Development of a Low-Cost, Small-Format Bioreactor for Bioluminescent Bacterial Cell Culture

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Introduction

Bioreactors are an essential technology utilized in the production of biologics, fermented products, and specialty chemicals.[1] Our team seeks to increase exposure to this technology at the undergraduate level. Thus, we have developed a low-cost, small-format bioreactor using non-restricted materials.

For the demonstration of the bioreactor, the marine bacterium, *Vibrio fischeri,* was cultured. *V. fischeri*, which is well-studied for its bioluminescence, is also widely-available for purchase, and is both non-pathogenic and recognized as safe for study in BSL-1 lab spaces.[2] The bioluminescence of *V. fischeri* served crucial for determining inoculation and life cycle timing within the bioreactor system.

Bioreactor Design

It was critical to the design of the bioreactor that all building materials were widely-available, and that the production of the bioreactor was low-cost in comparison to lab-grade or industrial bioreactors.

The core of the bioreactor is the cell culture vessel, which is a borosilicate glass beaker sealed using a rubber gasket. A magnetic stir plate and stir bar are used to agitate the contents of the vessel. A cylindrical shape for the vessel was chosen for the final design. Initial testing was performed with a round-bottom shape, but issues with stirring turbulence were encountered. The cell culture vessel is modular and can be removed for sterilization separate from the rest of the system. The total volume of the vessel is 1000 mL; considering headspace restrictions during culture, the liquid working volume of the vessel is approximately 500 mL.

The cell-culture vessel is jacketed by a water bath to maintain a consistent temperature. The main structure of the bioreactor system is laser-cut, clear acrylic. Low-cost temperature and pH probes are integrated into the cell culture vessel for real-time monitoring of temperature and pH. A Raspberry Pi 3 microcomputer is used for data output and control of instrumentation.

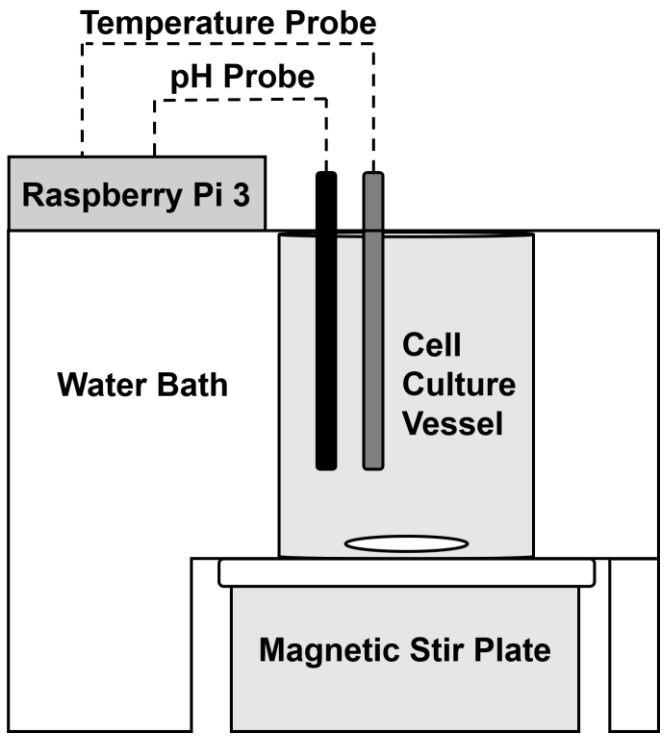

Figure 1. Final Design Diagram for the Bioreactor

Cell Culture Methods

The seawater-tryptone nutrient media used for experiments was sourced from the literature and was chosen due to its straightforward composition.[3] *V. fischeri* was sourced in the form of educational-grade starter cultures from Carolina Biological Supply Company. Cultures were maintained on solid agar media before scale-up.

For scale-up, 1-2 mL of liquid media was inoculated by a visible cell mass, with 1:10 dilutions of inoculum in fresh nutrient media occurring every 72 hours. Further work to assess scale-up viability was done at the 100 mL scale, with tests done with both shaker platforms and stir plates. Scale-up tests were performed at 22°C, and the target culture pH was approximately 7.2-7.5.[3] Observed luminescence of the bacteria in culture, which is triggered naturally by cell density, was interpreted as a qualitative indication of both stationary phase growth and of the need for further propagation.[3]

Pilot Testing

Test Run 1: The bioreactor was charged with 10% (v/v) inoculum in 500 mL fresh nutrient media. The culture was run for 72 hours. Culture pH and temperature was recorded electronically, but neither parameter was actively controlled. An increase in culture turbidity was observed after 24 hours, but luminescence was not observed during the test run.

Test Run 2: It was suspected that too large of an inoculum volume was used in Test Run 1. Thus, for Test Run 2 the inoculum was reduced to 1% (v/v) in 300 mL fresh nutrient media. The culture was run for 70 hours. After 22 hours the culture turbidity had increased, and after 45 hours luminescence was observed when the culture vessel was shaken.

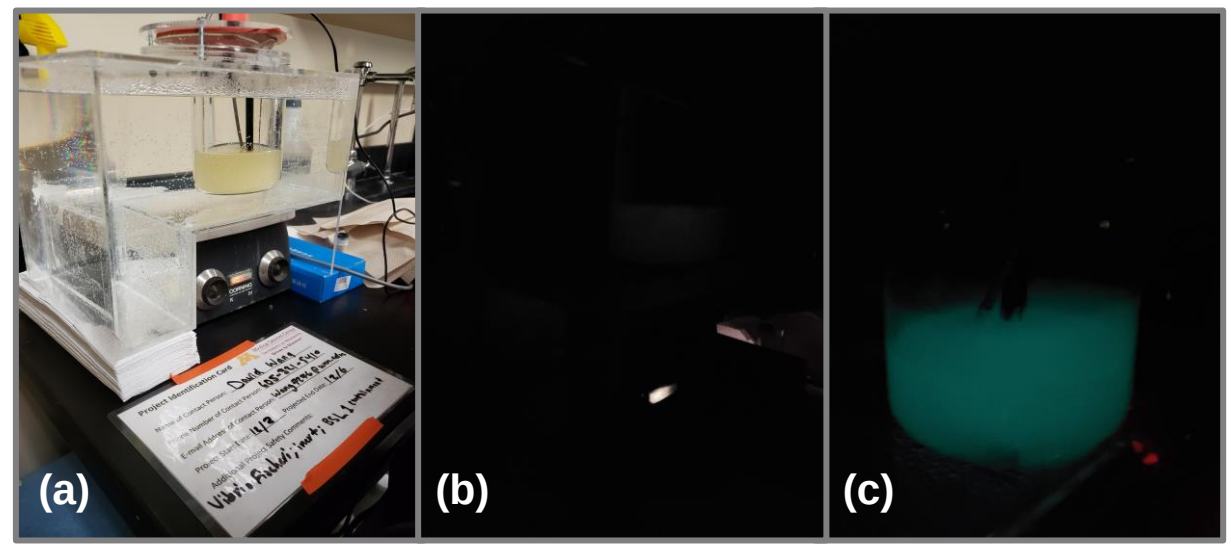

Figure 2. Test Run 2 at 45 hours: **(a)** stirred in ambient light, **(b)** stirred in total darkness, **(c)** shaken in total darkness

Future Directions

- Introduce a heating element for active temperature control; implement a touchscreen and graphical user interface for ease of use
- Pursue other cell culture targets, including hydrogen-producing bacteria, penillicin-producing molds, and cellular viticulture

References

[1]. Ward, O. P. Bioreactors in Bioprocessing. *Bioprocessing* **1991**, 37–54.
[2]. Ruby et al. Complete Genome Sequence of Vibrio Fischeri: A Symbiotic Bacterium with Pathogenic Congeners. *Proc. Natl. Acad. Sci.* **2005**, *102* (8), 3004–3009.
[3]. Madden, D.; Lidesten, B.-M. Bacterial Illumination: Culturing Luminous Bacteria. *BioScience Explained* **2001**.