Design and implementation of an affordable laboratory-scale bioreactor for the production of microbial natural products

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Large-scale bacteria culturing can present many challenges for small academic laboratories. To address these challenges a cost effective, laboratory scale bioreactor was designed and implemented. The constructed bioreactor addresses common problems that small or teaching-focused laboratories face when attempting scale up cultures. The design utilizes materials commonly found in standard chemistry laboratories that are easily assembled with minor modifications. The system was validated through the replication of natural product production in shake flasks and the bioreactor. Additionally, measurements were done to ensure the designed bioreactor had comparable $k_{La}$ values to common shake flask conditions. We anticipate that this design will be of use to other small academic natural product groups as well as teaching laboratories as it offers an economical way to undertake large scale culturing of microorganisms.

KEYWORDS
low-cost bioreactor, natural products, teaching laboratories, undergraduate education

An enduring struggle of bacteria-derived natural product isolation is the low titer of interesting compounds. This necessitates the culturing of large volumes of bacteria to obtain enough material to spectrally characterize an isolated compound. Currently, it is estimated that upwards of 90% of all culturing experiments in biotechnology are performed through shaken cultures.1 The benefits of using shaken cultures are numerous and include the ability to culture in high density, a relatively low-cost barrier, and the use of easily accessible equipment. There are, however, a number of drawbacks to using shake flasks. Primarily, shake flasks rely on surface aeration to transfer oxygen into the culture, resulting in low oxygen transfer compared to stirred bioreactors.2 For natural product researchers, the application of shake flasks for the large-scale cultivation of microorganisms for natural product isolation has additional challenges. Although high-capacity shakers capable of handling upwards of 100 flasks at one time are available, they are expensive and have a large footprint. This necessitates the use of smaller, cheaper orbital shakers that requires multiple rounds of culturing to generate enough material for compound isolation and characterization.

Despite their drawbacks, it is our observation that the large majority of microorganism culturing for natural product production is done in shake flasks. It would be far more economical, in terms of time and resources, to culture microorganisms on a larger scale instead of many shake flasks spread out over multiple culturing experiments. Although essential to the upstream industrial processing of live microorganisms, large bioreactors are often cost prohibitive for smaller
academic and teaching laboratories. The increased initial and continuing cost prevents research groups from realizing the full biosynthetic potential of the microorganisms they are investigating.

Commercial bioreactors are often unaffordable for smaller and teaching labs with costs reaching many thousands of dollars for large scale systems. Exploring the literature reveals significant work in the area of micro and miniature bioreactors, ranging in volume of low μL to 0.5 L. These options, however, were too small for our purposes. Others describe the use of larger, bench, and laboratory-sized bioreactors for natural product production. These reports, however, utilize commercial bioreactors. The lack of affordable commercial equipment and the need to increase our culturing capabilities led us to designing our own simple bioreactor. This bioreactor uses low-cost components and other equipment commonly found in chemistry laboratories to culture up to 20 L per setup. The design addresses the specific space and resource challenges associated with operating within an academic laboratory. Optimization experiments ensured that the system transferred oxygen as well or better than standard shake flask conditions typically employed by natural product chemists. Herein, we describe an economical design and validation of an efficient laboratory-scale bioreactor. The low cost of the individual components combined with the ease of assembly makes this bioreactor an affordable option for academic and other small laboratories without the space or funds needed for large commercially available fermentation systems.

The most common culturing apparatus for natural products research uses Erlenmeyer flasks on orbital shakers. Therefore, the primary concern in developing this laboratory-scale bioreactor was comparability to shake flasks in terms of metabolite production and oxygenation. The bioreactor was designed to meet a few basic conditions: aseptic and safe culturing, sterilizability, and the ability to maintain sterility throughout the experiment, mixing capability, sampling, and monitoring. As shown in Figure 1, the design combines low cost and readily available components in a way that is easy to assemble with minimal alterations. Design considerations are described below. A complete list of parts (Table S1), step-by-step instructions with images (Figures S1 to S8), and additional notes based on experience, are given in the supplemental information.

Primary considerations for the vessel include volume, construction material, and footprint. For the production of bacteria-derived natural products, volume is important as increased titer of compounds is the ultimate goal of culture scale-up. Additionally, it is often necessary to autoclave the entire bioreactor system. The chosen vessel must be made of autoclavable material and fit into the undersized autoclaves typically available to a smaller academic research laboratory. Polycarbonate and polypropylene carboys are both commonly available in large sizes; polycarbonate was chosen for its transparency. Standard bioreactor vessels are typically round. At just over 21 in in height, a standard round 20-L carboy did not fit into our autoclave. We anticipate other academic laboratories would have a similar issue. A rectangular 20-L carboy (12 × 16 × 24 in; W × D × H) did fit into the autoclave. Larger autoclaves would be able to fit a round carboy; however, a rectangular shaped vessel was ultimately preferred as described below.

A simple heated stir plate serves two functions: temperature regulation and mixing. A 10 × 10 in stirring hot plate was chosen as it affords a large surface area that closely matched the footprint of the carboy. A model with a digital display was chosen for a convenient and easy control of mixing and temperature. With the large mass of water in the

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**FIGURE 1** Bioreactor design for the low-cost and efficient culturing of microorganisms for natural product production and isolation. Notable components are highlighted.
carboy, the hot plate temperature needed to be set higher than the target temperature. A temperature relationship profile was prepared to correlate the setting with media temperature. For our specific hot plate, a setting of 60°C correlated to 30°C media temperature. Once set, the temperature remained constant and no temperature gradient was observed. The rectangle-shaped carboy was more efficient in thermal transfer to the liquid media than a standard round carboy. The bottom of the rectangle carboy was larger and slightly less concave than the round carboy, allowing greater surface area and better contact with the hot plate.

The cap, shown in Figure 2, must provide both continued aseptic culturing as well as sterile sampling and additions. Two 1/4 in barbed fittings, to allow the connection of air in and vent lines, were inserted into the lid by drilling a hole into the cap and securing the fitting with epoxy. For sterile access to the culture, a stainless steel Luer lock fitting was installed on the cap through the same procedure. A short section of silicon tubing was attached to the Luer fitting at a length just long enough to extend into the culture medium. When not in use, the port is kept tightly capped. When access is needed, a sterile Luer lock syringe is attached. A volume of culture can be removed or nutrients added with the syringe. A new, sterile cap is put on after sampling. The cap is designed with extra space for additional modifications. Depending on the application, such changes could include the installation of a temperature probe to obtain more precise measurements of temperature or a pH probe to track system pH. We encourage others to alter the design to fit their own needs.

Our system is designed to use a magnetic stir bar to mimic the impeller in commercial bioreactors. No additional drive motor is necessary, simplifying the design. Since a magnetically driven stir bar cannot exert the same force as mechanically driven impeller, numerous stir bars shapes and sizes were tested for maximum liquid movement: cross, round disk with ridges, dumbbell and varying lengths of straight stir bars. Decreasing the size of the stir bar allowed for a greater spin speed at the cost of efficient mixing. Using very large stir bars resulted in a decrease of circulation through a combination of increased friction, weight, and smaller drive magnet to stir bar size ratio. Ultimately, a midsized stir bar (3 × 1/2 in) allowed for moderately fast stirring (300 rpm) with significant circulation of the liquid medium. It was observed that the corners of the square vessel increased turbulence at the liquid-gas interface compared to the rounded vessel, increasing liquid turn over. This observation has been reported by others in the past and supported the choice of a square carboy.

Subsurface aeration is required to properly transfer oxygen into large-scale cell cultures. This design utilizes a 10-μm stainless steel filter, similar to the solvent stones used in many chemistry applications. The metal aerator is autoclavable and the pore size produces midrange bubbles. The tubing was attached to the underside of the cap and cut to a length that allowed the aeration stone to hang just above the stir bar for maximum distribution of air bubbles throughout the culture. An air regulator is attached between the source of compressed air and the bioreactor to control the flow rate into the culture. To ensure air introduced into the bioreactor was kept sterile, an autoclavable 0.3-μm air filter was installed between the bioreactor and air source. The system is vented through tubing attached to the cap of the bioreactor that terminates in a reservoir of bleach and water to prevent health and safety issues.

Long-term sterility of the bioreactor was demonstrated by autoclaving the complete system but not inoculating the culture medium. The bioreactor was set up to mimic a culturing experiment with stirring and air flow and left for one week. At the conclusion of the experiment, there was no detectable difference between the sample taken from the bioreactor
and an autoclaved blank sample of the culture medium beyond the variability of the instrument, suggesting that no cell growth was detected and the system remained sterile.

To demonstrate the hardiness of the system in the production of bacteria-derived natural products, an experiment was undertaken to explore the metabolites produced by a bacterium in shake flasks and the bioreactor culturing systems. A strain of *Photobacterium halotolerans* (M128SB283Ax), a Gram-negative bacterium known to produce a family of cyclic depsipeptides known as the kailuins, was chosen as a model organism. The bacterium was grown in both culturing systems using the same liquid medium. Organic extracts were generated and analyzed to compare the metabolic profiles of the bacterium grown in the two different systems. Figure 3 shows the structure of the metabolites of interest and an overlay of the chromatograms generated from the high-performance liquid chromatography–ELSD (HPLC-ELSD) data. Peak identities were confirmed through mass spectrum analysis. Kailuin H did not replicate in the bioreactor. However, we have found that kailuin H is difficult to replicate between consecutive shake flask cultures. The data suggest that the bioreactor did not significantly interrupt the production of the compounds of interest.

After verifying the bioreactor set up allowed for the isolation of similar natural products compared to shake flasks, the oxygen transfer efficiency was characterized. The goal of this system was to mimic shake flask systems currently employed by most natural product researchers. Published volumetric mass-transfer coefficient ($k_La$) values for 1-L and 2-L flasks containing 0.5-L and 1-L of culture medium range from 7 to 8.8 h$^{-1}$ respectively. The lowest compressed air flow rate that afforded a higher $k_La$ than literature values for shake flasks was 4 L/min. Table 1 shows the calculated $k_La$ values at 4 and 6 L/min of air flow versus published literature values of representative shake flask systems. At 4 L/min, the $k_La$ exceeds that of the shake flasks and increasing flow rates cause a relative increase in the $k_La$.

**TABLE 1** Comparison of calculated $k_La$ values for the designed bioreactor versus published data for common shake flask systems

| Vessel Type | Vessel Volume (L) | Liquid Volume (L) | Air (L/min) | RPM$^a$ | $k_La$ (h$^{-1}$) |
|-------------|-------------------|-------------------|-------------|--------|------------------|
| Literature Values | | | | | |
| Flask 1 | 1 | 0.5 | 0 | 110 | 7.5 |
| Flask 2 | 2 | 1 | 0 | 140 | 8.8 |
| Flask | 1 | 0.3 | 0 | 110 | 13.7 |
| This study | | | | | |
| Bioreactor | 20 | 20 | 4 | 300 | 11.4 ($\pm 0.5$) |
| Bioreactor | 20 | 20 | 6 | 300 | 15.1 ($\pm 0.6$) |

$^a$Note: RPM for shake flasks refers to the speed setting on an orbital shaker while RPM for the bioreactor system refers to the speed of the magnetic stir bar set on the stir plate.
As shown here, we have designed and implemented a low-cost, customizable, laboratory-scale bioreactor for the cultivation of microbial natural products. The design is able to aerate the culture medium as well and, in some cases, with a greater $k_{La}$ than a typical shake flask system. It can be assembled using readily available supplies that many chemistry laboratories may already have on hand. Although we recognize that more advanced systems exist, we anticipate that academic laboratories and those without access to more sophisticated fermentation facilities will benefit most from this design. Our experiences have shown that undergraduate students are able to independently assemble and implement this system, making this design particularly useful in a teaching laboratory setting.

1 | MATERIALS AND METHODS

1.1 | Temperature tests
To determine the correct setting for the hot plate, the bioreactor was filled with 20 L of deionized water and allowed to equilibrate for several hours while stirring. The temperature was recorded over the course of 1 hour. Multiple hot plate settings were tested to plot a temperature relationship profile to determine the correct setting for our specific hot plate. To test for temperature gradients, the temperature probe was placed at different depths and locations throughout the carboy.

1.2 | Sterility experiments
Twenty liters of Marine Broth was prepared and added to the bioreactor. Approximately 10 mL of media was removed, split into 2-mL aliquots, autoclaved in 15-mL centrifuge tubes, and subsequently stored in the refrigerator ($4^\circ$C) to be used as blanks. The bioreactor was fully assembled and autoclaved. The system was not inoculated with any microbial strain. After one week of operation ($30^\circ$C, 300 rpm, and 6-L/min air flow rate) a sample was removed from the bioreactor and immediately analyzed for bacteria growth. OD$_{600}$ was measured using a Bio-Rad SmartSpec Plus Spectrophotometer (system variability, ± 0.005 abs). Three replicates were taken.

1.3 | Comparison of metabolite production in shake flask and bioreactor
A Photobacterium halotolerans strain (M128SB283Ax) isolated by our laboratory was grown in MD 1 medium with SWS and 20 g/L XAD-7HP adsorbent resin for two weeks in shake flasks (2 L flask, 1 L of culture medium, 200 rpm, and $30^\circ$C) and in the designed bioreactor (20 L, $30^\circ$C, 300 rpm, and 6-L/min air flow rate). The cultures were extracted as previously described and dried under vacuum. The extracts were prepared at 20 mg/mL in a mixture of 90:10 methanol:water and analyzed by HPLC-ELSD-MS. Data were extracted and overlaid in postprocessing. Growth medium, extraction procedures, and HPLC-ELSD-MS method were all as previously described.$^{24}$

1.4 | Determination of dissolved oxygen and mass transfer coefficient in the bioreactor
All experiments were carried out in deionized water at room temperature ($20.5^\circ$C ± 0.5$^\circ$C). For determination of the oxygen mass transfer coefficients, the water was sparged with nitrogen gas until reaching less than 5% saturation, as measured with an oxygen meter (YSI dissolved oxygen probe) suspended approximately in the middle of the water. After sparging was complete, the bioreactor was connected to a source of compressed air and oxygen absorption was measured with readings taken at 15 s intervals. Measurements we taken until the water was above 90% saturation. The bioreactors were stirred at 300 rpm and the air flow rates varied in each experiment. Experiments were run in triplicate at each flow rate. The oxygen mass transfer coefficient, $k_{La}$, was calculated as described previously and corrected for probe response time.$^{26,27}$ Nonlinear regression (using the solver function in Microsoft Excel) was used to determine the best estimate of $k_{La}$ from each data set and is reported as the average of three trials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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REFERENCES

1. Büchs J. Introduction to advantages and problems of shaken cultures. Biochem Eng J. 2001;7(2):91-98. https://doi.org/10.1016/S1369-703X(00)00106-6
2. Betts JI, Baganz F. Miniature bioreactors: current practices and future opportunities. Microb Cell Fact. 2006;5:21. https://doi.org/10.1186/1475-2859-5:21
3. Bartholomew SR, Tansey JT. Cost-effective engineering of a small-scale bioreactor. Biotechnol Bioeng. 2007;96(2):401-407. https://doi.org/10.1002/bit.21118
4. Betts JI, Doig SD, Baganz F. Characterization and application of a miniature 10 mL stirred-tank bioreactor, showing scale-down equivalence with a conventional 7 L reactor. Biotechnol Prog. 2006;22(3):681-688. https://doi.org/10.1021/bp050369y
5. Chen A, Chitta R, Chang D, Amanullah A. Twenty-four well plate miniature bioreactor system as a scale-down model for cell culture process development. Biotechnol Bioeng. 2009;102(1):148-160. https://doi.org/10.1002/bit.22031
6. Doig SD, Diep A, Baganz F. Characterisation of a novel miniaturised bubble column bioreactor for high throughput cell cultivation. Biochem Eng J. 2005;23(2):97-105. https://doi.org/10.1016/J.BEIJ.2004.10.014
7. Gill NK, Appleton M, Baganz F, Lye GJ. Design and characterisation of a miniature stirred bioreactor system for parallel microbial fermentations. Biochem Eng J. 2008;39(1):164-176. https://doi.org/10.1016/J.BEIJ.2007.09.001
8. Isett K, George H, Herber W, Amanullah A. Twenty-four-well plate miniature bioreactor high-throughput system: assessment for microbial cultivations. Biotechnol Bioeng. 2007;98(5):1017-1028. https://doi.org/10.1002/bit.21484
9. Lamping S, Zhang H, Allen B, Ayazi Shamlou P. Design of a prototype miniature bioreactor for high throughput automated bioprocessing. Chem Eng Sci. 2003;58(3-6):747-758. https://doi.org/10.1016/S0009-2509(02)00604-8
10. Reis N, Gonçalves CN, Vicente AA, Teixeira JA. Proof-of-concept of a novel micro-bioreactor for fast development of industrial bioprocesses. Biotechnol Bioeng. 2006;95(4):744-753. https://doi.org/10.1002/bit.21035
11. Zhang Z, Perozzielo G, Boccazzi P, Sinskey AJ, Jensen KF. Microbioreactors for bioprocess development. J Assoc Lab Autom. 2007;12(1):143-151. https://doi.org/10.1016/J.JALA.2006.10.017
12. Amna T, Puri SC, Verma V, et al. Bioreactor studies on the endophytic fungus Entrophospora infrequens for the production of an anticancer alkaloid camptothecin. Can J Microb. 2005;51(3):189-196. https://doi.org/10.1111/j.1139-0607.2005.tb06437.x
13. Marwick JD, Wright PC, Burgess JG. Bioprocess intensification for production of novel marine bacterial antibiotics through bioreactor operation and design. Marine Biotechnol. 1999;1(5):495-507. https://doi.org/10.1007/PL00011806
14. Sarkar S, Saha M, Roy D, et al. Enhanced production of antimicrobial compounds by three salt-tolerant actinobacterial strains isolated from the Sundarbans in a niche-mimic bioreactor. Marine Biotechnol. 2008;10(5):518-526. https://doi.org/10.1007/s11012-008-9090-0
15. Chreptowicz K, Wielechowska M, Główeczyk-Zubek J, Rybak E, Mierzejewska J. Production of natural 2-phenylethanol: from biotransformation to purified product. Food Bioprod Process. 2016;100:275-281. https://doi.org/10.1016/J.FBP.2016.07.011
16. Li J, Jaitzig J, Lu P, Süssmuth RD, Neubauer P. Scale-up bioprocess development for the antibiotic valinomycin in Escherichia coli based on consistent fed-batch cultivations. Microb Cell Fact. 2015;14(83). https://doi.org/10.1186/s12934-015-0272-y
17. Zhang Y, Arends JBA, Van de Wiele T, Boon N. Bioreactor technology in marine microbiology: from design to future application. Biotechnol Adv. 2011;29(3):312-321. https://doi.org/10.1016/j.biotechadv.2011.01.004
18. Charles M, Wilson J. Fermenter/bioreactor design. In: Flickinger MC, ed. Upstream Industrial Biotechnology: Equipment, Process Design, Sensing, Control, and cGMP Operations. Vol. 2. Hoboken, NJ: John Wiley & Sons; 2013:1101-1136.
19. Garcia-Ochoa F, Gomez E. Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnol Adv. 2009;27(2):153-176. https://doi.org/10.1016/j.biotechadv.2008.10.006
20. Matthews G. Fermentation equipment selection: laboratory scale bioreactor design considerations. In: McNeil B, Harvey LM, eds. Practical Fermentation Technology. Chichester, UK: John Wiley & Sons, Ltd; 2008:3-36.
21. Kato I, Tanaka H. Development of a novel box-shaped shake flask with efficient gas exchange capacity. J Ferment Bioeng. 1998;85(4):404-409. https://doi.org/10.1016/S0922-338X(98)80084-6
22. Marks DM. Equipment design considerations for large scale culture. Cytotechnology. 2003;42(1):21-33. https://doi.org/10.1023/A:1026103405618
23. Harrigan GG, Harrigan BL, Davidson BS. Kailuins A–D, new cyclic acyldepsipeptides from cultures of a marine-derived bacterium. Tetrahedron. 1997;53(5):1577-1582. https://doi.org/10.1016/S0040-4020(96)01136-2
24. Theodore CM, Lorig-Roach N, Still PC, et al. Biosynthetic products from a nearshore-derived gram-negative bacterium enable reassessment of the kailuin depsipeptides. J Nat Prod. 2015;78(3):441-452. https://doi.org/10.1021/np500840n
25. Nikakhhtari H, Hill GA. Modelling oxygen transfer and aerobic growth in shake flasks and well-mixed bioreactors. Can J Chem Eng. 2008;83(3):493-499. https://doi.org/10.1002/cjce.5450830312
26. Tribe LA, Briens CL, Margaritis A. Determination of the volumetric mass transfer coefficient (kLa) using the dynamic gas out-gas in method: analysis of errors caused by dissolved oxygen probes. *Biotechnol Bioeng.* 1995;46(4):388-392. https://doi.org/10.1002/bit.260460412

27. Van’t Riet K. Review of measuring methods and results in nonviscous gas-liquid mass transfer in stirred vessels. *Ind Eng Chem Process Des Dev.* 1979;18(3):357-364. https://doi.org/10.1021/i260071a001

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