Bringing Pasteur Back to Life: Studying the Biochemistry of Yeast Fermentation Through Discussion Groups and an At-Home Lab

Kimberly A. Wodzanowski\(^1\), Madison V. Anonick\(^1\), Lauren A. Genova\(^1\), April M. Kloxin\(^2,3\), and Catherine L. Grimes\(^1,4\)*

\(^1\)Department of Chemistry and Biochemistry, University of Delaware
\(^2\)Department of Chemical and Biomolecular Engineering, University of Delaware
\(^3\)Department of Materials Science and Engineering, University of Delaware
\(^4\)Department of Biological Sciences, University of Delaware

Abstract
This hands-on, student-centered biochemistry lesson introduces beginner biochemistry students to the techniques of effectively reading and discussing primary literature, identifying fundamental biological concepts, and applying that knowledge to design their own experiments. Students begin by reading Louis Pasteur’s article on the discovery of fermentation, a key biochemical concept in metabolism. Using guided questions while they read the primary literature, students dissect the key biochemical concepts of fermentation in student-led discussion groups. Following the group discussion, students “act like Pasteur” by designing their own lab experiment to collect similar data to that in the paper. For the lab activity, students utilize standard home-kitchen techniques and food-grade reagents to grow yeast in different microenvironmental conditions, such as temperature, pH, presence of oxygen, and substrate concentration in water. Here, students apply key laboratory skills such as designing experiments with proper controls, keeping a lab notebook, and communicating results. Students are given the opportunity to pursue variables they find interesting by performing experiments in their own home. Student understanding is assessed through group discussion, completion of learning issue questions, multiple choice quiz questions, midterm questions, and a lab report. This lesson features a diverse array of activities: reading and understanding the primary literature, participating in scientific discussion in small groups, and designing and performing experiments, all essential skills for any future biochemist.

Learning Objectives
Students will be able to:

◊ read primary literature, identify fundamental concepts such as equilibrium/ Le Châtelier’s principle, and translate science into everyday examples.
◊ develop a testable hypothesis and identify the appropriate experimental observations and control variables.
◊ predict outcomes, ask questions, analyze data, design and conduct experiments, gather data, make observations, and relate results back to original hypotheses.
◊ predict the growth behavior of microbes based on their growth conditions, e.g., temperature, available nutrient, aeration level, etc.
◊ describe how very high (or low) temperatures, pH, or salt concentration inhibit growth (e.g., membrane stability, enzyme activity, proton motive force, etc.).
◊ explain the importance of microbial fermentation products to food/beverage production (e.g., bread, cheese, yogurt, wine, beer, etc.).
◊ provide examples of essential microbe-microbe or microbe-host relationships related to Pasteur’s work.
INTRODUCTION

Introduction to the Pedagogy

The French chemist and microbiologist Louis Pasteur discovered that microorganisms cause fermentation. Pasteur is also well known for his discovery of pasteurization in food preparation and storage, development of germ theory, and vaccines for anthrax and rabies. Not only did Pasteur's discoveries have instrumental implications in the fields of health and preservation, but they also opened new doors and research directions in the field of biochemistry as we know it today. Understanding and deconvoluting complex biochemical pathways, such as fermentation, are key to any biochemistry student's education (1).

Previous lessons have been developed to demonstrate different types of fermentation, such as preparation of yogurt to study lactic acid fermentation and isolation of yeast—a eukaryotic, single-celled, fungal microorganism—to utilize for brewing (2, 3). For lessons studying alcoholic fermentation, collection of carbon dioxide in vessels relies heavily on access to laboratory equipment (4, 5). With the switch to online learning due to the COVID-19 pandemic, we desired to create an effective lesson that engaged students to think critically about biochemistry and allowed them to do the experiments without the use of a formal laboratory space. This lesson focuses on the discovery of fermentation by Louis Pasteur utilizing primary literature, student-guided group discussion, and at-home labs where students can try out critical experiments in this discovery for themselves in their own kitchens.

This lesson is novel due to the incorporation of several layers of student-centered pedagogical techniques. We first build on Dr. Herman Epstein's ideas of utilizing primary literature to drive group discussions (6). Traditional teaching of biochemistry has relied on lectures and reading of biochemical concepts from a textbook. However, being able to read and understand primary literature is extremely valuable for a career in biochemistry. By critically reading and analyzing the primary literature, students begin to understand the nature of research, scientific communication, and scientific ethics. These are equally as important skills for a successful biochemist as understanding the biochemical concepts interwoven throughout the literature (7). Students who read the primary literature have benefited in their ability to understand the process of science, think critically, and evaluate evidence (8). This lesson also incorporates problem-based learning (PBL), where students collaborate in small groups to answer complex open-ended questions about fermentation. By pairing the primary literature reading with PBL student-guided discussion, students are able to dissect the concepts related to biochemistry in the traditional lecture-style classroom (7). Finally, this lesson allows students to apply what they have learned in self-designed at-home experiments. This at-home lab provides students with the opportunity to further engage with the literature and these biochemistry concepts by promoting higher-order thinking (as students explore the top levels of Bloom's Taxonomy: creating, evaluating). Moreover, the at-home lab component of this lesson provides students with agency (i.e., freedom in the decision-making process) by allowing them to choose which variables they would like to explore when investigating fermentation. A plethora of research suggests that promoting student agency in the classroom can help improve students' engagement and motivation in the sciences (9, 10).

Introduction to the Science

All life requires energy to survive. Respiration, the chemical breakdown of molecules such as carbohydrates, is a common mechanism by which cells release energy. Indeed, respiration can be broken down into two major categories: aerobic and anaerobic. Aerobic respiration requires the presence of oxygen to break down carbohydrates like glucose to create adenosine triphosphate (ATP) energy for cellular processes. However, when there is a lack of oxygen, cells go through an anaerobic respiration process called lactic acid fermentation, in which glucose is broken down into lactic acid instead of fully degraded to produce energy. This process can be commonly found in the production of yogurt and sourdough bread using microbes and in our own muscle cells during exercise. In some microbes, the final product of their fermentation is not lactic acid, but instead ethanol in a process commonly known as alcoholic or ethanol fermentation. This occurs when glucose is broken down into alcohol and carbon dioxide molecules and is commonly used in the production of beer and wine.

Pasteur's fermentation discovery was read at a meeting on August 3, 1857, of the Scientific Society of Lille and published in Annales de chimie et de physique. In this lesson, students read the translated transcript of this discovery entitled “Pasteur’s Memoir on Fermentation,” from James Bryant Conant’s “Harvard Case Histories in Experimental Science” (11). Here, Conant points out that, in the 1857 article, Pasteur details lactic acid fermentation and his discovery of a “new yeast” that produces lactic acid, which is different from the ethanol that Brewer's yeast produces. Pasteur analyzes the differences he observes in fermentation by the inclusion of a nitrogenous plastic substance, presence or absence of oxygen, inclusion of chalk (or calcium carbonate) as a buffering system, and changes in pH. In this lesson, students “act like Pasteur” by designing their own experiment with variables such as temperature, pH, and substrate concentration to see how they affect yeast fermentation.

Intended Audience

This lesson was implemented in an introductory biochemistry course for sophomore undergraduate biochemistry majors that combines elements of two independent courses: a lecture component and a laboratory component. The “lecture” component of this course met synchronously twice a week over Zoom in 75-minute sessions for group discussions centered on the assigned primary literature readings and the major biochemical concepts covered within those articles. Although listed by the registrar as a “lecture” class, this portion of the class was primarily focused on student-led group discussions with short (10–15 minute) lecture presentations by the instructor to jumpstart the discussion topics. If desired, the instructor's lecture regarding the introduction to the paper and its biochemical concepts could be pre-recorded and watched asynchronously prior to the lecture class to provide more group discussion time in the synchronous portion. The laboratory component of this course consisted of a weekly three-hour laboratory period, where students completed hands-on lab experiments as individuals or partners. Most of the students were enrolled in both portions of this course, but
some students took only the lecture or the laboratory portion. Although targeted at sophomore biochemistry students, this course is of interest to other life science majors of varying levels (sophomores to seniors), including biology and chemistry majors and those interested in attending medical school. For almost all of the students, this course is their first college biochemistry class and laboratory.

Required Learning Time
The total time for this lesson and the accompanying at-home lab activity is approximately (~) 6–8 hours over the duration of the synchronous and asynchronous parts (Table 1). Students will need about 30–60 minutes to read the paper and answer the discussion guide prior to the in-class discussion. The in-class discussion aspect is designed for a 75-minute lecture class period, with about 30 minutes for an introductory lecture and 45 minutes for student group discussions, but can be altered to span multiple lecture class periods of shorter lengths with group discussion over two class periods (Supporting File S2). Students will then take about 25 minutes to plan out their experiment and 45–90 minutes to collect their data in the at-home lab activity. Finally, writing the laboratory report will take about 2–3 hours total.

Prerequisite Student Knowledge
Students are expected to have been introduced to foundational biology and general chemistry concepts in those respective courses. Students should be familiar with writing chemical equations, equilibrium and buffer calculations, and the basics of fermentation. Students should also be familiar with basic experimental design from their introductory science laboratory courses, ensuring the use of proper controls, how to collect and analyze data, proper lab safety and etiquette, and how to write a lab report.

Prerequisite Teacher Knowledge
Instructors with a graduate degree in biochemistry, biology, or chemistry or related disciplines will be able to teach this lesson. Instructors should have a basic knowledge of chemistry and biology including metabolic pathways of glycolysis and fermentation (both lactic acid and alcoholic), chemical reactions, and equilibrium. Instructors should also be familiar both with reading and discussing primary literature as well as basic laboratory skills (e.g., setting up a laboratory notebook, good experimental design, and safe laboratory practices).

SCIENTIFIC TEACHING THEMES

Active Learning
Before the lecture class, students are provided a copy of the Conant translation of Pasteur’s paper, “Translation of Portions of Pasteur’s Memoir on Lactic Fermentation,” (11). They are encouraged to read the article on their own, write down questions, and attempt to look up topics that they do not recognize; they also should take detailed notes on the information they gathered (Supporting File S1). Then, during the in-person or synchronous online lecture class, the instructor gives a brief lecture on Pasteur (Supporting File S2), and students assemble into small, assigned groups to discuss the main aspects of the article and clarify any questions they have with their group members. During this time, the instructor and any teaching assistants rotate among groups to facilitate discussion. If this is done in electronic format, breakout rooms can be utilized. Students are guided in their discussion using Supporting File S3. Students then share their findings and main discussion points with the entire class in a large group setting. Outside of lecture class time, the students work independently at their own pace to design their own experiment/hypothesis for the at-home laboratory portion of the lesson (Supporting File S5). This hands-on laboratory activity allows the students to expand their knowledge in the classroom and apply what they read in a creative manner.

Assessment
Before the lecture class, students individually answer a question sheet outlining the main concepts of the paper, which is uploaded to the course Canvas site to check for completion at the beginning of the class session (Supporting File S1). Students then work in small groups to discuss the article and answer a set of “learning issue” questions for submission (Supporting File S3) (12). Learning issues are instrumental components of PBL courses that are critical for self-directed learning (13, 14). These learning issues are questions that students identify as gaps in their knowledge while reading an article they need to resolve in order to better understand an article (12). Additionally, students work individually or in groups to plan their at-home experiment based on what they learned in the paper and from the in-class discussion, execute the experiment, and write a lab report. The focus of the lab report is to provide students practice in designing and executing their own experiment as well as writing all the necessary sections common in scientific publications, including an introduction, materials and methods, data figures, and discussion/conclusions. Student learning is also assessed with an online, asynchronous multiple-choice quiz that was then retaken in groups in the synchronously online class and an online, asynchronous, open ended midterm exam. The questions in both the quizzes and midterm focus on the core biochemical concepts discussed from the paper such as equilibrium, Michaelis-Menten kinetics, pH, and chirality as well as designing experiments “like Pasteur” to produce data for different types of fermentation.

Inclusive Teaching
In the lecture class, students are placed in heterogeneous discussion groups that are organized with a balance of GPA, gender, race/ethnicity, and graduation year. At the beginning of the semester, students answer an informal questionnaire regarding career goals, prerequisite knowledge about certain course topics, and hobbies. These responses help the instructor balance the groups to include a mixture of different students and support students’ varied learning interests and/or personal needs during the semester. Students typically work in groups of four to five with the instructor and teaching assistant(s) jumping from group to group (in various breakout rooms on Zoom when taught online) to support discussion. In this format, attention can be focused on groups that need more individual support. Small group discussions are led by the students, allowing them to focus on the concepts they find most interesting in the paper. Small group discussions also allow students to be more comfortable in sharing their ideas as compared to feeling that they are required to report out to
the entire class (15). Students assign roles within their groups (such as discussion leader, scribe, and reporter to the main group) and can rotate these roles as the course moves through different primary literature papers. At the end of the course, each student provides a written evaluation of the contributions of their group members throughout the course, which is incorporated into an overall class participation grade.

For the at-home laboratory, students are able to design their own experiment from a list of suggested variables, allowing students to focus on areas more interesting to them when creating a hypothesis. This increases the motivation and engagement of the students with the lesson (16). There is no time limit to the at-home experiment, which removes the pressure of requiring labs to be completed in a specific time frame that may not be suitable for all students and their learning preferences/needs. Students are assessed on the material in the form of group discussions, written lab reports, a multiple-choice quiz, and a midterm exam, providing the students multiple avenues of showing their understanding. This combination of formative and summative assessments provides a more holistic view of students’ understanding as opposed to oral or written presentations only, where some students may struggle based on the different ways in which they prefer to demonstrate their knowledge.

LESSON PLAN

Summary

There are three components to this lesson: (i) a pre-lab discussion of primary literature (during lecture), (ii) the independent lab activity, and (iii) the post-lab report assessment (Table 1). The first part of this lesson is completed in a synchronous manner, during one 75-minute lecture period in which students discuss the literature. The at-home lab portion of the activity is performed asynchronously and is flexible in the time constraints (though it typically takes about 45–90 minutes depending on set-up time). After performing the lab activity, students have one week to write and submit their final lab report.

Synchronous Primary Literature Discussion

To teach this lesson, students first read Pasteur’s fermentation paper on their own, writing down key findings and questions they had while reading the paper using Supporting File S1. This summary sheet guides the students through recording the most important points from the paper and noting areas of confusion or unknown concepts to bring to class discussion. Note that this is most likely the first time these students are reading primary literature like this, so using the discussion guide will help orient discussion in the groups. We recommend providing students with Pasteur’s fermentation paper at the end of the lecture class preceding this lesson, where students have until the next lecture class period (i.e., the start of this lesson) to read through and complete the study sheet (approximately two days for a lecture class that meets 2–3 times each week). In a synchronous lecture class, the instructor briefly presents an introductory lecture to Pasteur, and the main findings of Pasteur’s paper are then discussed as an entire class (use Supporting File S2 as a guide of potential lecture points). Then, students assemble into their assigned smaller discussion groups of 4–5 students to discuss the paper in greater detail. In particular, we encourage students to explore the different experimental variables Pasteur tested and what effects they had on the fermentation he observed. The instructor and any teaching assistants can rotate around from group to group to help guide discussion around learning issue questions and other questions/concerns the groups may have (Supporting File S3).

Asynchronous At-Home Lab Activity

Prior to beginning the setup for the experiment, students should design their experiment for examining the effects of oxygen concentration and then select at least one other variable to test. The first week of the experiment will include an explanation of the assignment during the weekly scheduled lab time and signing of the safety contract (Supporting File S4). Students will then have a week to complete the experiment, during which students can email any questions to the instructor/teaching assistant or attend the instructor/teaching assistant’s office hours for any clarifications prior to beginning the lab activity on their own. The following week’s synchronous lab time will then be dedicated to discussing experimental results, troubleshooting for those wishing to repeat the lab on their own time, and providing guidelines for the lab report.

For the lab activity, students utilize standard home-kitchen techniques and food-grade reagents collected on their own to grow yeast with different microenvironmental conditions (e.g., temperature, pH, oxygen, substrate concentration in water) (Figure 1). The students can work collaboratively in small groups (of 2–3 students that they select) for the at-home lab activity (although, due to COVID-19, most of our students chose to work individually). Students should follow the laboratory instructions, which are described in brief here (Supporting File S7) and were inspired by science activities within the public domain. When students are ready to perform their experiments, they should clear a surface and place appropriate secondary domain.

Figure 1. Overview of the at-home experiment workflow.
contamination on it (e.g., large sheet pan). To protect themselves and the experiments, students should clean all surfaces before and after use of a microorganism (baker's yeast, in this case). Students should then gather relevant supplies and bring them to the laboratory space they set up. Students who do not have access to supplies can reach out to the instructor for loaner supplies, or instructors can opt to provide a kit of supplies (e.g., disposable plastic cups, measuring devices) for each student to take home. For each experimental condition the students set up, they should utilize the same amount of (i) water per glass container (approximately ½ cup [c.] of water is suggested per glass container) and (ii) yeast per glass container (approximately 1 teaspoon of yeast per glass container is recommended to conserve this reagent).

Each student must test the effects of oxygen concentration on yeast growth and then select at least one other variable such as temperature, substrate concentration, or pH for testing its effects on yeast growth over a short timescale of about 45–90 minutes. For measuring the oxygen concentration, an open glass container can be considered an aerobic condition and a covered glass container can be considered an anaerobic condition. Each student will then select an additional variable to test from the following: (a) Temperature, (b) Substrate concentration, or (c) pH.

(a) Temperature: Students can collect tap water into an appropriate larger glass container and place it (i) on the countertop for an extended period of time to ensure room temperature (RT ~20–25 °C) or (ii) in the refrigerator (~4 °C). If students have access to appropriate equipment for safely heating water (e.g., tea kettle, stove and pot, microwave), water can be heated to achieve different temperatures. Water can be boiled using an appropriate apparatus and then allowed to cool slightly for ~5 minutes before carefully transferring it to a desired container for future use (see Supporting File S5). Different ratios of hot (~80 °C) and RT water can be gently combined for achieving a range of temperatures. If students have an appropriate food-grade thermometer available, they can use it to measure temperature. Alternatively, students can estimate temperature for different ratios of hot water and RT water mixed together using this rough calibration that we have prepared: RT ~20 °C; ¾ c. RT + ¼ c. hot ~36 °C; 2/3 c. RT + 1/3 c. hot ~40 °C; ½ c. RT + ½ c. hot ~46 °C; 1/3 c. RT + 2/3 c. hot ~59 °C; ¼ c. RT + ¾ c. hot ~65 °C; and hot ~80 °C at sea level. Students should consider ways to maintain temperature (e.g., water bath or insulation of container) and monitor temperature over the experimental time course.

(b) Substrate concentration: ~1 teaspoon of sugar in ~½ c. of water can be considered a standard growth condition. It is recommended the students use standard table sugar, but students can utilize different sugar types, such as artificial sweetener, to investigate how the changes in sugar structure could affect the fermentation rate (17). Higher and lower concentrations of sugar can be achieved by controlling the amount of sugar added to water.

(c) pH: The pH of water varies based on its source. Tap water typically varies from pH 6.5 to 8.5, and distilled water has a pH ~5.8 when exposed to room-temperature air. Further, tap water from different municipalities or well sources may contain different trace contaminants. Consequently, the source of water used can be considered a variable. Food-grade distilled white vinegar purchased from the grocery store typically has 5% w/v acetic acid and a pH ~2.4; pH resulting from its dilution was considered relative to a control at a neutral pH. Students who choose this variable should be aware of an important safety note that stronger non-food-grade forms of vinegar are NOT permitted and should NOT be used; stronger concentrations are extremely hazardous and can ONLY be used in a laboratory setting (e.g., in a chemical fume hood and only when wearing appropriately increased levels of personal protective equipment). As discussed in class, chalk has limited solubility in water; therefore, an approximate ¼-inch piece of chalk can be broken up (with a hammer or the side of a tin can) and added to the solution of yeast and sugar in water. In addition, students were given the option to use baking soda as a readily available household replacement for chalk. Students should only test one variable at a time, keeping in mind that they should not mix acidic and alkaline components, such as vinegar and baking soda or chalk. This is an excellent opportunity to discuss the nuances of experimental design.

When the students start their experiment, water of a desired temperature should be placed in the appropriate glass container within secondary containment. Then, the sugar and variable alkaline or acidic components should be added followed by addition of the yeast. The container can be kept or covered, as desired. As the yeast grows, carbon dioxide is produced, yielding a foam on top of the surface of the water. Using a ruler or similar measurement tool, students will measure the height of the foam over time (e.g., every 5 minutes for up to 30 minutes) as an indirect measure of yeast growth and record the data on their observation sheet (Supporting File S6). These data can be analyzed to compare the effects of different experimental conditions on the rate of yeast growth. Since all reagents are food-grade for in-home use, once the experiment is complete, aqueous suspensions of yeast in the water at RT can be poured down the drain, and glass containers and other apparatuses can be washed with dish soap and then cleaned with water, as appropriate.

Students should carefully consider factors that impact yeast growth when selecting variables to probe individually or in combination. For example, when probing effects of variables besides temperatures, students should use a sufficiently ‘warm’ temperature (RT to 40 °C) to promote sufficient yeast growth for measurable foam height over the experimental time frame. Further, students should select containers with consistent and appropriate cross-sectional area for the fluid volume to give sufficient foam height for easy measurement. Students should consider ways to minimize error and enable sufficient replication for drawing conclusions. Only linear data should be fit to determine approximate growth rate for these short time experiments. It is important to use this opportunity to discuss the validity of data and best practices for determining if the data can accurately report on the variables being tested.

Post-Lab Report Assessment

Following completion of the laboratory experiment, students are tasked with writing a manuscript-style lab report to explain their findings. Prior to instructing students to write the lab report, the instructor/teaching assistant will walk students through their expectations and the rubric (Supporting File S7). In addition to submitting their lab report, each student is also required to turn in their observation sheet (Supporting File S6). Students are graded on the quality of the lab report based on
I think the best way to design this course is to use the PBL structure was a very cool way to investigate the development of biochemistry as a field and introduce research areas very cool way to investigate the development of biochemistry as a field and introduce research areas.

Many students also enjoyed the PBL format utilized in this lesson to solve these open-ended questions in biochemistry by guiding themselves and their groups through the learning process. Students continued to use this PBL format throughout the rest of this course. One student said, “I think the best part of this course is the problem-based-learning format. It allowed me to learn material in ways that I didn’t know were possible and definitely prepared me for formatting like this in the future. I pretty much learned how to teach myself, in a way, which I know will be extremely valuable in the future.”

Other students commented on how “the PBL structure was a very cool way to investigate the development of biochemistry as a field and introduce research areas.” They noted that “I thought the problem based-learning format was a great aspect to this course because I’ve never experienced anything like this and I found new ways to learn, study, and prepare for exams that I can use in the future,” and that “Problem based learning worked tremendously for me because retention isn’t one of my strengths. Instead of memorizing facts, [the instructor] encouraged understanding of the material by helping us think about manuscripts in different ways.” Another student remarked that “more classes should be designed this way. There is a time and place for lectures (survey courses, etc.), but this course was perfect for a PBL format. Education should be expanded beyond the course concepts to more general skills about working in teams, communicating, and critical thinking. This course was excellent at that.”

This lesson can be modified to adapt to both in-person and online learning with synchronous and asynchronous components. Prior to the COVID-19 pandemic, this course and this lesson were taught in person. Having access to a classroom with round tables instead of traditional desks has promoted productive group discussion for the primary literature aspect of the lesson. Instead of the fermentation lab being completed at home, the laboratory component of this course could take place with the same equipment or more advanced equipment in a traditional general chemistry or biology lab. In our at-home version of the lab, students were asked to collect the materials on their own. However, we encourage the instructor to consider providing kits to the students, including all of the materials students may need to standardize the reagents they choose to use. This approach will make the lab more accessible in low-resource settings where students may not be able to afford the materials for the at-home lab. The instructor can also opt to allow for students to submit revisions to their original lab report, based on the feedback they receive.

This lab activity can be paired with mathematical descriptions and even simulations of yeast fermentation (e.g., for courses educating engineering or applied science students). For example, in a lab for senior chemical engineering students during the pandemic (Fall 2020), this at-home lab activity was paired with mathematical descriptions of yeast growth and simulations of the operation of a yeast fermentor for bioethanol production, as described in a manuscript by Nagy (18) and publicly-available Simulink model by Hedengren. To close the gap between at-home activities and lectures and experiences with professional laboratory equipment, virtual labs can also be used (such as Labster), where students—through an avatar—use a micropipette, microscope, and fermentor amongst other tools in batch lab-scale fermentation (19). Further, microbes and fermentation are increasingly being used to make ‘non-traditional’ products, including engineered proteins and materials, providing an opportunity for bridging this course into biopharmaceuticals or materials science and engineering with complementary topical lectures and discussions (20, 21).

As mentioned above, the lesson blends elements of two individual biochemistry courses: a “lecture” and lab section, and students may not be taking both. Instructors who wish to teach this lesson can opt to teach either the lecture portion with the primary literature reading and discussion or the at-home lab portion. The at-home lab alone can be adapted and applied to other related courses such as biochemical engineering or microbiology courses. The hands-on lab portion can also be paired with online lab simulations such as that of a fermentor, which was done in a previous iteration of this lab. Overall, such at-home lab activities encourage resourcefulness and provide a new perspective on both the natural world and design of experiments toward fostering lifelong learners and citizen scientists.
Bringing Pasteur Back to Life: Studying the Biochemistry of Yeast Fermentation Through Discussion Groups and an At-Home Lab

Conclusions
In summary, this lesson strives to expose students to reading primary literature to learn the biochemistry involved in alcoholic and lactic acid fermentation, discuss in groups the biochemical principles behind the data, and apply that information to their own at-home experiment to replicate Pasteur's findings. Students are introduced to a PBL format, where they answer complex open-ended questions while working together in diverse groups to digest how Pasteur was able to discover fermentations. This lesson also allows students to design their own experiments from home, where they can embrace agency and creativity to study variables they find interesting. Students reacted positively to the PBL format, the reading of the primary literature, and lactic acid fermentation, discuss in groups the biochemical activity.

SUPPORTING MATERIALS

- S1. Biochemistry of Fermentation – Summary Sheet
- S2. Biochemistry of Fermentation – Pasteur Lesson Outline
- S3. Biochemistry of Fermentation – Primary Literature Learning Issues
- S5. Biochemistry of Fermentation – Instructions for Lab Activity
- S6. Biochemistry of Fermentation – Observation Sheet for Lab Activity

ACKNOWLEDGMENTS
We would like to thank the Cottrell Scholars, Camille and Henry Dreyfus Foundation, the National Science Foundation (NSF) CAREER Award (1554967), and the NSF University of Delaware Materials Research Science and Engineering Center (DMR-2011824) for funding related to this project. Thank you to Dr. Susan Groh, Dr. Raul Lobo, and UD Environmental Health and Safety for their helpful discussions around safety for the at-home activities. KAW would like to thank the National Institutes of Health (NIH) for support through the Chemistry-Biology Interface (CBI) training grant, T32GM133395. KAW would also like to thank Erin Vinson and the Fall 2021 Faculty Mentoring Network (FMN) Online4Bio group for helpful discussion and editing of this manuscript. Lastly, we thank the students of CHEM 342: Introduction to Biochemistry for engaging in this lesson and completing all associated activities.

REFERENCES


Table 1. Timeline overview.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Estimated Time</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparation for Synchronous Class</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Read Pasteur’s Fermentation Paper</td>
<td>Have students download and read the translated Pasteur's paper on fermentation</td>
<td>~60 minutes</td>
<td>Students should fill out the summary sheet (Supporting File S1) provided with main concepts as well as questions that came up while they were reading.</td>
</tr>
<tr>
<td><strong>Synchronous Class Session(s)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Introductory Lecture/Full Class Discussion</td>
<td>Pasteur’s Fermentation</td>
<td>45 minutes</td>
<td>Instructor should refer to Supporting File S2.</td>
</tr>
<tr>
<td>Small Group Discussion</td>
<td>Have students discuss in small groups (4–5 students) main concepts from the paper and their learning issues.</td>
<td>60–75 minutes</td>
<td>Students will discuss the main concepts from the paper in small groups with the instructor/teaching assistants to aid in discussion and questions (Supporting File S3).</td>
</tr>
<tr>
<td>Recap of Group Discussion</td>
<td>Have each group share major discussion points and clear up any remaining conceptual difficulties</td>
<td>15 minutes</td>
<td></td>
</tr>
<tr>
<td><strong>Preparation for Asynchronous Lab Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Designing the Experiment</td>
<td>Students should plan out all variables for their experiments</td>
<td>~20 minutes</td>
<td>This estimate does not include the potential time needed to buy or find all of the materials required to conduct the experiment. That can vary depending on whether materials are provided to students or (are already) accessible.</td>
</tr>
<tr>
<td>Read Safety Contract</td>
<td>Have students read and sign the safety contract for the lab</td>
<td>~5 minutes</td>
<td>Instructor should distribute Supporting File S4.</td>
</tr>
<tr>
<td><strong>Asynchronous Fermentation Lab Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Fermentation Lab Activity         | 1. Students will test the effects of oxygen concentration and at least one other variable (temperature, substrate concentration, pH, etc.) on the effects of yeast growth 2. Students will measure carbon dioxide generation by measuring the height of the foam on the surface of the water in the containers 3. Students will record their observations in the observation sheet | ~45–90 minutes | • The time frame is variable, and students can choose for how long they want to perform the experiment.  
• Students will perform this experiment at home on their own time in their kitchens or other available space.  
• The lab protocol explains more details about selecting variables and suggestions for measuring variables (see Supporting Files S5 and S6). |
| **Post-Lab Report**               |                                                                             |                |                                                                                                                                 |
| Experiment Summary Lab Report     | Manuscript-style lab report that emphasizes an experimental design, safe lab practices, and results-driven approach to communicating findings | ~2–3 hours     | • Students are given a suggested outline for the lab report as well as a rubric used for grading (Supporting File S7).  
• Students have one week to write the report. |
Table 2. Assessments used to examine student understanding of fermentation arranged by learning objective. Checkmarks indicate which learning objectives are targeted in each assessment.

<table>
<thead>
<tr>
<th>Learning issues</th>
<th>Quiz questions</th>
<th>Midterm exam questions</th>
<th>Lab report</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Learning Objective 1:</strong> Read primary literature, identify fundamental concepts such as equilibrium/ Le Châtelier's principle and translate science into everyday examples.</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td><strong>Learning Objective 2:</strong> Develop a testable hypothesis and identify the appropriate experimental observations and control variables.</td>
<td>-</td>
<td>-</td>
<td>✔</td>
</tr>
<tr>
<td><strong>Learning Objective 3:</strong> Predict outcomes, ask questions, analyze data, design and conduct experiments, gather data, make observations, and relate results back to original hypotheses.</td>
<td>✔</td>
<td>-</td>
<td>✔</td>
</tr>
</tbody>
</table>