

At-Home Yogurt Making to Investigate Microbiology Concepts: A Remote Biology Laboratory



RECOMMENDATION

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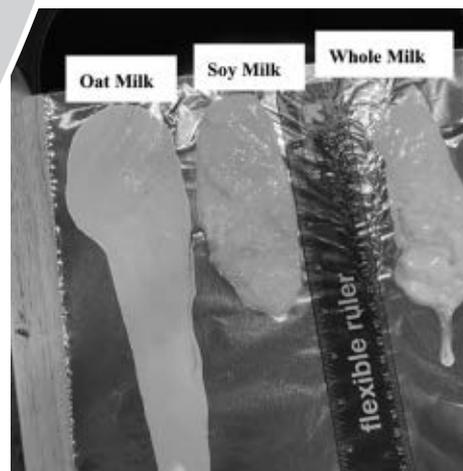


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ABSTRACT

The global COVID-19 pandemic has forced many educators to move their courses to the online environment with little time to adjust. It especially affected undergraduate biology laboratory courses that rely on on-campus facilities to provide students with meaningful laboratory-type experiences. Here we describe a multisession, at-home, and hands-on laboratory activity that utilizes yogurt culturing to explore microbiology concepts. We also summarize the findings of 219 undergraduate students who successfully performed this lab remotely. In small virtual groups, students learned how to make yogurt at home, formulate a testable hypothesis, run an experiment on conditions necessary for yogurt fermentation, analyze experimental results, and present their results to peers in an oral scientific talk. Practical considerations include the use of low-cost and accessible materials, low-tech yet effective quantification approaches, and online note-taking and data management tools to coordinate group work and provide informal and formal assessment.

Key Words: inquiry-based learning; microbiology experiment; remote lab; yogurt fermentation.

○ Introduction

Hands-on learning experiences in undergraduate biology courses can lead to greater confidence in students' academic outlook, higher learning outcomes, and better student retention (Beck & Blumer, 2012; Gasiewski et al., 2012; Beck & Bliwise, 2014; Freeman et al., 2014). In undergraduate biology programs, students typically enroll in introductory laboratory courses in their first year with the understanding they will receive hands-on learning experiences with the techniques and tools used in their field. In contrast to traditional lecture courses where information runs the risk of being transferred unilaterally and received passively (Bransford et al., 2000), hands-on practical learning stimulates multiple senses simultaneously,

creating more neural connections and easier memory recollection later (Willis, 2007). Although there are benefits all students gain from hands-on laboratory experiences, pretest/posttest research has shown the greatest improvements are conferred to the least-prepared students (DeHaan, 2005; Beck & Blumer, 2012). The global COVID-19 pandemic resulted in closure of physical schools, which required students, teachers, and support staff to pivot from in-person to distance learning and to experiment with a range of modalities from asynchronous television (Kohli & Blume, 2020) to synchronous online learning (Esquivel et al., 2020). This effort includes a growing number of distance laboratory exercises for undergraduate biology students (Hanzlick-Burton et al., 2020; Noel et al., 2020; Gya & Bjune, 2021).

Here we describe a multiweek, at-home, and hands-on laboratory activity in which students culture yogurt to explore microbiology concepts. This “basics of microbiology” module aimed to guide students through the process of learning to make yogurt at home with the materials and tools available, formulating a testable hypothesis about the conditions necessary for the yogurt fermentation process, designing an experiment to test that hypothesis, analyzing and interpreting experimental results, and ultimately giving a 10-minute oral scientific presentation in groups. We selected milk fermentation, or yogurt making, for our remote lab because the materials are easily accessible, regardless of the geographic location where a student resides (Figure 1). Additionally, during fermentation, milk undergoes some noticeable physical changes that could be easily measured and evaluated by students at home without using special lab equipment. We provided training to all students on Microsoft OneNote and Microsoft Teams to coordinate note taking and data management among groups and

scheduled weekly synchronous group work and whole class times using Zoom. Microsoft OneNote and the learning management software Brightspace (D2 L) were utilized to track and evaluate students' progress and provide informal and formal assessment.

“I enjoyed the yogurt-making lab the most. We learned about biological processes and ran experiments to see which yogurt-making method worked the best.”
—Student

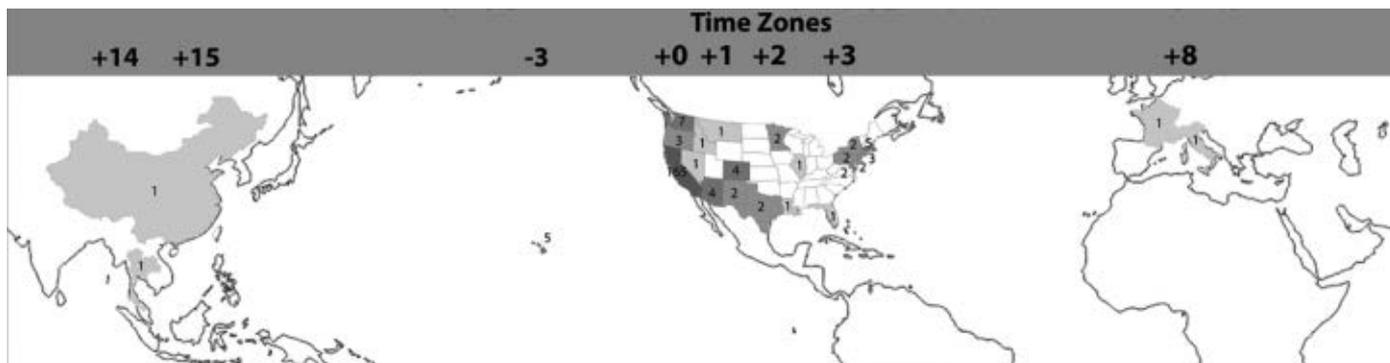


Figure 1. Geographic location of students in the course who participated in the yogurt-making activity based on 219 survey responses.

Though their small size often keeps them hidden from us, microorganisms are the most diverse type of living organism on the planet. Many people fail to appreciate the important role that microorganisms play in shaping the environment and our daily lives. As a species, we evolved and live in an environment shared with bacteria; thus, recognizing those connections is crucial for developing a holistic, biological world view. A prime example is that people have been using bacterial metabolic processes, such as lactic fermentation, in food preservation for at least 4000 years (Rotar et al., 2007). Long before the discovery of microorganisms, people noticed that fermentation alters the physical, bioactive, and nutritive properties of food, providing health benefits and enhancing flavor. Now, building on the tools and techniques that have advanced our scientific understanding of bacterial fermentation, we are equipped with hands-on approaches that can be leveraged in the classroom to introduce students to microbiology in a relevant and accessible way.

The modern yogurt-making industry is carefully monitored and standardized to maintain the quality and safety of the fermented products that contain live cultures. For example, the U.S. Food and Drug Administration (FDA) specifies that yogurt should be made only with *Lactobacillus bulgaricus* and/or *Streptococcus thermophilus* with one or more other optional ingredients allowed (US FDA 21 CFR 131.200). Therefore, the differences in taste and texture come from differences in the yogurt-making procedure, substrate source, and bacterial strains used (Pakpour & Hussain, 2020). Other probiotic bacteria such as *L. acidophilus* and *Bifidobacterium longum* may also be added to yogurt for gastrointestinal health benefits (Linares et al., 2017; Rezac et al., 2018).

Yogurt bacteria break down lactose in milk to glucose and galactose. Glucose eventually is metabolized into lactic and acetic acids. Those acids are the major byproducts of the yogurt fermentation process: commercial yogurts contain 1.2–1.4% of the acids and generally have a pH around 4.6, although it can range from 3.0 to 5.5. In such an environment, milk protein casein aggregates together forming a familiar yogurt gel structure (Yildiz, 2016). Additionally, heating the milk above 70°C leads to the denaturation of whey proteins that further thickens the yogurt (Ozcan et al., 2015). Another important compound produced by bacteria is EPS, or exopolysaccharides, a slime-like substance that gives yogurt its smooth cream-like texture (Yildiz, 2016; Zhang et al., 2011). Thus, lactic acid bacteria change the physical properties of milk, such as texture, viscosity, and smell, allowing students to evaluate microbial activity by measuring physical properties of yogurt without using special lab equipment. This multiweek, inquiry-based activity was run in a first-year undergraduate general biology laboratory course at Loyola Marymount University in 2020. Participants included 219 students from a range of life

science and non-science majors and academic levels. The activity was conducted over four 4-hour synchronous laboratory sessions utilizing Zoom and included dedicated times for opening lecture-style instructions, group work in “breakout rooms,” and ad hoc question and answer / troubleshooting sessions with a faculty or teaching assistant. To develop data management skills, each group maintained a digital, shared-access notebook using Microsoft OneNote and presented their findings in a cumulating 10-minute group oral presentation (see Supplemental Material for rubrics, available with the online version of this article). The objectives of this activity are to

- learn and apply basic microbiology techniques, including aseptic technique, to successfully culture yogurt.
- observe microbial growth in action through the transformation of milk into yogurt, and explore the effect of various factors on the process of fermentation and yogurt thickening.
- assess preliminary assay results and design a hypothesis-driven follow-up experiment
- synthesize generated data and communicate activity findings in an oral scientific talk.

Student Geographic Distribution & Technology Survey

Students were asked to take a precourse survey in which they informed the faculty of their geographic location, familiarity with different technological tools, and any concerns about technology and/or internet access. Students attended the class via Zoom from across the globe (Figure 1). While most students lived within the Pacific time zone, some students did have to cope with a large time difference. Approximately 10% of students were concerned about participating in synchronous class, and 20% were worried about having reliable internet access or other technology issues. Those students were contacted individually to make any necessary arrangements for Zoom recordings and asynchronous work options; however, all the students who completed the course chose to work synchronously with their group mates and attended the Zoom meetings. Students were also asked to name technological platforms used for academic communication that they were familiar with (Table 1). Zoom was the most popular answer. The concerns were addressed at the beginning of the course by providing students with instructions to communicate any technical difficulties immediately to their TA and instructor. The solutions to more common issues like OneNote not syncing were also made available so students could attempt troubleshooting on their own as well. This allowed us to resolve the majority of difficulties in the first weeks of the semester, and the rest of the course went very smoothly.

Table 1. Student familiarity with technology. Five most commonly cited platforms are reported based on 219 student responses.

Platform	Percent (Number) of Students Familiar with Each Platform
Zoom	80.4% (176)
Email	74.9% (164)
Google Classroom	29.2% (64)
Teams	5.9% (13)
Brightspace	4.6% (10)

Yogurt-Making Method

In the first week of the experiment the students were introduced to laboratory aseptic technique principles (via video and Kahoot quiz, which is in the Supplemental Material) and the general procedure of making yogurt. Students were instructed to practice aseptic techniques, like cleaning all the tools with soap, while preparing their yogurt using the following method. One cup of milk was heated uncovered until it reached 85°C–90°C or small bubbles began to form on the surface. It's recommended to wipe the bottom of the pot with ~1/4 teaspoon of oil to prevent scorching of milk. If film formed on top of the milk, it was removed by skimming the top of the milk with a clean spoon. The milk was allowed to cool to approximately 55°C, a temperature at which a student can comfortably hold their hand on the side of the pot. One tablespoon of yogurt was then mixed into the milk. The pot was covered and placed in a constant temperature environment at 43°C–46°C for four to eight hours. An oven with the light on can provide such an environment. Starting at the fourth hour of incubation, the yogurt was checked hourly. Pictures were taken, and students recorded any qualitative data on smell and visual appearance during these checks. After eight hours of incubation or after the yogurt solidified, the pot was moved in the refrigerator for either eight hours or overnight before performing final evaluations.

We have created two versions of the video instructions, depending on the kitchen supplies available for the students.

- Demonstration video of yogurt fermentation setup using oven by T.A. Grace Riggs: <https://vimeo.com/455557587>.
- Demonstration video of yogurt fermentation setup using Instant Pot by Instructor Tatiana Kuzmenko: <https://vimeo.com/455633935>.

After the first round of yogurt making, students met on Zoom, shared their observations on the qualitative data (smell, thickness, appearance, color, whey separation, etc.), and discussed the similarities and differences.

Then students were introduced to the following concepts of experimental design.

- Testable hypothesis—a proposed explanation for an observation/process/phenomenon that may be supported or rejected as you make observations, run experiments, and draw conclusions.
- Independent variable—a parameter/variable that is not changed by other variables.
- Dependent variable—a parameter/variable that is changed by the independent variable.

- Control—an experimental unit that is not altered/held constant during an experiment.
- Treatment—an experimental unit that is altered during an experiment.
- Replicate—multiple copies (biological replicate) or measurements (technical replicate) of a control or treatment.

Below is the detailed description of an example experimental setup for the yogurt fermentation.

- Positive control: 1 cup of whole cow milk heated to 72, inoculated with 3 tablespoons of cow milk yogurt culture, and incubated at 44°C for 6 hours.
- Negative control (optional): The milk you used for your experiment, untreated. (If you decide to heat and incubate it without adding yogurt, keep in mind bacteria may still be introduced from the environment as we can't maintain sterility in the kitchen.)
- Treatment/alteration: Add 1 tablespoon of culture.
- Treatment/alteration 2 (optional): Add 2 tablespoons of culture.
- Replicates: Technical replicates help us estimate the accuracy of our measurements (you use the same batch of yogurt) and calculate an average reading, and biological replicates help us see if the treatment leads to a consistent effect (you use the different batches of yogurt treated in the same manner). Biological replicates can be set up by different group members. If group members' control measurements are noticeably different, it is recommended to use percentage difference between control and experimental group as a less biased way to compare biological replicates. The following video illustrates this approach: <https://www.youtube.com/watch?v=AeN7iXoiqOo>.

Technical replicates should always be set up by the same person. In order to perform statistical analysis, students would need at least three replicates. To check students' understanding of the experimental design process, the class played Kahoot (link provided in Supplemental Material) and discussed difficult questions.

Next, the TA provided students with some ideas of what independent variables can be modified in this experiment:

- Different yogurt starters (regular, Greek, French)
- The amount of the starter (# of tablespoons)
- Type of milk used (whole, 2%, almond milk, coconut milk, etc.)
- Incubation temperature (oven with light on or off)

Afterward, the section was divided into groups of three to four students, and they were sent into Zoom breakout rooms to discuss their unique experiment and work together on the OneNote collaboration page, created in advance by the TA (Figure S1) filling out the following prompt:

- We hypothesize that
- We will use the following materials:
- Our independent variable(s) is/are:
- Our dependent variable(s) is/are:
- Our control(s) is/are:
- Our sample size is (amount of replicates):

- Our experiment will be conducted by (state how long it will run, under which conditions, etc.):
- We will quantify our results by (what will you measure?):
- OPTIONAL -We will use the following statistical test:
- We predict (state your predicted outcome(s) for this experiment [if/then]):

Before proceeding with their experiment, students sent their proposals to the TA or instructor for approval. Figure 2S demonstrates several examples of the student yogurt-making setups. An example of a TA Zoom meeting agenda is provided on Figure 3S.

Yogurt Viscosity Assays

As described earlier, the production of acids by fermenting bacteria leads to milk protein conglomeration and, as a result, the thickening of yogurt. Thus, yogurt viscosity evaluation was the central assay for the estimation of fermentation success. Students and instructors developed a number of tests for quantifying differences in viscosity among batches of yogurt. Below we describe the two that have shown the most consistency among technical replicates—the Run Test and the Splat Test (Figure 2).

Run Test: The Run Test was used in 23 out of 61 experiments. This test consisted of allowing the yogurt to travel down an inclined surface and measuring its speed and, therefore, its viscosity: more viscous yogurt traveled slower. The speed was calculated either by measuring the time it took yogurt to travel a set distance or by measuring the distance traveled in a set time. An example setup is illustrated in Figure 2A where the student places a leveled tablespoon of yogurt from each jar while the surface lays flat (1), then one side of the surface is lifted at a 45-degree angle (2), and in 30–90 seconds (the optimal time may vary between the groups depending on how quickly the thinnest yogurt travels) student records the distance covered by each sample (3). The surface can be cleaned and the test repeated with another spoon of yogurt to create multiple technical replicates. The students in a group were responsible for keeping the surface as consistent as possible within a group. For example, all group members would agree to use foil, glass, or baking sheet as a surface.

Splat Test: The Splat Test was utilized in 16 out of the 61 experiments. An example setup is illustrated in Figure 2B where the student drops a level teaspoon of yogurt from a specific height, like 30 cm, onto a flat surface such as a cutting board or countertop. Again, the group members coordinated to keep the landing surface as consistent as possible. The widest diameter of the “splat” was measured with a ruler. A larger diameter signified a less viscous yogurt. The surface can be cleaned and the test repeated with another spoon of yogurt to create multiple technical replicates. For all viscosity tests performed, it was emphasized that the yogurt should be mixed thoroughly beforehand, and all the surfaces should be cleaned between trials to ensure consistency for all three technical replicates.

pH Assay: For those students that happened to have access to a pH meter or pH paper that could detect the changes between ~6.5 and 4.5 pH, this assessment was quite successful and accurate in the evaluation of the fermentation process. Thus, if possible, we recommend that instructors consider supplying students with a pH measuring tool. This would enhance their fermentation exploration experience.

Data Analysis and Statistical Tests

Students were introduced to basic statistical analysis such as calculation of average and standard deviations for building graphs using Excel (see the video <https://www.youtube.com/watch?v=AeN7iXoiqOo>) as well as *t*-test and one-way ANOVA for determining if the differences observed are statistically significant. We used <http://vassarstats.net/> to run the statistical tests.

Background Literature Search Supporting Experimental Outcomes

Students were encouraged to use Google Scholar or PubMed to search for publications related to their project and explore the background information of how factors and variables that they manipulated may affect the fermentation process. At a minimum, two scientific publications were required to be cited in the presentation. Based on the conclusions that students made from their data and

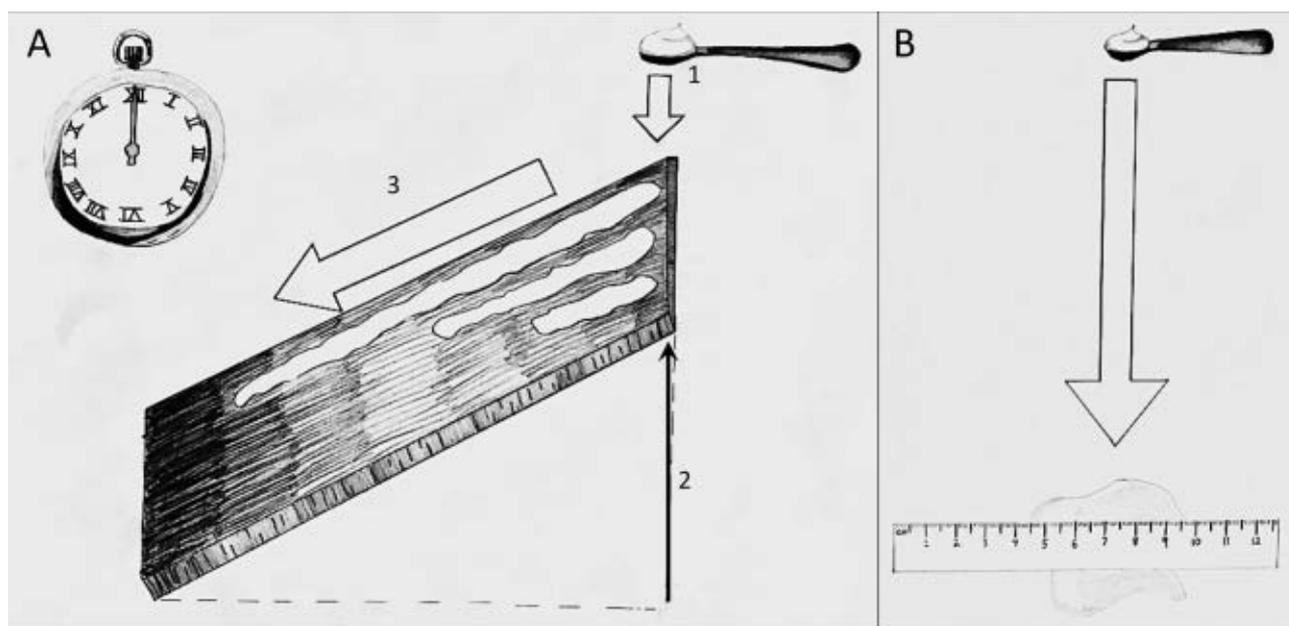


Figure 2. Schematic representation of yogurt viscosity evaluation assays: (A) run test and (B) splat test.

their literature review, students formulated and presented to their class a follow-up future experiment that would potentially address the questions left unresolved.

Group presentations. Students presented their data in the 10-minute group PowerPoint presentation, delivered via Zoom. We allowed students to prerecord their presentation and share the video with the TA ahead of time if they anticipated any technical issues for the synchronous delivery. The link to the instructions and the grading rubric for group presentation are provided in section 1 of the Supplemental Material.

Experimental outcomes. In the first week of the exercise, almost every student set up a trial batch of yogurt. In the following Zoom meeting, students discussed the factors that may have contributed to the differences in physical appearance in their yogurt or the time it took for their yogurt to thicken. For example, many have observed that variation in the incubation temperature had a strong effect. In the second week of the experiment, each group used observations from the initial trial to brainstorm and design an experiment that included changes on one of the independent variables that all group members could reliably measure and compare. TA provided feedback on their proposal and approved it before they could proceed. In the third week of the experiment, students met on Zoom to collect qualitative data, describing the smell, color, and textural appearance of their yogurt and taking pictures, as well as quantitative data, measuring yogurt viscosity or in some cases pH. The students were allowed to use one of the suggested viscosity tests described earlier or come up with their own. The data was recorded in the personal OneNote notebooks. Next each group member's data (pictures, descriptions, and measurements) was combined on the shared collaboration space, and the analysis was performed as

a group activity in breakout rooms during the Zoom session (Figure S1). Students worked on their presentations in Google Slides. Those collaborative platforms have been extremely useful in involving every group member in the process of data analysis. Additionally, this type of group work often resulted in peer-to-peer teaching and active exchange of knowledge between students. Student peer evaluations (Figure S4) and course evaluations (see section 4 in the Supplemental Material) confirmed that students were able to build productive relationships with each other.

We recognize that this yogurt-making activity could be simplified and adapted for smaller classes, younger students, or shorter than four-week activities. We have summarized our recommendations in Table 2. For example, if the students are not ready for statistics or if the group work is logistically problematic, those components could be excluded without losing the benefits of exploration on how microbes alter their environment. We have described the advanced level of the activity in this paper that worked very well for the first-year undergraduate laboratory course and would likely be successful in the upper division microbiology courses as well. For the benefit of other educators, we analyzed all 61 student experiments, removed setups with experimental design flaws (for example, no proper replicates were set up), and organized the remaining 35 experiments into 6 major categories, providing examples of factors altered and indication of how often the changing factor affected the viscosity of the final product (Figure 3). Thus, you can appreciate that multiple factors affect yogurt viscosity, including changes in the substrate, starter type, addition of sugar, and the duration of preliminary milk heating. The full list of all significant factors with the specific examples is summarized in Table 3.

Table 2. Suggested modifications for the yogurt-making activity to provide flexibility on the level of complexity required for a specific course.

Activity Components	Level of Complexity		
	Basic	Intermediate	Advanced
Experiment Designed by Students (Rather than Instructor)		X	X
Make Yogurt	X	X	X
Technical and Biological Replicates		X	X
Test Various Yogurts	Select 1 setup	Select 1 setup	Select 1 setup
Test Various Substrates			
Test Sugar Differences			
Test Fermentation Temperature			
Test Heating Time			
Test Acidity			
Qualitative Observations	X	X	X
Viscosity Assay	X	X	Select 1 or both assays to measure fermentation
pH Assay			
Group Work		X	X
Data Analysis and Statistical Tests		X	X
Research for Explanations			X
Presentations		X	X
Design Follow-up Experiments			X

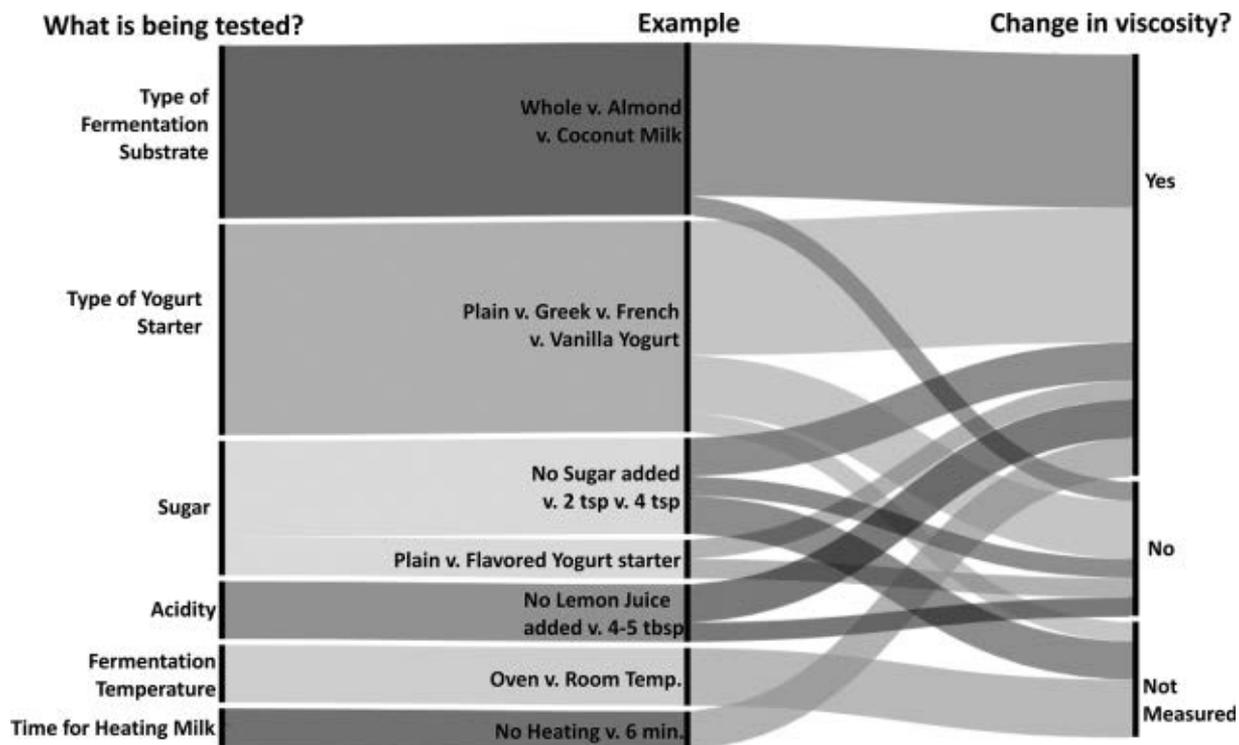


Figure 3. Summary of 35 yogurt fermentation experiments arranged by the variable tested, an illustrating example, and its effect on yogurt viscosity. Note, metrics besides viscosity, such as pH and time taken to solidify, were measured but are not shown here.

Table 3. Summary of factors that affected yogurt viscosity in student-developed experiments with specific examples that have shown statistically significant differences.

What Is Being Tested	Effective Experiment Examples
Type of Fermentation Substrate	Whole milk versus coconut milk
	Coconut milk versus almond milk
	Whole milk versus oat milk
	Whole milk versus goat milk
	Oat milk versus goat milk
Type of Yogurt Starter	Greek yogurt versus plain yogurt
	French yogurt versus plain yogurt
	Yakult* versus plain yogurt
	Kefir versus Yakult
	Chobani versus Lucerne
	Activia versus Yoplait
Sugar	No added sugar versus 4 teaspoons of added sugar
	Various flavors of Chobani versus plain Chobani
Acidity	4–5 tablespoons added lemon juice versus no added lemon juice
Fermentation Temperature	23.9°C versus 48.9°C
	28.3°C versus 48.9°C
Milk Heating Time	0 minutes versus 6 minutes
	6 minutes versus 9 minutes

○ Conclusion

The high level of variation among students' preliminary results should be expected given the variety and largely uncontrolled environment of their homes and the diversity of tools and quality of materials used. Inspiringly, these results motivated students to compare their data and look for factors they did not initially consider, such as the amount of added sugar. Students were challenged to actively discuss their findings within their groups and collaboratively think through how to modify activity components in their follow-up experiments (Table 2). As a result, students produced clever and unexpected approaches to examine their data. One commonly encountered challenge was optimizing their methods on measuring yogurt viscosity to produce consistent results across different kitchen environments. In cases where the control measurements were quite different between the group members (biological replicates), the group used the percent change compared to the control instead of an absolute value, making sure that each member collected the measured viscosity multiple times (technical replicates). Importantly, in this new remote lab we saw a higher-than-average proportion of meaningful follow up experiments designed by the students, as compared to observations made in Kuzmenko et al. (2021), indicating an increased level of involvement in the outcome of the project.

In general, students indicated in the end-of-the-semester feedback that they appreciated the hands-on collaborative approach of this exercise. Notably, we conducted other remote laboratory activities through the fall 2020 semester and students singled out this yogurt lab as the one they enjoyed and learned the most from. As one student wrote in their course evaluation, "I enjoyed the yogurt-making lab the most. We learned about biological processes and ran experiments to see which yogurt-making method worked the best. That lab felt the most like what I expected a bio lab to feel like."

On the downside, many students pointed out a considerable time commitment for this lab, particularly to gather necessary materials and to fully run their experiments, which sometimes extended beyond the dedicated four-hour weekly lab period.

Although this is our first attempt at conducting a massive (200+ students, 15 sections) remote lab, we are confident that this activity provides a unique opportunity for students to do a hands-on collaborative microbiology experiment using common household materials and tools in the settings of a remote lab environment. While the current remote learning circumstances associated with the COVID-19 pandemic will subside and in-person instruction will return, we are encouraged by the outcomes of this activity, and we aim to leverage lessons learned into future laboratory activities.

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