While developing a laboratory course to reinforce topics covered in our second-term biochemistry lecture, which is focused on metabolism, we identified a need to augment available metabolic experiments in glycolysis, fermentation, and xenobiotic metabolism (1–3) with additional application-oriented exercises. Most of the students in our biochemistry labs are biology majors and tend to be more engaged by experiments involving live organisms and environmental or human health applications. Considering their interests, this lab exercise has been developed as a real-world lesson in metabolism using a herbicide to inhibit growth and amino acid biosynthesis in live cultures of microalgae. The experiment exemplifies blockade of a metabolic pathway and demonstrates the impact of a herbicide on non-target aquatic organisms while inspiring active learning in metabolism.

The herbicide we used in the experiment is sulfosulfuron (from the commercial formulation, CERTAINTY®), a representative of the sulfonylurea class of herbicides that has been demonstrated to specifically inhibit acetolactate synthase (ALS, E.C. 2.2.1.6), the common enzyme in the synthesis of the branched-chain amino acids (BCAAs): leucine, isoleucine, and valine (Fig. 1) (4). The inhibition of ALS in plants results in the depletion of BCAAs, followed by dysfunctional protein synthesis, and eventually, death (4).

Like plants, many species of microalgae express the ALS enzyme and are therefore sensitive to herbicides that target ALS (5). Herbicide sensitivity is widespread among freshwater and marine algae, and microalgae have been used to identify herbicides that may be harmful to non-target organisms or processes (6). The hypothesis tested in this exercise is that the ALS enzyme produced in selected species of microalgae is inhibited by sulfosulfuron, as reported for the ALS enzyme in plants, and that the inhibition will cause microalgae to grow more slowly and exhibit lower concentrations of BCAAs relative to cultures without sulfosulfuron treatment.

This laboratory exercise offers unique advantages for undergraduate curricula in biology and microbiology, including 1) the use of microalgae as economical model organisms that can be obtained commercially or from field sources and be used with this protocol; 2) both scalability and adaptability, since the class of herbicide, the type of assay, and the level of focus on environmental and metabolic topics can all be adjusted to the class level and interests; and 3) the use of a microplate spectrophotometer, a commonly used instrument in laboratory settings, which necessitates the application of a standard curve and permits more timely data acquisition for multiple student groups. Through this experiment, students also get practice 1) handling live microorganisms in sterile culture, 2) preparing cell extracts, 3) performing enzymatic quantitation of a substrate, 4) preparing and using a standard curve, and 5) performing spectrophotometric analyses using a plate reader.

**PROCEDURE**

**Instructor preparation**

The exercise requires preparation time of two to three weeks for growing the stock algal cultures, and can be performed with marine or freshwater microalgae (Appendix 1). Growth media recipes are readily available from.

![Diagram of the biosynthesis of isoleucine, valine, and leucine (branched-chain amino acids, BCAAs), and inhibition by sulfosulfuron.](image-url)
most culture collections (Appendix 1), and a clean hood is preferred for working with algal cultures but is not required. Cultures can be grown in the lab classroom on a benchtop orbital shaker (for aeration) and irradiated with low-wattage fluorescent lamps controlled by a timer, or they can be grown on a windowsill in non-direct light with filtered air bubbled in using a small aquarium air pump. Procedural details, including culture source information and optional procedures, are provided in the supplemental materials (Appendix 1).

Student procedures

Eight students, working in pairs, have successfully completed the exercise in two 150-minute class periods in consecutive weeks using a single plate reader and a two-part procedure: 1) addition of a known ALS inhibitor, sulfosulfuron (Appendix 1), to cultures of microalgae with manual counting of algal cells in treated and untreated cultures, and 2) determination of BCAA concentration in crude extracts from treated and untreated cells using a colorimetric assay method. Cell counting is performed using a hemocytometer, and crude cell extracts are prepared by mechanical disruption using a bead mill (Appendix 1). Other methods for either step should be adaptable to the exercise (7). Detailed student instructions are provided in the supplemental materials (Appendix 2).

First laboratory session. Two algal cultures, one with herbicide (treated, 150 mL) and one without herbicide (untreated, 150 mL), are prepared from a stock culture of algae by each student pair. The cultures are initiated and grown in sterilized Erlenmeyer flasks with vented closures. Each student group prepares the growth media appropriate to their organism. Students are introduced to manual cell counting with a hemocytometer and practice the technique on their two starting cultures (untreated and sulfosulfuron-treated). Students are introduced to the use of a standard curve in spectrophotometric analyses.

Second laboratory session. Students follow the detailed procedure provided in Appendix 2 of the supplemental materials. Each culture is sampled three times for manual counting, and from each culture, three samples (1 mL each) are prepared as crude cell extracts (CCEs). If optical density (OD) measurements are used in place of cell concentrations, the OD_{650} is performed with three separate 100-μL samplings from the culture. A commercially available kit for BCAA detection and quantitation has been applied to this exercise for convenience (Appendix 2). However, other available protocols for the coupled enzymatic detection of BCAAs may be used (8).

RESULTS

An example of student data demonstrating the effect of sulfosulfuron treatment on growth and BCAA concentrations in four species of freshwater algae is presented in Appendix 3 of the supplemental materials. In lab reports detailing the experiment, students interpreted the class data in terms of 1) the most and least sulfosulfuron-sensitive species, 2) the implications of insensitivity to ALS inhibition, and 3) the correlation between cell growth and BCAA depletion in each species.

CONCLUSION

Biochemistry courses play an increasingly central role in the education and development of undergraduates in biology and microbiology. Laboratory exercises like this one hold student interest and encourage the use of problem-solving skills, analytical reasoning, and adaptability. In addition, this experiment can be applied to many species of algae and can also be expanded to include other ALS inhibitors or an unknown herbicide. Finally, this experiment inspires curiosity about algae and concern for the environmental impacts of herbicide use, sparks peer learning through discussion and collaboration, and exposes students to instrumentation and techniques that will be useful for their future participation in research at the graduate and undergraduate levels.

SUPPLEMENTAL MATERIALS

Appendix 1: Instructor resources
Appendix 2: Student instructions
Appendix 3: Student data example

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