A Model Research Experience for Undergraduate Biology Labs Using Microalgae

Abstract

Problem Based Learning (PBL) or Course-embedded Undergraduate Research Experience (CURE) projects challenge students to problem solve using scientific literature and teamwork while investigating solutions to a real problem. Through scaffolding, students learn how to conduct peer-reviewed literature research, formulate research questions, co-design experiments, collaborate in groups, conduct experimental protocols, troubleshoot lab protocols, and report their findings. This paper discusses a PBL/CURE project introduced to Microbiology students at Dalton State College in Spring 2023. The project begins by discussing global problems including concerns with greenhouse gas, scarcity of crude oil, and human waste management. Students watch an inspirational TED talk presented by NASA's Jonathan Trent on Energy from floating algae pods. Students explore the topic of algae biodiesel fuel, the potential types of microalgae and how to grow, harvest, and extract lipids for bio-diesel conversion. Student groups explore peer-reviewed literature research on the assigned algal organisms: *Nannochloropsis oculata*, *Porphyridium cruentum*, or *Chlorella vulgaris*. Then, student teams collaborate with the professor and co-design experiments in attempts to obtain the final product which are algal lipids. Students are relatively successful in growing, harvesting, and obtaining dry weight of algae in 10 weeks of the 15-week semester. Due to the insufficient amount of dried algae mass obtained, extracting algal lipids proves to be unmanageable for this project. Students create a written research paper and present the paper in the course symposium: DSC's Algae Biodiesel Research Symposium. Students complete a post-survey pertaining to their PBL/CURE project experience, and express that this research experience has provided a unique opportunity that they would not have experienced outside this course. This paper shows a model for experiential learning that provides a valuable and enriching research experience for undergraduate students in science lab courses.

Keywords

STEM Education, CURE/ PBL, Microbiology, Biotechnology, Biodiesel

Introduction

Experiential learning such as Problem Based Learning (PBL) or a Course-embedded Undergraduate Research Experience (CURE) provides an opportunity for students to explore and apply the scientific process [1]. Students co-create a research project that incorporates learning objectives for the course including safe lab techniques and culturing microorganisms [2]. Students practice science lab skills and utilize scientific creative thought processes in an open-ended research project. Typical undergraduate science labs learn concepts using delineated lab activities with known outcomes [3]. The process of authentic exploration and improvisational troubleshooting is typically not modeled in these types of labs. Although popular and effective in learning basic science skills, this approach to learning excludes students from engaging with the professor in real-time collaboration on an open-ended research project where outcomes are unknown [4]. Using PBL/CURE in the science labs offers students a forum to gain experience on how to plan and implement research. This gives learners agency in their education and empowers them to make choices in research design such as the consideration of sample sizes and time [5]. This experience provides students a forum to develop and practice 21st century competencies including critical thinking, problem solving, creativity, innovation, communication, and collaboration [6]. This paper describes a project conducted in Spring 2023 in a General Microbiology course at Dalton State College (DSC), Department of Life Sciences in Dalton, GA. The title of the project is *Optimizing Algal Lipids for Biodiesel Production.* The project's driving question is, "How can students at DSC contribute to the global solutions for the obstacles described by NASA's Jonathan Trent?" Students conduct peer-reviewed literature research on algae biodiesel production and the methods for dewatering the algae. The three types of algae available for the project are *Nannochloropsis oculata*, *Porphyridium cruentum*, and *Chlorella vulgaris.* Students collaborate in teams with the professor to design experiments for culturing and dewatering the algae. Three research questions are derived from literature review:

1) Which type of algae is more suitable for biodiesel production?
2) Which vessel is more suitable for upscaling the algae culture?
3) Which chemical flocculant results in obtaining higher yield of biomass?

The experiential learning project concludes with a course symposium DSC's Algae Biodiesel Research Symposium where students present their research using oral PowerPoint presentations.

Methodology

Students participated in a whole class discussion with the professor as an introduction to the project *Optimizing Algal Lipids for Biodiesel Production*. The discussion involved global concerns pertaining to sustainable energy resources, growing human population, food resources, and human waste management. Students watched a TED talk by NASA's Jonathan Trent which defines the interconnected global problem along with proposed solutions. The TED talk describes the use of microalgae to produce biodiesel fuel that competes with fossil fuels and proposes solutions for sustainability, food production and human waste management [7]. The students were presented with the PBL driving question, "How can students at DSC contribute to the global solutions for the obstacles described by NASA's Jonathan Trent?" After discussion, students received written information about the project where expectations were defined. Students were instructed to conduct peer reviewed research on two aspects: (1) the characteristics of the algae assigned and its role in biodiesel production (*N. oculata*, *P. cruentum*, or *C. vulgaris*) and (2) dewatering the algae using flocculation.

Culturing Algae: Each student obtained an algae culture kit corresponding to the algae assigned for the literature review. The kits were purchased from Algae Research Supply and the students followed the instructions to begin culturing [8]. The 50 ml flasks containing algae were incubated in the windowsill for photosynthesis using natural sunlight where temperature ranged from 17-21°C using an alcohol thermometer in a beaker of water. Algae cultures were shaken periodically for gas exchange. After three weeks, culture flasks were moved to an incubator set at 25°C with 10-watt bench lights purchased from Algae Research Supply for one week. After four weeks of growing algae, *C. vulgaris* did not grow sufficiently. Thus efforts focused on upscaling the culture volumes of *N. oculata* and *P. cruentum*. Students prepared new media and sterilized in an autoclave. In a 1:4 ratio (algae culture: medium), students inoculated *N. oculata* and *P. cruentum* into 50 ml conical tubes at full capacity, 500 ml Beaker Bags at half capacity or 500 ml

Erlenmeyer culture flasks with a stir bar at half capacity. They placed the beaker bags and tubes in an incubator set at 25°C with 10-watt bench lights. These vessels were manually shaken daily for gas exchange. The flasks were grown in the windowsill using a hot plate stirrer set at 25°C. After two weeks, we added media to the beaker bags and culture flasks for full capacity and grown another two weeks. Each team measured the final volume of *N. oculata* cultured. The initial optical density (OD) of pre-flocculation of the cultures were measured using Secchi disk depth (SDD) in millimeters (mm).

Dewatering algae: Based on literature research, we decided to use ferric chloride, zinc chloride and ferric sulfate comparing two concentrations [9]. Each team measured 2 x 500 ml of algae culture into two flasks with stir bars then added the corresponding flocculant and stirred. After 30 minutes, students transferred the mixtures to graduated cylinders to settle the flocs for 30 minutes. They measured the OD for each reaction using a SDD after flocculation. The following equation was derived to calculate flocculation efficiency: A is the initial OD and B is the OD after flocculation: Flocculation efficiency % = (1- (A/B)) x 100) [9]. Flocculated algae cultures were filtered using a Buchner funnel, Whatman #4 filter paper, and vacuum filtration. Students placed the filter paper and algae in a drying oven at 80°C for 18 hours and measured the dried algae mass using an analytical balance.

Student Research Paper and Oral Presentation: Students received written instructions on components to include in creating group research papers and oral presentations including the Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, and References. Using scaffolding, the instructor provided examples on how to write the paper. The rubric delineated the point values for each component of the paper and presentation including student participation. Students submitted rough drafts two weeks prior to the final draft for professor feedback. The student products were presented at the course symposium titled: DSC's Algae Biodiesel Research Symposium.

Student Post-Survey: A student post survey was conducted seven months after the students completed the project. This survey was exempt through the internal review board (IRB) committee at DSC. The survey used the survey tool Qualtrics. Questions on the survey included: *Did the CURE's project enhance your lab learning experience?*; *Did the CURE's project provide an opportunity to collaborate with other students?*; *Do you feel the research experience has provided a unique opportunity that you would not have experienced outside this course?*; and *Did you find that the CURE's project provided a valuable and enriching research experience for undergraduate students in science lab courses?* Students were given an opportunity to provide any additional feedback on the CURE's experiential learning project. Depending on the question, the possible responses were as follows: definitely yes, probably yes, might or might not be, probably not, definitely not; or strongly agree, somewhat agree, neither agree nor disagree, somewhat disagree, and strongly disagree.

Results and Discussion

The opening event created excitement for the research project and explained the components of the project, and as a result, students were interested in experiencing research. Students were assessed on completing a peer reviewed literature search on two aspects: (1) the type of algae assigned on its role in biodiesel production and (2) dewatering the algae via flocculation. All students submitted a total of four articles including primary and secondary research articles. This component of the project offered experience in searching and reading primary and secondary peer-reviewed research literature. The research literature proved a springboard for classroom discussions and project collaboration. This assignment offered students low stakes assessment by simple completion and offering discussions were the assessment. In completing this assignment, the professor became aware that students may benefit from learning how to take research notes; thus, guided research notes were implemented to scaffold note taking.

Culturing Algae: N. oculata and *P. cruentum* grew slowly in the windowsill with natural light. *C. vulgaris* did not thrive. Due to slow growth and cooler temperature in the lab room, troubleshooting led to setting up 10-watt bench lights in an incubator set at 25°C where all the cultures were transferred from the windowsill. After a week under the new growth conditions, *N. oculata* and *P. cruentum* showed significant growth and were used to upscale the culture volume. *C. vulgaris* did not revive in the enhanced growth conditions and was eliminated from the project. After two weeks, all *P. cruentum* cultures created large clumps of algae and growth appeared to cease. *P. cruentum* was eliminated from the project. The *N. oculata* thrived under all growth conditions. Students collected all the algae culture from the various vessels and measured the volumes. The volumes of *N. oculata* liquid cultures grown in the 50 ml tubes, the 500 ml beaker bags and 500 ml Erlenmeyer flasks were 0.46 L, 4.0 L and 3.5 L. The initial OD of the cultures were 17 mm, 15 mm, and 10 mm, respectively. The Erlenmeyer flasks were stable and the use of stir bars for gas exchange was convenient. The beaker bags were unstable, easily fell over in the incubator and spills occurred. The 50 ml tubes were not practical for large volumes of culture were unmanageable for large scale production.

Dewatering algae: Flocculation experiments offered students the opportunity to use analytical balance, measure volumes, take OD measurements with SDD, and observe the flocculation reaction occur while the algae clumps after adding the reagent. Troubleshooting occurred where the SDD was too long for a vessel or not long enough to measure the OD. Solutions occurred by transferring the culture to a larger vessel or taping two SDD together. Filtering the flocculated culture using the Buchner funnel apparatus provide opportunities to troubleshoot because the flocs clogged the pores of the filter paper or the filter paper did not cover the pores, so the culture passed through to the filtration funnel. The flocculation efficiencies and mass of dried algae collected were as follows: 0.2g and 0.4g of ferric chloride were 77% and 86% and 0.83g and 0.92g; for 0.2g and 0.45g of ferric sulfate were 92% and 93% and 0.59g and 0.69g; and for 0.2g and 0.4 g of zinc chloride were 74% and 77% and 1.0g and 0.8g, respectively.

Student Research Paper and Oral Presentation: Student groups co-created a written research paper and delivered outstanding oral presentations. Students were successful in following the rubric guidelines and incorporated all the components including Abstract, Introduction, Material and Methods, Results and Discussion Conclusions, and References. Students successfully completed the instructed assignments to compare the results with at least two literature resources which were scaffolded for the students. Colleagues from UTC acted as the stakeholders by attending the DSC's Algae Biodiesel Research Symposium in our final laboratory session. UTC colleagues presented questions which ignited discussion for students to receive immediate feedback on the project.

Student Post-Survey: There were three responses to the survey out of thirteen students that completed the project. The students that completed the survey anonymously agreed that their information can be used for publication. All students answered “strongly agree” to the question: *did the CURE project enhance your lab learning experience?* All students responded “strongly agree” to the question: *did the CURE project provide an opportunity to collaborate with other students?* All students responded “definitely yes” to the question: *do you feel the research experience has provided a unique opportunity that you would not have experienced outside this course?* All students responded “definitely yes” to the question: *did you find that the CURE project provided a valuable and enriching research experience for undergraduate students in science lab courses?* The following two statements were student’s additional feedback on the CURE's experiential learning project in the survey: “I loved the skills and knowledge I acquired during this project. It was a great experience and a great introduction to lab research!” and “It was especially great to be able to present at the end of the semester (presenting research is just as important) and if done again, it would be great to have more people :).” Although the response rate was low for the post survey, the information gathered was deemed valuable to include in this paper. In future endeavors, more timely surveys should be administered to improve response rates.

Conclusion

This paper describes a PBL/CURE experiential learning project where students gain research experience embedded into their undergraduate Microbiology lab. The driving question for the PBL/CURE, “How can students at DSC contribute to the global solutions for the obstacles described by NASA’s Jonathan Trent?” is answered by students learning that they are the stakeholders. A subjective assessment is that students can contemplate the complexity of global problems using this model PBL/CURE to show how everything is interconnected on our physical planet. Our discussions include that we as a global society are responsible, and that it is a global effort to derive solutions. The solution for the problem proposed in the PBL/CURE involves education, training, practicing sustainable practices and a global shift which is in progress based our research reviews. The research project conclusions for the parameters we set are as follows. (1) *N. oculata* is the heartiest of all algae explored. *N. oculata* is most suitable for our lab’s attempt to grow algae for biodiesel production on the premise that it was the only culture that thrived throughout the project. Our experience shows that the algae growth parameters are not optimal in this project, and this is where improvements can be made next time implementing this project. (2) The optimal culture vessels for upscaling are the Erlenmeyer flasks. They are stable, come in assorted sizes and can support stir bars for gas exchange. (3) Zinc chloride at a concentration of 0.2 g of in 500 ml of algae culture proves to be the optimal flocculation condition because the highest algae mass was recovered. Many improvements can be made in the implementation of this project, yet overall, the imperfections in the project offers more interesting experience to engage thought provoking situations to learn basic biology concepts such as ecological niches and temperature’s importance in organismal growth. There are numerous improvements that can be made for this project which provides problem solving questions to introduce in future implementation. The next offering of this project plans to include growth tents to help regulate temperature for larger growth vessels and improve lighting system. Student survey responses show that students agree that this experience is valuable and enriching, the project enhances their lab learning experience, and they learn new skills not covered in the regular lab.

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