - Most sugar sources will work well including both granulated (e.g., white, brown) and syrups (e.g., molasses, agave, maple, simple syrup).
        - Avoid honey, which is more likely to get contaminated, and sweeteners like stevia and sucralose, which contain sugars that microbes can't use.
    - Choose a minimal set of dyes or colorants (e.g., red, blue, yellow) that visitors can use and combine creatively to produce intermediate colors.
        - Standard food coloring and gel food colorants both work well, but feel free to explore other options. Sticking to food-safe items is recommended, however, to decrease the chances of them accidentally impacting the health of the culture.
        - Avoid mineral pigments or things with oils as they can disrupt biomaterial formation.

2. Prepare ingredients for visitor use.
    - Clean all activity ingredient bottles well and sanitize with 70% alcohol before filling them.
        - This is important for minimizing contamination growth in reagents that will be added to visitor-designed kombucha cultures.
    - Brew large batches of each type of tea using an electric kettle or other hot water source and fill labeled dispensing bottles with the respective teas.
        - Refrigerate teas at all times when they are not in use to reduce unwanted microbial growth. Check for and dispose of contaminated ones before every use.
    - Dissolve any granulated sugars in water to create a solution and fill labeled dispensing bottles with the respective sugar sources.
        - We recommend diluting commercially prepared syrups by at least half to help keep biomaterial from becoming very, very sticky. It's easy for visitors to add too much sugar with concentrated syrups.
        - Refrigerate sugars at all times when they are not in use to reduce unwanted microbial growth. Check for and dispose of contaminated ones before every use.
    - Put dyes into their own dispensing bottles or combine them with other ingredients to create additional design constraints for visitors.

Technical Tip: Minimizing Contamination in the Visitor-Facing Activity

Microbe food ingredients are the most likely source of contamination in the activity. Unwanted bacterial growth in tea often looks like long, floating opaque tendrils or flakes that settle at the bottom, while mold may be visible on top of the tea or as small spheres inside the liquid. In syrups, contamination often grows on the surface. Empty out any containers with signs of contamination and clean them thoroughly with soap and alcohol before reuse. This will decrease the number of visitor-made cultures that fail to grow biomaterial and are wasted due to contamination issues.

Selecting and Preparing Culture Growing Station Supplies

The setup of the visitor-facing Culture Growing Station requires some advance preparation and coordination with the backend kombucha starter culture supply chains and systems being used. Ensuring that the provided source of living microbes is robust and healthy is critical for successful growth of visitor-made cultures designed at the Microbe Food Mixing Station.

Materials

Reusable Equipment	
Item	**Notes**
Individual growing containers	One per activity participant
Starter culture dispenser	A pump that can deliver a pre-measured volume is ideal

Consumable Supplies	
Item	**Notes**
Kombucha starter culture	Produced by staff according to the instructions above

Procedures

1. Select culture growing containers for the visitor-facing activity.
    - Individual visitor-made cultures can be grown in containers of any size or shape, but for stable ongoing operations the volume of these cultures should be matched to the visitor throughput and the size of your backend kombucha starter.
    - Plastic containers work well and are long-lasting, but glass or glazed ceramic containers can also be used if they are thoroughly cleaned between uses.
    - Avoid metal containers, as acid in the culture will cause metal to corrode.
2. Prepare living microbes for visitor use.
    - Remove active kombucha starter culture from the backend system (produced by staff according to the instructions above). Visitors will combine this with their microbe food to create an active culture.
        - Remove enough volume to support the visitorship you expect for a session of the activity (e.g., for 100 visitors and 5mL of culture each, remove at least 500mL).
        - Leftover starter culture can be stored for future use.
    - Put active kombucha starter culture into a dispenser.

Equipment Highlight: Pre-Measured Volume Pumps

Using a kombucha starter culture dispenser that delivers pre-measured volumes not only improves the visitor experience and simplifies facilitation for staff but also streamlines backend operations. This tool can help ensure that each visitor-made culture gets the appropriate volume of living microbes added at the Culture Growing Station. Stabilizing this variable improves overall biomaterial growth rate and dried biomaterial quality. These two elements are key for achieving a relatively self-supporting and low-maintenance visitor-supported assembly line.

Growing Visitor-Made Cultures

After each activity session, staff should collect the individual visitor-made cultures left at the Culture Growing Station and store them in a growing location for at least 7 days. During this time, the microbes (assuming happy cultures) will produce a layer of floating biomaterial that can be used by future visitors at the Biomaterial Drying Station. Staff can prepare and grow supplemental cultures as needed.

Materials

Reusable Equipment	
Item	**Notes**
Growing chamber or location	Setups can vary a lot

Procedures

1. Identify an appropriate backend growing chamber or location.
    - Since kombucha is alive, certain basic conditions must be met for these cultures to successfully produce biomaterial layers.
        - The culture must be kept at room temperature or a bit warmer.
        - Use a stable surface where the cultures won't be moved or jarred, as too much sloshing of the culture liquid can interrupt biomaterial formation.
        - Cover individual containers (e.g., individual lids, cheesecloth, enclosed growing cabinet) to reduce contamination and fruit flies.
    - Consider a spot with good ventilation or airflow to help disperse fermentation odors.
2. Allow the visitor-made cultures to grow for at least 7 days.
    - The exact length of time it takes for them to produce a sufficient layer of biomaterial will depend on the specific growing containers, environment, and backend kombucha starter culture being used as well as the ingredients in each unique visitor-made food mixture.
        - To identify the optimal growing period for your specific conditions and location, consider running a test and check biomaterial production at various time points.
    - Discard any cultures that have no obvious growth after one week.
3. After a full growing period, visitor-made cultures with a nice layer of biomaterial can be put into the activity for visitors to use at the Biomaterial Drying Station.

Technical Tip: Seedling Mats for Warmth

A relatively cheap and easy way to create a slightly warm growing environment for kombucha cultures without needing to have an incubator or full growing chamber setup is to use plant seedling mats. Culture containers can be set on top of or near them to get some supplemental heat if the environment overall is a bit too cool.

Drying Grown Biomaterial

At the Biomaterial Drying Station, visitors remove wet biomaterial from a culture made by a previous visitor and lay it out on a drying surface. As needed, staff should relocate these laid out pieces to a defined drying location. Depending on the drying setup and the thickness of the biomaterial, pieces will need a day or more to fully dry out before they are ready for visitors to use at the Crafting Station.

Materials

Reusable Equipment	
Item	**Notes**
Flexible drying surfaces	Silicone or plastic with a texture is ideal
Fan	Optional but speeds drying

Procedures

1. Select biomaterial drying surfaces.
    - For ease of removing dried biomaterial from the surface it was dried on, opt for flexible materials (e.g., silicone, plastic) that can be peeled away from the dried biomaterial.
    - Dried biomaterial will retain the texture of the surface it dries on. Provide a variety of surfaces with different textures to support further visitor choice and customization.
    - Avoid rigid and metal surfaces to prevent tearing during removal.
2. Move wet biomaterial laid out by visitors to a backend location for drying.
    - Depending on the thickness of the biomaterial and the exact drying setup being used, biomaterial can take up to several days to fully dry out.
    - Biomaterial will dry faster with consistent airflow, so use fans to speed up the process.
    - Reduce the space needed by only drying out sturdy, fully-formed pieces of biomaterial.

Common Backend Questions

Standard operating procedures for this activity will vary based on the unique context of a given institution. Factors such as physical spaces, programming frequency, equipment availability, staffing models, and audience characteristics will introduce constraints and preferences that the general procedures above can be adapted to accommodate. Below are answers to the most common operational questions and insights from our experience running the activity in the Biotinkering Lab.

Question	Information
What is a good range of ingredients to offer visitors?	*We offered three teas (white, black, and oolong) and three sugar sources (sugar and agave syrups and molasses).* These tea and sugar options provided a wide array of base color variety (from light to dark) for visitors to choose from. Additionally, in our own test, we found that varying these ingredients had different impacts on the growth of biomaterial in cultures as well as the look and feel of the dried biomaterial.
How can we manage the amount of dye visitors add to their food mixtures?	*Dilute the dyes in a light-colored tea.* We chose to integrate dyes (red, yellow, and blue) into the white tea that we provided visitors, which allowed for color customization of microbe food by visitors but required them to think creatively and make trade-off decisions during mixing. From an operational perspective, this helped staff avoid the mess of providing concentrated dyes directly to visitors and also allowed us to limit the total amount of dye added to each culture to keep the microbes happy.
How can we help visitors make purple cultures?	*Use a red dye that skews maroon.* Since teas have a natural yellow tint to them, mixing purple from a set of primary-colored dyes can be a bit difficult, especially for younger visitors. This can be offset by choosing a red dye with a more bluish undertone for balance.
Send help! How do we manage the fruit flies?	*Keep growing cultures covered or in an enclosed location as much as possible.* Fruit flies are attracted to the smell of fermenting kombucha cultures. The best way to keep flies out is to cover or enclose growing cultures as much as you can either with individual lids or inside a cabinet. Remember, kombucha needs air to grow, so make sure that lids or covers are not fully airtight.
Is there a way to make growing cultures smell less bad?	*Fermentation will always have an odor, but culture ingredients can impact it.* The smell profiles of individual kombucha cultures vary a bit depending on the type of culture and what ingredients are used. Consider exploring different ingredient options for the backend kombucha starter culture until you reach a version that has the most palatable smell. We found that our staff preferred oolong tea as the base.
Why is the biomaterial so sticky? Can that be fixed?	*Excess sugar in the initial culture can make the final biomaterial sticky.* The yeast in kombucha break down and use the sugar in sweet tea. If a huge amount of sugar is added to a food mixture, however, a normal week-long growth window might not be enough time for them to work through it all. Large amounts of unused residual sugar can coat biomaterial and make it very sticky. Strategies for minimizing and remedying this in visitor-made cultures include: ● Use water to dilute the concentration of sugar sources provided to visitors, thereby allowing larger volumes to be added without an issue. ● Grow cultures for more time to allow yeast to break down more sugar. ● Wash sticky biomaterial in soapy water before drying to remove sugar.

Supplemental Resources

Full Materials List and Recommendations from The Tech

Reusable Equipment		
Item	**Notes**	**Specific Recommendations**
Refrigerator	If storing ingredients long-term	
Kettle	Any hot water source works	We use an instant hot water tap.
Mixing containers	Heat-resistant for brewing tea	We use Cambro 4 quart containers.
Large culturing container(s)	Glass or plastic with a spigot is ideal	We use an Anchor Hocking 2 gallon beverage dispenser with a metal spigot.
Culturing container cover(s)	Can use cracked-open lids or porous materials like coffee filters with rubber bands	We use a glass lid with a homemade spacer to leave an air gap.
Ingredient bottles	Condiment or squeeze bottles work well	We use hard plastic condiment or travel bottles to control the amount that comes out when squeezed. This is easier for younger visitors to control and helps to manage the mess.
Individual growing containers	One per activity participant	We use plastic STORi Stackable 3” x 3” Drawer Organizers. These fit efficiently on standard-sized bun pan for both transport to and storage in our growing chamber.
Starter culture dispenser	A pump that can deliver a pre-measured volume is ideal	We use BrandTech seripettor® Bottletop Dispensers, which can dispense pre-measured volumes up to 25 mL.
Growing chamber or location	Setups can vary a lot	We used an enclosed bun pan rack to create an enclosed space and regulated the temperature with seedling mats. We would choose a non-metal growing chamber solution in the future for better long-term durability.
Flexible drying surfaces	Silicone or plastic with a texture is ideal	We mainly use textured silicone trivets and cake fondant molds cut into squares sized to match our individual growing containers.
Fan	Optional but speeds drying	
Measuring cup and spoons	Optional as amounts don’t need to be too precise	

Mixing containers	With a clearly defined volume for visitors to fill with microbe food	We use small plastic pitchers with a max-fill line made out of electrical tape.
Mixing tools	Provide a variety that supports visitor pouring, measuring, stirring, and mixing	We use lab spatulas and scoops, mini whisks, pitchers, beakers, graduated cylinders, test tubes, and funnels.
Liquid waste container	For visitors to dispose of unwanted microbe food and grown culture liquid	We use flasks for the Microbe Food Mixing Station and plastic bins for the Biomaterial Drying Station.
Drying tray	To hold laid out wet biomaterial	We use bun pan trays with wire cooling racks.
Scissors	For visitor crafting	
Hole punches	For visitor crafting	We provide normal circular ones as well as some fun other shapes.
Markers or pens	For visitor crafting	We provide colors that will show up on the biomaterial and include some paint-style ones for extra emphasis.

Consumable Supplies		
Item	**Notes**	**Specific Recommendations**
Gloves	All sizes	
Kombucha beverage or starter	Can be purchased from grocery stores or various online sources	We have successfully used GT's Kombucha (original flavor), Poseymom, and Joshua Tree kombucha starters.
70% alcohol	Rubbing alcohol	We use spray bottles to make cleaning easier.
Water		
Teas	Provide several options - black, green, white, and oolong all work well	We use black, white, and oolong teas.
Sugar sources	Provide several options - granulated and syrups work well	We use sugar syrup, agave, and molasses.
Dyes or colorants	Food-safe recommended	We use the primary colors (red, yellow, blue) to support color wheel exploration. For a red that enables visitors to better make purple, we use Americolor Crimson Gel Food Coloring.
Crafting supplies	Provide a variety of supplies that support cutting, coloring, and attaching	We use string, yarn, and keychains.