JOURNAL OF MICROBIOLOGY & BIOLOGY EDUCATION, December 2015, p. 286-288
DOI: http://dx.doi.org/10.1128/jmbe.v16i2.934

Tips & Tools

Powerful Soil: Utilizing Microbial Fuel Cell Construction and Design in an Introductory Biology Course†

Craig D. Jude and Brooke A. Jude*
Biology Program, Bard College, Annandale on Hudson, NY 12504

INTRODUCTION

Microbial fuel cells (MFCs), batteries powered by electron output of bacterial cells in a mud/soil substrate (reviewed in Refs. 5, 6), are currently the basis of a large number of studies pertaining to power production (2), bioremediation (4), and a coupling of these two processes (1). The concrete application of this technology, and the accessibility of technology in a college classroom provide a rich introductory laboratory and classroom experience for biology majors and non-majors alike. Microbial fuel cells built by students serve as an instructive tool that can be used throughout the semester. Microbial fuel cells demonstrate varied ecosystems and microbial communities, can be examined for evidence of microbial reproduction and microbial community development, and demonstrate cellular metabolism, among other related topics.

In this tool, we develop a semester-long laboratory by initially building a commercially available MFC kit (Keego Technologies http://www.mudwatt.com). This experience is followed by an independent bio-design project, charging students with designing and building a battery with sourced materials that will produce more power than the original battery they constructed. This strategy allows students to repeat laboratory techniques, leading to increased comprehension and success, and provides an opportunity to work independently early on in their coursework. Students are provided detailed instructions on how to build the initial MFCs, as well as how to culture microbes from a source, and biochemical- and molecularly-based species identification. Data collected in these experiments are submitted for grading in the form of figures and/or tables and legends, appropriate for a peer-reviewed journal article. This tool can be adapted for non-majors courses, and a truncated version of the tool has been successfully executed in one-hour workshops for local middle school students.

*Corresponding author. Mailing address: Biology Program, Bard College, 30 Campus Road, P.O. Box 5000, Annandale on Hudson, NY, 12504. Phone: 845-752-2337. E-mail: bjude@bard.edu.
†Supplemental materials available at http://jmbe.asm.org

PROCEDURE

The MFC construction lab was developed for an introductory biology course, Environmental Microbiology, one of several majors-level introductory biology courses offered in the curriculum. This lab course takes place over a 16-week semester, meeting for two 80-minute lectures and one three-hour laboratory session per week. In the first laboratory meeting, pairs of students were provided with a MudWatt MFC/battery construction kit (Keego Technologies) and accompanying instructional materials for microbial care and culture (Fig. 1A, Appendix 4). Using soil collected from various on-campus sites, students built the microbial fuel cells, reserving a small amount of soil for culturing dilutions on LB agar plates or R2A agar plates (Appendix 7) at 25°C and 30°C. In the subsequent seven weeks, students monitored the power output of the MFC using a standard laboratory grade voltmeter and accompanying resistors in MudWatt hacker board kit (Appendix 4), and were trained to isolate and grow bacteria in pure culture (Appendix 4), perform Gram-stain and bright-field microscopy (3), obtain antibiotic resistance profiles using Kirby Bauer analysis techniques (3), and isolate genomic DNA for 16S rRNA sequencing (Appendix 4). Following the completion of each laboratory procedure, students were required to assemble data into an appropriate figure and legend or table and legend and were graded according to a rubric designed for this assignment (Appendix 6).

For the remaining eight weeks of the semester, pairs of students worked independently to design and build a new MFC, aiming to construct one that was more powerful than the original MFC built earlier in the semester. Variables that could be altered included: soil and water sources; nutritive additions to the soil and water mixture; the size and shape of the vessel; size, shape, and layout of the anode and cathode graphite felt material; and configuration of the titanium wire (Fig. 1, Appendix 1–3). To prepare for this variability, and to allow for handmade construction of these parts, various sized containers were purchased and recycled (Appendix 3), and graphite felt (Appendix 1, 7), titanium wire, and wire coating (Appendix 1, 7) were purchased. Blinker circuit boards, capacitors, and LED lights from MudWatt MFC kit were recycled from the first MFC for this second round of MFC construction. In subsequent iterations of

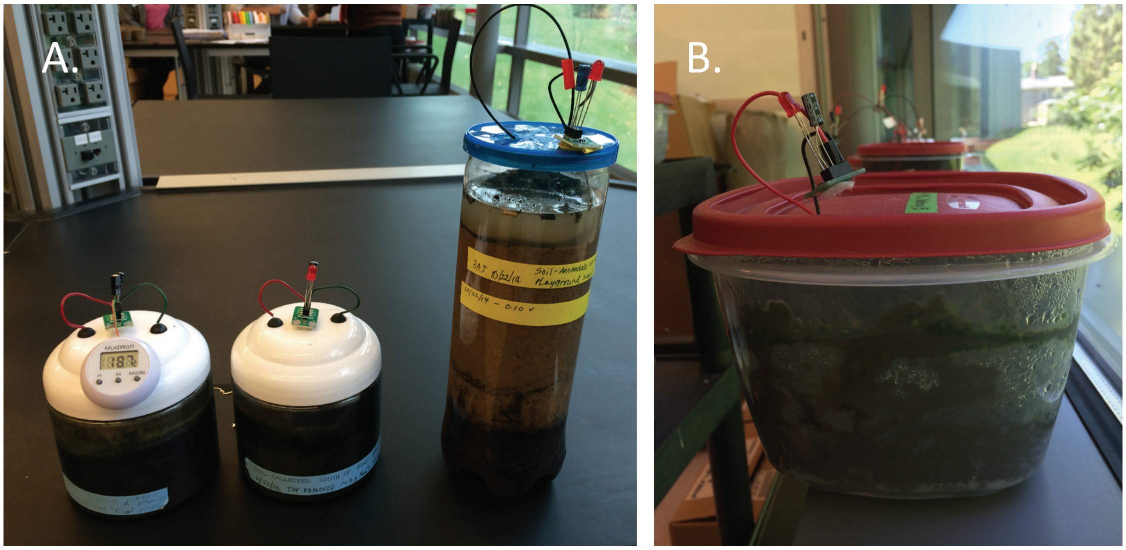

FIGURE 1. Completed microbial fuel cells. (A) MFCs constructed using MudWatt kits (left) as well as a recycled tennis ball canister (right). MudWatt kit batteries pictures are displaying either the thermometer/clock or a red LED bulb. (B) MFC constructed using a rubber food storage container.

this activity, empty tennis ball cans and caps replaced the plastic containers (Appendix 1–3) to house the MFC. This type of container had the benefit of being freely available as a recycled material and required a smaller overall surface for the anode and cathode graphite felt. Appendix 5 includes step-by-step instruction for MFC construction.

During the MFC design portion of the laboratory, students were required to identify two microbes from their soil/water source used to power the MFC. Microbial strains isolated included *Bacillus amyloliquefaciens* and *Bacillus halodurans*. The students completed all of the assays they had learned in previous weeks in consideration of the new MFC construct. All materials required to execute each of the assays were made available to the students throughout the duration of the semester, allowing students to take ownership over the design and pace of the experimental procedures. Throughout the laboratory, students were required to conform to proper microbial handling and disposal of cultures. Wescodyne treatment of more than 10 minutes was used to decontaminate all liquid cultures, and all solid waste was disposed of via biohazard waste bags and autoclaved for 60 minutes on a dry/waste cycle. Images of plates were obtained using a flat bed scanner (Epson) dedicated for imaging of biological samples. The final laboratory assignment was to produce all figures and legends from the data produced from this laboratory (sample schedule, Appendix 6).

In an adaptation of this tool, visiting eighth grade students built tennis ball can construction MFCs in a 60-minute period. Multiple groups of students from regional middle schools annually visit the college for one-day workshops, participating in five various science and mathematics courses throughout the length of the visit. During each biology unit of this workshop, approximately 20 students, supervised by four or five trained college students, built two MFCs to take back to their classrooms. Students were provided a protocol to follow for the MFC construction and subsequent power production analysis (Appendix 5). Classroom teachers were provided with educational materials to use for follow-up activities.

CONCLUSION

Microbial fuel cell construction serves as an effective anchor for a number of curricular points throughout the semester of introductory biology. General learning goals of this course included learning how to read and analyze primary literature while learning to write portions of scientific manuscripts. The students were able to juxtapose reading papers with the task of interpreting data and creation of their own succinct and clear figures, tables, and accompanying legends. Throughout this activity, students are provided with the chance to learn laboratory protocols and repeat them throughout the semester for clarity and to gain expertise. This laboratory experience also allows for creativity and ingenuity on the part of the students when designing a more powerful MFC and leaves a positive lasting impression on introductory biology students.

SUPPLEMENTAL MATERIALS

Appendix 1: Supplemental Figure 1: Set up of materials required for MFC construction
Appendix 2: Supplemental Figure 2: Construction of MFC
Appendix 3: Supplemental Figure 3: Sample MFCs
Appendix 4: Protocols for culturing MFC microbes and amplifying 16S rRNA
Appendix 5: 8th Grade Workshop MFC Construction Protocol
Appendix 6: Sample schedule for final lab assignment

Appendix 7: Figure and Legends/Table and Legends grading rubric
Appendix 8: Material ordering information and recipes required for MFC Lab

ACKNOWLEDGMENTS

Purchase of course materials was supported by Bard College. Eighth grade activity visits were supported by the Bard Center for Civic Engagement and the Bard College Science, Mathematics and Computing Division. Students from Bard College BIO145 courses in 2013 and members of the Jude Laboratory helped to develop protocols critical for this manuscript. A portion of this data was presented as a poster at the June 2014 NEMPET meeting (http://www.nempet.rutgers.edu/2014.htm). BAJ has been interviewed and filmed by a company hired by Keego Technologies to promote an upcoming Kickstarter campaign to raise funds for production of an educational version of the original MudWatt. This participation was voluntary and unpaid, based on positive experiences with the original MudWatt product.

REFERENCES

1. **Doherty, L., Y. Zhao, X. Zhao, and W. Wang.** 2015. Nutrient and organics removal from swine slurry with simultaneous electricity generation in an alum sludge-based constructed wetland incorporating microbial fuel cell technology. Chem. Eng. J. **266**:74–81.
2. **Fang, Z., H.-L. Song, N. Cang, and X.-N. Li.** 2015. Electricity production from Azo dye wastewater using a microbial fuel cell coupled constructed wetland operating under different operating conditions. Biosens. Bioelectron. **68**:135–141.
3. **Lammert, J.** 2007. Techniques in microbiology. Pearson/Prentice Hall, Upper Saddle River, NJ.
4. **Li, W.-W., and H.-Q. Yu.** 2015. Stimulating sediment bioremediation with benthic microbial fuel cells. Biotechnol. Adv. **33**:1–12.
5. **Lovley, D. R.** 2006. Microbial fuel cells: novel microbial physiologies and engineering approaches. Curr. Opin. Biotechnol. **17**:327–332.
6. **Lovley, D. R.** 2006. Bug juice: harvesting electricity with microorganisms. Nature Rev. Microbiol. **4**:497–508.