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Implementing the Elements of Course-Based Undergraduate Research Experiences (CUREs) in a First-Year Undergraduate Chemistry Laboratory with Bioremediation Relevance

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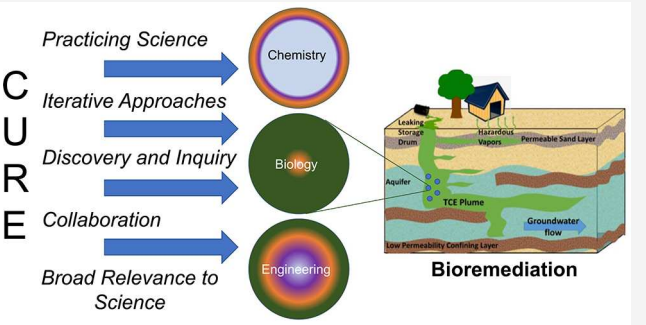
ABSTRACT: General chemistry laboratories are a core requirement for nearly all STEM (Science, Technology, Engineering, and Mathematics) majors and have the greatest breadth in disciplines and audience of any STEM course at a university. A bioremediation Course-based Undergraduate Research Experience (CURE) for first-year undergraduate students was developed to capture and engage student interest for this diverse group. In this multiweek laboratory exercise, students joined an NSF-funded research project designed to enhance the bioremediation of chlorinated aliphatic hydrocarbons. Students explored various biocompatible polymer blends and cross-linkers for encapsulation to create protection for bioremediation microbes. In this "guided research" model, students constructed the measurement apparatus, made hydrogel blends, and then monitored the diffusion of acid via pH measurements using a custom instrument. Herein, we describe how CURE elements were implemented within the bioremediation research experience culminating in student teams presenting posters at our university's undergraduate research symposium. An open-laboratory format facilitated an active research group experience and recitation "group meeting" provided flexibility and needed time for reflection and discussion. Student survey data and course evaluations indicated that students saw value in this genuine research experience and enjoyed the freedom and time to practice and hone skills as both a scientist and teammate in a laboratory setting.



KEYWORDS: *First-Year Undergraduate/General, Interdisciplinary/Multidisciplinary, Undergraduate Research: Bioremediation, Laboratory Instruction, Collaborative/Cooperative Learning*

INTRODUCTION

The well-documented successes of Course-based Undergraduate Research Experiences (CUREs) in the undergraduate classroom are a testament to their classification as a high impact practice (HIP) for education.[1] Indeed, CUREs have been developed and offered to a wide variety of audiences and disciplines.[2−5] This work follows the significant efforts made in bringing the CURE methodology to chemistry courses, and there are five specific elements that are key: *Practicing Science*, *Iterative Approaches*, *Discovery and Inquiry*, *Collaboration*, and *Broad Relevance to Science*.[2,6] Each element requires the practice of essential STEM (Science, Technology, Engineering, and Mathematics) and "soft" skills that are critical for near term and long-term student success, while serving to enhance student engagement. The implementation of this CURE follows the land grant mission of our university by teaching valuable skills that students can use to improve their career prospects such as problem solving, time management, and teamwork.

Enrollment in general chemistry courses is typically composed of a large and diverse group of students from various disciplinary focuses and major subject areas. Understandably, most traditional general chemistry laboratory courses surrender to less engaging "scripted" or "cookbook" educational models as research-type problems do not have a precise outcome.[2,6] Additionally, capturing interest and implementing CUREs for this audience can be challenging. Here, we discuss how an interdisciplinary topic, bioremediation, was used to anchor the second semester of the full year general chemistry laboratory curriculum for a 24 student Honors cohort. The development, implementation, and initial assessment of a semester-long CURE in the area of bioremediation[7] provided a truer research experience for students while addressing the five key elements. This effort follows on previous work at the University of Idaho, through a Howard Hughes Medical Institute (HHMI) funded grant,

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focused on a cooperative effort between biology and chemistry to re-envision first-year laboratory curriculum.[8,9] The learning objectives for this CURE aligned with core objectives of the general chemistry laboratory curriculum, which include (1) apply the scientific method to chemical problems; (2) explain the relationships between atomic/molecular structure to chemical and physical properties; and (3) identify the types of intermolecular bonding and explain relationships.

Learning by inquiry, as exemplified by Process Oriented Guided Inquiry Learning (POGIL), is another exciting developmentary pedagogy that drives students to take leadership of their own education, as they "connect the dots" to learn fundamental science principles.[3,10,11] This is undoubtedly a powerful exercise for students, but we would not typically expect there to be a publishable or "Eureka!" moment, as is popularly associated with scientific research. Conversely, among some faculty, there is resistance to providing undergraduate students an *authentic* research experience because of risks associated with delays, grotesque failures, back-tracking, and "back-to-square-one" reboots that can occur when pursuing research. A middle option in inquiry driven learning may include "guard rails" with scientific best practices, allowing an undetermined end point for an activity without bearing all the risk associated with authentic research, giving students opportunities to learn, assimilate, practice, and hone their STEM skills. Additionally, this type of course could be tailored to faculty research efforts, generating cohorts of students with experience in areas precisely where long-term undergraduate research opportunities exist at the host institution, providing an on-ramp for students into authentic research.

Toward this goal, as part of a larger NSF-funded research effort to engineer bioremediation, a semester-long CURE was developed and implemented to test a combinatorial aspect of the overarching research project. Engineering bioremediation is interdisciplinary and presents topics that involve students from a variety of STEM and non-STEM majors. For this course specifically, students are taught and discuss the U.S. National Priority list (superfund sites) and the major contaminants at these locations, including chlorinated aliphatic hydrocarbons (CAHs), particularly trichloroethylene (TCE) and vinyl chloride (VC), which are #16 and #4 most prevalent, respectively.[12] Microbial anaerobic reductive dechlorination is a known remediation process that breaks down TCE to 1,2-*cis*-dichloroethylene, to vinyl chloride, and then into nontoxic ethylene sequentially, but acid is generated at each degradation step as shown in Figure 1, which presents a challenge since

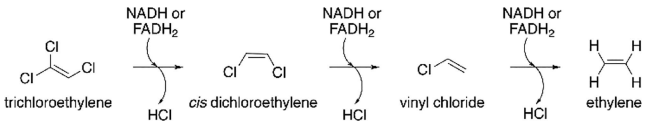

Figure 1. Biodegradation mechanism of trichloroethylene through anaerobic reductive dechlorination.

acid build up may inhibit[13] the dechlorination process or kill microorganisms.[14] The goal of this project is to understand mass transfer in hydrogels to aid in the design of hybrid materials for bioremediation applications. This aspect is the core of our CURE's Broad Relevance to Science.

DISCUSSION

Broad Relevance to Science

CAHs, specifically TCE, were popular, but toxic, lubricants, dry cleaning solvents, and degreasers from 1910 to 1980.[15] Because of their volatility and toxicity (regulated in the low part-per billion levels), CAHs present a particular challenge to remediate. These CAHs are more dense than and poorly soluble in water, which when released, causes them to accumulate at the bottom of aquifers and become a source of contamination for decades if left untreated.[16] Bacterial consortia that can degrade TCE to ethylene via three sequential reductive dechlorination steps have been commercialized. Both CAH and acidic byproducts are toxic to the microorganisms at elevated concentrations.[14,17] A protective coating of hydrogel (also known as "biobeads") can provide a controlled environment for microbes to carry out remediation and was explored in this project.

CAH breakdown to ethylene requires a balance of reaction rate and mass transfer, referred to in this project as the "Goldilocks" problem. When nutrients or CAHs are not provided to the microorganisms at a sufficient rate, or their consumption level is high relative to supply, all processes occur in the outer shell section of the biobead and the inner microbes perish (see Figure 2A). When mass transfer exceeds

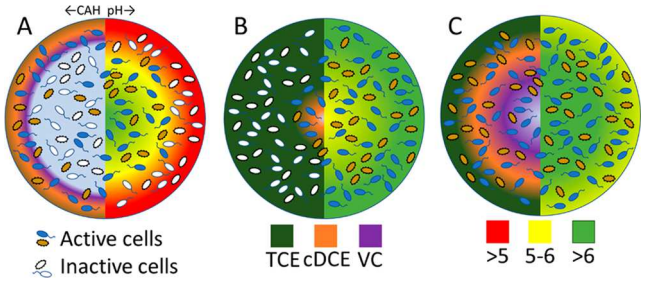

Figure 2. Illustration of different mass transfer to microbial reaction rates in spherical "biobeads". Each sphere is graphically divided in half reflecting CAH concentrations on left and pH on right. Since mass transfer of each (CAH or H^+) can be independent, more complex examples could occur than are shown here. Active cells are colored, inactive and/or dead cells are white. CAHs: TCE, dark green; cDCE, orange; VC, purple. pH: pH > 5, red; pH 5−6, yellow; pH > 6, light green. (A) Mass transfer limited system where CAHs are rapidly degraded and internal microorganisms starve or where pH builds up and exceeds microorganism tolerance. (B) Reaction limited system where contaminants exceed microorganism tolerance, leading to cell death. Likely no negative effects are expected if rapid mass transfer of H^+ occurs. (C) Balanced reaction and mass transfer system.

the ability of organisms to degrade contaminants and becomes inhibitory or concentrations of acid build up in the system without being removed, conditions within the biobead may be lethal to microbes[14] (see Figure 2B). In an ideal or balanced "Goldilocks" scenario, CAHs are supplied at a rate that does not inhibit microorganisms, while waste acids are removed (or neutralized) from the system (Figure 2C). Students were introduced to the idea of balanced mass transfer of nutrients (and CAHs) and a modulated outward diffusion of wastes and acids to protect microbes. As CAHs are inherently toxic, students participated in the project by monitoring diffusion of acid over time through a series of self-made polymeric hydrogel membranes.

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Targeted as an undergraduate CURE, first-year students were asked to explore the mass transfer of acid using biocompatible polymer blends and hydrogel cross-linking methods as variables. Polymer hydrogels evaluated were blends of poly(vinyl alcohol) (PVA), sodium alginate (Alg), and chitosan (Ch). Cross-linking agents included $CaCl_2$,[18] boric acid,[19] and NaOH.[20] Data were collected in triplicate using a custom, student assembled pH measurement instrument called a "GelipHish" (build instructions in the Supporting Information (SI)). The combination of inexpensive and readily transferrable hardware (Raspberry Pi) and a well-explored measurement (pH) limited complexity and failure opportunities.

To determine the mass transfer of acid within hydrogel membranes, students employed the diaphragm cell method[21] within GelipHish to monitor pH over time. Each GelipHish was composed of a custom hydrogel holder that fit directly into poly(vinyl chloride) pipes and separated a chamber of high concentration of acid from a low concentration (see Figure 3,

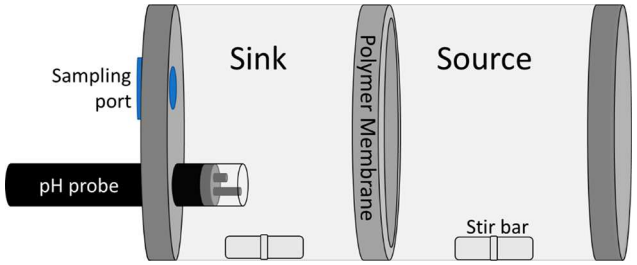

Figure 3. Depiction of an example diaphragm cell. The GelipHish apparatus is made with three sinks and one source mimicking this setup.

dimensions in the SI). A pH probe monitored over time provided mass transfer information for acid, and data was collected and displayed from a custom-built graphical user interface onboard the Raspberry Pi. Results were analyzed using customized Microsoft Excel spreadsheet macros developed by the overseeing principal investigators (PIs) and graduate students.

As time is one of the most important and crucial elements of laboratory classrooms (and progress toward research results), this course started as one 3 h laboratory period per week plus one 1 h recitation for initial student instruction and then moved to an independent, open laboratory setting. Students reported data using a shared online platform (Microsoft Teams) or other learning management system (LMS) for the instructor, teaching assistants (TAs), graduate student mentors, and PIs to evaluate. The class utilized the prescheduled recitation period as a "group meeting" time to discuss the design of experiments, analyze data and results, and reflectively review and plan future experiments based on new knowledge gained between and within student groups. This period also served as a time to synthesize results in context and improve communication skills by educating students in writing abstracts and creating posters. As a requirement for the course, all student teams presented posters at the University of Idaho's Undergraduate Research Symposium, to provide students a more authentic research experience.

This CURE was implemented in the second semester of a two-semester general chemistry Honors course over two years' of student cohorts (AYs 2019−2021). Honors sections (two of the 14 total laboratory course sections with 12−24 students per section) were selected to provide a smaller cohort to test the new CURE. In the first year, the students alternated weekly laboratory periods with rote prescriptive skill development exercises and the CURE exercise. Initial student evaluation data showed that students did not enjoy switching between the research and the nonresearch exercises; therefore, in the second year of evaluating the CURE, students had a consistent 10 weeks of research culminating in the presentation of their work at the symposium (see the Supporting Information, SI). This first year implementation was also interrupted by the global COVID-19 pandemic, and many experiments and replicate runs of the GelipHish were not completed.

Given the new challenges of real research experience in a research laboratory (also amid the COVID-19 pandemic), in the second year, cohort's students registered for 1 additional earned credit hour in undergraduate research. For this CURE, owing to its research structure, students were housed in our newly built (2017) Integrated Research and Innovation Center (IRIC), which boasts multiple bays in an open-laboratory format. To gain access to the research laboratory (via student ID keycard), students were required to take a laboratory safety course (offered in the first recitation section) by a campus Environmental Health and Safety officer and complete the training. The University of Idaho campus was open at reduced capacity in AY2020−2021, so the "open laboratory" format was particularly suitable to social distancing protocols. The new safety course and associated quizzes are now offered online through the university LMS.

Materials. Sodium alginate was purchased at low viscosity and very low viscosity from Alfa Aesar; poly(vinyl alcohol) MW_{avg} 146−180 kDa 99+% hydrolyzed was purchased from Millipore-Sigma; chitosan (>90% deacetylated) and sodium alginate (100 mPa·s) were purchased from AK Scientific; calcium chloride, boric acid, hydrochloric acid, glacial acetic acid, and sodium hydroxide were purchased from Fisher Scientific.

Potential Hazards. Hydrochloric acid is a strong acid and requires proper PPE be used and care taken during dilutions. Sodium hydroxide is a strong base, care is required during dilutions.

IMPLEMENTATION OF CURE ELEMENTS

Practicing Science

The CURE Bioremediation project was deliberately placed in the second semester of first-year general chemistry course to (1) fulfill educational objects common to second semester topical material (e.g., acids and bases; pH, rates and kinetics, and equilibrium) and (2) utilize a cohort of students that already found success in the first semester of the course. This CURE not only offered a unique opportunity for students to try their hand at research in a class setting but also allowed them to contribute to a real project with real world significance. We hypothesize this is beneficial for both the student, who may be more engaged and interested in a course with real world applications, and PIs, who can use these CURE laboratories to obtain preliminary data for future project goals.

Introductory recitation periods discussed the bioremediation project. Subsequently, two introductory laboratory periods with exercises in POGIL derived experiments, such as hand rub sanitizing gels[9] and cross-linking of alginate with calcium chloride, gave students experience with nonscripted procedures

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and open-ended experiments. Students were then switched from the teaching laboratory to the research laboratory space with introductory knowledge of hydrogels and diffusion. Initially, students were split into three member teams and given a laboratory notebook to make all data recordings. This notebook always stayed in the laboratory, allowing for TAs and PIs to check on the notebooks daily, which is the first indication of authentic research.

Instructions on how to assemble the GelipHish apparatus as well as how to make 10% PVA 2% Alg hydrogels were first led by the TAs and graduate and undergraduate research assistants. Students were taught how to pour the polymer blends into the molds, how to properly label samples, and where to store the blends as they froze. As the process relies on >3 freeze/thaw events to obtain gels with sufficient strength, TAs or graduate students assisted for the first few cycles, updating the laboratory notebooks, and making comments in the LMS. At the following recitation meeting, students discussed the experiments and the steps and were refreshed regarding the next tasks.

Upon returning to the laboratory, the students evaluated their hydrogels to look for leaks and measured gel thickness using calipers. The GelipHish was each loaded with three similar hydrogels, the students began the experiments, and data was collected over a two to four day period, saved, and loaded to the LMS system. Data management was then discussed at group meetings. Diffusivity calculations were performed using an automated Microsoft Excel sheet. The students simply had to copy and paste data gathered from the GelipHish software and input measured values in the designated cells.

The diffusion coefficient was calculated using eq 1.[21] Where L_m is the thickness of the membrane, V_{sink} is the volume of the sink chamber, A_m is the cross-sectional area of the membrane, $C_{H+,source}$ is the acid concentration of the source, and $\frac{dC_{H^+}}{dt}$ is change in acid concentration overtime in the sink and is equal to the slope of the $[H^+]$ vs time graph at pseudosteady state as shown in Figure 4.

$$D_e = \frac{L_m V_{sink}}{A_m C_{H^+,source}} \frac{dC_{H^+}}{dt} \quad (1)$$

Discovery and Inquiry

Following this discussion, student teams were given an experimental variable to explore for impact on the acid diffusion. Each research team was assigned various concentrations of PVA, alginate (Alg), and chitosan (Ch) for the hydrogels and were advised on previous structure/property relationships that affected the effective diffusivity (Table 1). This way, three-person student teams were combined later in the semester to make "super teams" for their poster presentations (example posters with student names redacted can be found in the SI). The super teams were made between teams with similar hydrogel blend ratios and different cross-linking methods (e.g., teams 1 and 6, Table 1), so the students could compare their results with respect to cross-linking methods as well as composition.

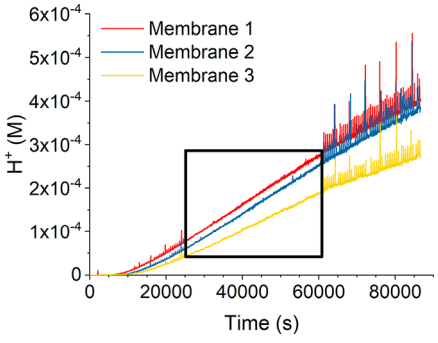

Figure 4. Example student data gathered with outliers removed from the pseudosteady state region. Three membranes of the same composition were tested at one time in the GelipHish apparatus. Slopes differ between membranes of the same composition due to variations in membrane thickness.

Collaboration

After training on prescribed GelipHish setup, data collection, and data analysis, students were allowed to enter the laboratory at their own schedule using keycard access. Student keycard access was granted during Monday to Friday and 7 am to 7 pm to optimize time when a person (TA, UG or graduate researcher, or PI) was available in case they needed assistance.

In addition to their unsupervised laboratory access, TAs were always present during formal laboratory course hours and graduate and undergraduate researchers provided times and availabilities throughout the week. Most often, students chose to setup the GelipHish instrument while TAs or researchers were present yet utilized and expressed positive responses to the keycard access for hydrogel creation, equilibrating hydrogels in new DI water solutions, disassembling GelipHish apparatus, saving data, and cleaning up. Each recitation period, on Friday afternoons, the student groups met with the PI and TAs to discuss the week's progress and determine action items for the following weeks' tasks. These sessions also served as abstract writing sessions for the undergraduate research symposium and as poster creation sessions. As this is a guided research project, the research team uploaded previous posters for the students to look at and templates for poster creation (size and shapes). These recitation-based group meetings solidified ideas and data as well as served as guidance for students needing assistance. This preexisting "recitation" hour for the course was a necessity as it allowed for the students to still be compliant with the expectations in the laboratory and it gave us time to fully discuss the overarching goals of the project with the teams as we worked with each team, in turn, to make sure they were on task.

Iterative Approaches

After beginning the laboratory, multiple groups found that their prompted hydrogel blends would not work and needed to be revised. Students were guided through troubleshooting the issues, which ultimately lead to either decreasing the mass fractions of polymer material in the hydrogel blend or changing the ratio of prepped solutions in the cases where chitosan was used.

This level of troubleshooting forced the students to repeat and retry gel preparations, which allowed them to better understand the nuances of the technique. This exercise, which continued for the majority of the project, gave students an opportunity to enhance their troubleshooting skills and improve their overall teamwork. Due to the timing requirements of various steps in the experimental procedure, teamwork was often vital to accomplish tasks in a timely fashion.

Initially, teams worked together in the laboratory to prepare hydrogels and run experiments, but by the end of the semester,

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Table 1. Team Hydrogel Blend and Crosslinker Assignments

teams	gel components	blend ratios	cross-linker
1	low and very low viscosity Alg and Ch	10% Alg/2% Ch 2% Alg/10% Ch 5% Alg/5% Ch	1.5 M NaOH
2	low and very low viscosity Alg and PVA	5% Alg/10% PVA 10% Alg/5% PVA 10% Alg/10% PVA	2% (w/v) $CaCl_2$
3	low viscosity Alg, Ch, and PVA	2% Alg/2% Ch/10% PVA 5% Alg/2% Ch/10% PVA 10% Alg/2% Ch/10% PVA	2% (w/v) $CaCl_2$ in saturated boric acid
4	low viscosity Alg, Ch, and PVA	2% Alg/2% Ch/10% PVA 5% Alg/2% Ch/10% PVA 10% Alg/2% Ch/10% PVA	1.5 M NaOH
5	low and very low viscosity Alg and PVA	5% Alg/10% PVA 10% Alg/5% PVA 10% Alg/10% PVA	2% (w/v) $CaCl_2$ in saturated boric acid
6	low and very low viscosity Alg and Ch	10% Alg/2% Ch 2% Alg/10% Ch 5% Alg/5% Ch	2% (w/v) $CaCl_2$ in saturated boric acid

they coordinated their efforts to accomplish goals with fewer students at a time to share responsibilities and maximize time efficiency. Students were able to recognize the necessity of time management and coordinated within their teams to complete tasks, both skills that are important for professional development.

From this experience the PIs, TAs, and researchers on the project became well-acquainted with the students and could see them grow as researchers or commend their research prowess. This experience served the students well in that they can point directly to research experience when they request to join a faculty member's independent research group. In turn, this allowed the PIs to be frank with our colleagues and comment directly on their abilities as researchers without speculation or biased by grades.

Assessment

Survey data (see the SI) was collected following the final symposium presentation using a five-point Likert scale. The survey indicated (as promoter) that students appreciated the independence of the open laboratory format and the repetition of the project. Repetition allowed for multiple attempts toward skill mastery instead of the scripted one-off exercises. One of the key take-aways was that students had a better understanding of a research laboratory (Q10). Nearly three-quarters of students thought that the group size was appropriate (Q6), which fell to roughly half of students when considering the merged "superteams" to be the appropriate size (Q7). Thus, the joining of groups into larger teams was not as well-appreciated, and it may simply reflect that smaller project groups were more comfortable and familiar. This is an area that can use refinement.

Another key take-away was that managing student expectations was critical and communication was crucial. For instance, at the end of the semester, only half of the students agreed that the CURE was at a doable skill level (Q4). Clearly students were challenged, which is a positive for an honors student cohort, however, the introduction of those challenges led to clear and significant frustration for two-thirds of students about the clarity of course expectations (Q5). Roughly half of the students could see that the laboratory experience was beneficial to their future career; however, this metric lacks a control response from noncohort students for comparison (Q2).

CONCLUSIONS

The goal of this paper has been to outline the use of a CURES approach to the first-year general chemistry laboratory, leveraging an ongoing PI research effort at our institution. In particular, students with existing interest in scientific research discovered an opportunity to learn about the workings of academic research groups. Thus, many specific details of this effort will not fit the wide variety of students, instructors, and institutions around the world; however, the approaches used to organize student efforts, scheduling, and teamwork are broadly applicable. The application of this approach in larger class sizes would require extensive resources (TA training, open laboratory space, etc.) and engagement from research active faculty. While not limited to a specific institution or class size, this idea benefitted most from a limited cohort of self-selected students.

Therefore, the goals of and lessons learned in this project can be of value to others seeking to rewrite core curriculum at their own institutions, as we all continually strive to advance and improve education opportunities for undergraduate students. Some key takeaways have already been stated, but additional considerations are worth discussion. First, the efforts of the attending research students (graduate and undergraduate) were absolutely essential to train TAs and oversee student progress, so facilitating their efforts is critical and can be an exciting opportunity for graduate students aspiring to pursue faculty positions.[22] Additionally in the open-laboratory format, TA responsibilities may shift from specific student sections to timeslots to focus TA efforts and improve student safety and time management.

In a forward-looking consideration, multiyear surveys may provide additional insight into the long-term opinions of students on CUREs. While the experiments were at the time difficult and often frustrating in the moment, specific students in this cohort acknowledged the benefits of learning both time management and research skills a year later, indicating follow up surveys may be beneficial after the course has completed to obtain additional insight. Such studies are already conducted

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with Alumni, but "closing the loop" earlier when the experience is fresh may provide better insights for course correction. As assessment metrics are key elements to understanding impact, future work must include tighter integration of assessment into the planning and implementation processes, ideally with the assistance of on-campus course-design facilities.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.2c00360.

GelipHish assembly instructions and the materials used, assignments, experimental setup, syllabi comparisons, course syllabus, end of semester survey, and example student posters (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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