Low-Cost Bioreactor to Enable Microbe Productivity Optimization

Design Team
David Christianson, Elizabeth Duffy
Bryan Keen, Andrew Mazzotta
Jameson Stark

Design Advisor
Prof. Jeffrey Ruberti
Email: j.ruberti@neu.edu

Abstract
The production of biofuel is necessary to reduce the global dependence on diminishing fossil fuels. Joule Unlimited, Inc., a biotechnology company, has developed a strain of microbes that convert solar energy to useable fuels, such as biodiesel and ethanol. To increase the cost efficiency of bioreactors which house these microbes, Joule has tasked the team to design a low-cost lab-scale bioreactor. The requirements from Joule are to have control over the following environmental parameters: culture depth variation, temperature, sparging of air/CO₂, pH level, and light intensity. The design of the bioreactor and the individual components along with the mathematical analysis done is explained in the following report. To control the required environmental settings this bioreactor is equipped with a radial sealed piston cylinder allowing culture depth variation, a thermoelectric device for thermal management, a sparging orifice plate with CO₂ and air supply, pH level and RTD sensor with control system, and an LED light board array for light intensity. Due to the cost of the complex manufacturing required to meet the design specifications, the team presented the solution to Joule and received positive feedback. Joule is currently evaluating the final design for fabrication.
The Need for Project

To optimize microbe productivity of biofuel, a low-cost bioreactor is needed with variability in environmental settings. Joule Unlimited, Inc., the project sponsor, has developed a strain of specialized microbes that convert solar energy to useable fuels, such as biodiesel and ethanol. These biofuels can be used in place of environmentally harmful and depleting fossil fuels. In the field, the microbes are housed in outdoor photo bioreactors (PBRs), where they are suspended in a solution and through photosynthesis produce biofuel. In order to make this process cost-efficient, a lab scale bioreactor is needed to test various environmental settings within the bioreactor to optimize microbe productivity. The lab test unit Joule currently uses costs $50,000 and does not satisfy all control parameters desired. A new bioreactor has been designed with a lower cost and greater environmental variability and control.

The Design Project Objectives and Requirements

**Design Objectives**

The design objective of this project is to design, fabricate, and demonstrate a lab-scale bioreactor. In order to increase productivity of the microorganism, the bioreactor they are housed in needs to provide an optimal environment. The bioreactor developed is flexible in design, allowing variation of parameters including light intensity, temperature, pH level, sparging (air and CO2 supply), and variability in culture depth. Such systems do not exist that satisfy all of these constraints. Joule has called upon the group to develop a bioreactor that is much more suitable for their needs and will provide them with a more cost effective and efficient way to optimize their microorganisms’ productivity.

**Design Requirements**

A very stringent set of design requirements was assigned from Joule. A temperature range from 25°C-60°C (±0.5°C) is required as different strains of culture prefer to live at different temperatures. Light intensity will vary from 100-3000µE/m²/s (where an E is one mol of photons). The incoming gas will be sparged from 0.1-4 VVM (volume of gas/volume of liquid/minute). A depth range from 5mm-40mm is required with a volume of 1L of culture at 10mm depth. Materials selection was a critical part of the design because the bioreactor is required to be an autoclave-biocompatible vessel.
Design Concepts considered

Each subsystem of our reactor required individual design concepts, but the compatibility between these components, particularly the sparging, depth variation, and thermal management proved to require the most consideration.

For a culture depth variation method, initially distinct, “stepped” depths were considered for mechanical simplicity. A disadvantage is that stepped solutions involve complicated sealing and assembly, and create stagnant flow and no-light zones. The lighting system could have been constructed from an array of blue and red LED’s, and while this provides adequate wavelengths for cellular absorption, it is not an accurate representation of sunlight. For a heating solution, resistive heaters deliver adequate heating capabilities in a small area. However, they are incapable of providing the necessary cooling as well (Rep. 3.2)

The sparging concepts are driven by the method of depth variation. A series of perforated tubes could provide the adequate gas flow rates, but would require reassembly for varied depth. A method for adjusting the sparging depth to accommodate culture depth was also necessary. Consideration was given to a piston-cylinder assembly, which would vary the sparge reservoir depth as the culture depth was changed. However, the combination of sealing requirements and manufacturing methods turned the team away from this solution. For materials selection PTFE was considered because it is machineable, autoclavable, and biocompatible. However, it lacks a practical thermal conductivity necessary for heating requirements (Rep. 3.2). A vital aspect to the system’s design is its overall shape, and a cylindrical shape was originally thought to provide simpler manufacturing and sealing methods, but would cause difficulties in sparge implementation.

Recommended Design Concept

A 316-L stainless steel piston-cylinder vessel houses the culture, where it is heated, sparged, and lighted in variable parameters meeting the design specification.

Design Description

Depth Variation

The final design settled upon is a rectangular piston-cylinder vessel machined in tight tolerance to utilize a radial double o-ring seal to mate the piston and cylinder (Rep. 3.1) for continuous variable culture depth. The entire cylinder is to be machined from a single billet of 316-L stainless steel to hold the necessary precision. A glass front plate sandwiched between a stainless steel bezel lined with a compliant PTFE buffer material and an o-ring face seal on the
cylinder encloses the front side of the vessel. All o-rings are standard AS-568 sizes and are made of FKM (fluoroelastomer) for biocompatibility with the culture and autoclave sterilization. The piston is actuated by a leadscrew assembly and vessel depth is tracked visually with a scale mounted on the backside of the piston.

**Material Selection**

Both the piston and cylinder are machined from 316-L to maintain autoclaving and biocompatibility. This also prevents a compromise of the o-ring seals due to thermal expansion over the 60° temperature change possible in the bioreactor. Using stainless steel for a vessel of this size, which must be moved to an autoclave, leads to a heavy and cumbersome final product. However, in order to meet all design requirements, this issue was addressed by removing any unnecessary material from the cylinder and piston to reduce weight.

**Thermal Management**

The culture is heated and cooled using a thermoelectric device (TED) mounted on the back of the piston. It applies a heat flux (positive or negative) based on the gains set in the PID controller to maintain the desired culture temperature, and conducts through the stainless steel piston. Modeling the sparged culture as a turbulent, fully-developed flow allows for accurate mathematical prediction of how much heat flux will be required for any given desired temperature (Rep. 3.2).

**Sparging Air/CO₂ and pH control**

Air and carbon dioxide gas sparge mixture is introduced to the culture through 0.2mm diameter orifices organized in an array located centrally on the bottom face of the cylinder bore to control the pH of the culture. This orifice size was calculated to produce 2mm diameter bubbles which are ideal for mass transfer of CO₂ to the culture. The bubbles also provide adequate mixing to the culture by generating two adjacent cyclic flows in the vessel (Rep. 3.3). The sparge array also allows for uniform gas introduction to the culture regardless of vessel depth. For whichever culture thickness is being tested in a given experiment, a base plate corresponding to the desired depth is bolted onto the bottom face of the cylinder which uses a PTFE sheet gasket face seal to block off sparge orifices not exposed to the culture. A series of baseplates is included with the bioreactor, covering the full
range of vessel depths. Air is constantly sparged to the culture, while CO₂ is intermittently added based on feedback from a pH sensor. When the acidity is too high, a solenoid valve controlling the CO₂ is opened until pH returns to desired levels (Rep. 3.6). Due to the difficulty in drilling these tiny 0.2mm holes, plunge EDM microdrilling must be used, and this requires the sparging plate to be manufactured as a modular assembly. A pocket is machined in the cylinder where the sparge plate will be laser-welded in place after the holes are drilled, and the critical surfaces are post-machined to tolerance afterwards.

**Light Intensity**

Light is provided to the culture by three white LED arrays mounted to a panel parallel to the glass face of the vessel. White LED arrays closely simulate sunlight and deliver the desired light intensities by varied duty-cycles. Both the lighting and pH regulation systems are controlled in a LABVIEW VI file (Rep. 3.5) and a solid state relay.

**Analytical Investigations**

Analysis on the thermal expansion of the vessel was done for seals. Heat transfer analyses was done to determine how the vessel would be thermally managed for culture temperature control based off of a fully developed turbulent internal flow (Rep. 3.2). Sparge orifice geometry and array dimensions were determined based on theoretical calculations from fluid flow dynamics through orifices, research, and experimental verification in a test rig (Rep. 3.3).

**Key Advantages**

This final design concept satisfies all design specifications set forth by the project sponsor, Joule Unlimited Inc. It is also at a lower cost bioreactor compared to the $50,000 unit in use right now. The advantages this bioreactor has over the existing one is variable culture depth, uniform sparging, the desired volume of culture needed, and varying light intensity. All of these features make this bioreactor capable of more environmental variability and will lead to more efficient optimization of microbe productivity.

**Financial Issues**

Due to the cost of the complex

By meeting all of the design requirements the cost in materials
and machining is inherently expensive. Weight reduction added to the machining costs. The EDM sparge hole drilling and laser welding processes are both expensive micro-fabricating procedures. Quotes have been gathered from various machine shops that show long lead times and relatively high costs. It is estimated that machining of the complex parts will cost a total of about $10,000. The cost of additional parts and accessories such as sensors, heating, cooling, and lighting totals an additional $5,000. A total estimate of $15,000 to fabricate this bioreactor is still significantly less than the $50,000 system currently used by Joule. For these reasons production has been delayed, however, Joule is currently evaluating the final design for fabrication.

**Recommended Improvements**

*With more time, manufacturing methods can be simplified in order to reduce cost.*

Given a longer development timeline it would be possible to investigate different manufacturing processes in order to reduce the complexity of the manufacturing and lower the cost. Casting of certain parts is an option that should be explored in the interest of higher production runs. With more funding, investigating alternative bio-compatible materials can reduce the weight of the system and simplify manufacturing. Another viable improvement of the bioreactor system would be a custom LED lighting array to optimize light distribution.