A Novel Single-Use Perfusion Bioreactor Without a Mixing Impeller that Operates with Reduced Shear and Lower Cost

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ABSTRACT

CP Biotools LLC presents progress to date in development of a Single-Use Bioreactor designed to operate in a perfusion mode for continuous cell culture. Based on U.S. Patent No. 7,875,448, the design incorporates a novel liquid diffuser located at the top of the liquid level that creates a fountain type of flow of the culture returning from an external circulation loop.

Both the counter-current flow of gas rising from the sparger and liquid flowing to the bottom of vessel and the air liquid interface of the fountain enhance mixing and kLa.

So far, extensive design testing and optimization have been completed using powerful engineering and scientific tools such as 3D printing, computational fluid dynamics (CFD), water runs to measure kLa, and extensive physical measurements with different sensors. In addition, three CHO cell culture runs have been completed over the different design iterations in conjunction with Jefferson Institute for Bioprocessing at Thomas Jefferson University.

Design Parameters for CP Biotools' Single-Use Bioreactor (SUB) Include:

- Simple bag-style design with a liquid diffuser and a gas sparger
- Eliminates mechanical mixing
- Ability to utilize single-use pH and dissolved oxygen probes that eliminates the need for an autoclave

CP Biotools LLC has made significant progress to date in development of a Single-Use Bioreactor designed to operate in a perfusion mode for continuous cell culture. The design incorporates a novel liquid diffuser located at the top of the liquid level that creates a fountain type of flow of the culture returning from an external circulation loop. In the external circulation loop, the culture leaves the vessel through the conical shaped bottom of the vessel and is circulated via a single-use, low-shear centrifugal pump[1] through a hollow-fiber filter that acts as the cell retention device and the flow and pump energy provides the mixing as the culture returns to the vessel via the liquid diffuser. The fountain effect of the returning liquid creates a liquid -gas interface that enhances the bioreactor kLa. (Figure 1)

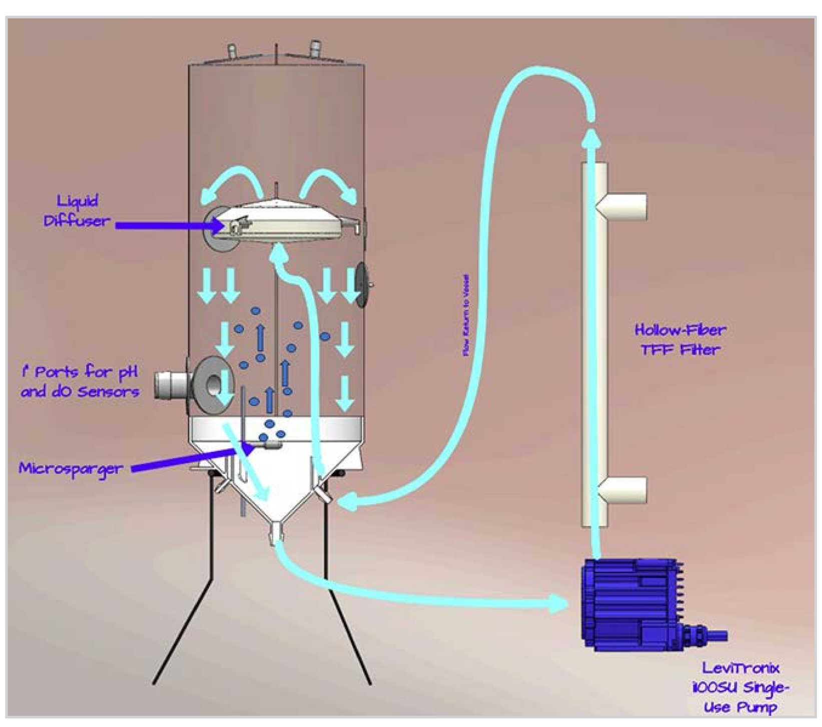

FIGURE 1

Iterative Research & Development Steps Leading to Final Design:

1. Completed crude testing with different container shapes that were 3D printed along with 3D printed liquid diffusers with different configurations based on a 1 LPM flow rate. This flow rate was selected based on the eventual cross flow required though the hollow fiber filter sized for this volume of bioreactor with perfusion rate of 1 VVD. Diffusers were made with various hole diameters and varying number of holes. Performed kLa studies to confirm optimal diffuser design and 2 to 3 cm fountain height. Mixing observation indicated very rapid dye dispersion.

2. Completed Computational Fluid Dynamic studies (CFD) with a contracted firm using the Ansys software to confirm efficient mixing and no dead zones. The software was also able to simulate the kLa of the gas dissolution. Results were very good with excellent rapid mixing when gas was flowing through the sparger. (Figure 2 and 3).

3. Detailed kLa testing was completed to determine the optimal sparger design and selection. This was completed by testing a variety of single use spargers that are manufactured of materials compatible with gamma radiation. The testing consisted of purging oxygen from purified water with nitrogen. Following that procedure, air was introduced at a constant flow rate and the percent saturation was graphed and the data logged. After saturation was achieved, the data was processed to calculate the kLa.

4. A "Diffuser Functional Test" was completed with CHO Cells November 2023. In this non-sterile run, with a 3D printed vessel Somos® BioClear USP Class VI material, the diffuser was being evaluated for the first time with cells. The kLa was estimated with only the diffuser fountain (no sparger) and was 8.05 h-1 which is substantial. Since the viability reading both for the bioreactor and shake flask were high (97 - 98%) and similar, the conclusion is that there was not any specific condition in the bioreactor causing cell death. Additionally, the liquid diffuser showed no evidence of clogging and this was also measured by logging the back pressure throughout the experiment.

5. For the first sterile batch run with CHO cells, a 3D printed BioClear vessel was designed with a lid sealed with a large O-ring and a series of plastic bolts and was completed in July 2024. The complete assembly was gamma radiated. A Mettler Toledo Single Use pH probe in a flow cell from PendoTECH and a custom made flow cell with a Single Use Mettler Toledo DO2 probe were in the circulation loop in this design. The run was a success with the control and bioreactor cell density and viability results being similar when accounting for a loss of control event overnight due to foam and a power outage, and discovery that temperature was controlling to about 35°C instead of the desired 37°C.

6. The first bag (film version) with CHO cells in a batch mode was completed in December of 2024. It consisted of an industry standard biocontainer film with port plates sealed to it which was then sealed to a machined custom-made base from polyethylene. This material is required to enable the sealing of the film with the custom-made heat-sealing tool. The run was successful in terms using the first bag version and performed well for sterility and with virtually no back pressure at the liquid diffuser with the pH and DO probes out of the circulation loop. However the machined base had a different slope in its bottom that leads to the vessel outlet versus the 3D printed base in the previous run, and roughness from machining and cells started to settle and accumulate as the density increased. Up to that point the bioreactor and control were performing similarly with matching glucose consumption.

Next Steps in the Bioreactor Development:

The final design is a simple bag-style design with a liquid diffuser and gas sparger and no internal moving parts. The bag is sealed to the plastic rigid base via a custom designed film to plastic sealing tool and the bag portion has port plates sealed into it. The bag portion incorporates a number of key components including ports to receive the single use pH and DO probes, ports to support the liquid diffuser, and addition ports. The rigid base prototype design has the correct slope which is the same as used in the first sterile run and contains the thermo-well for a temperature probe, the liquid drain, and the gas inlet to the gas sparger. The final design includes the hollow fiber for operation in a perfusion mode and four PendoTECH Single Use Pressure Sensors- three for measurement of the filter performance and one on the overlay tube to measure vessel pressure (Figures 7 and 8). The bioreactors have been sterilized via gamma radiation and the first perfusion run is scheduled for Q4 2025.

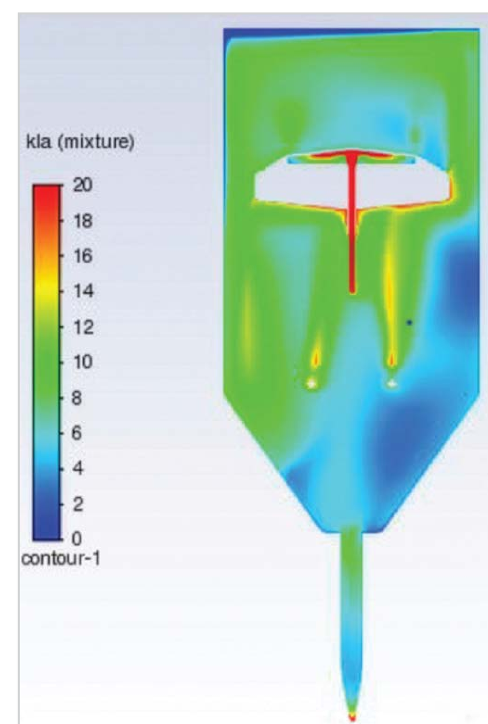

FIGURE 2 / CFD Output for kLa

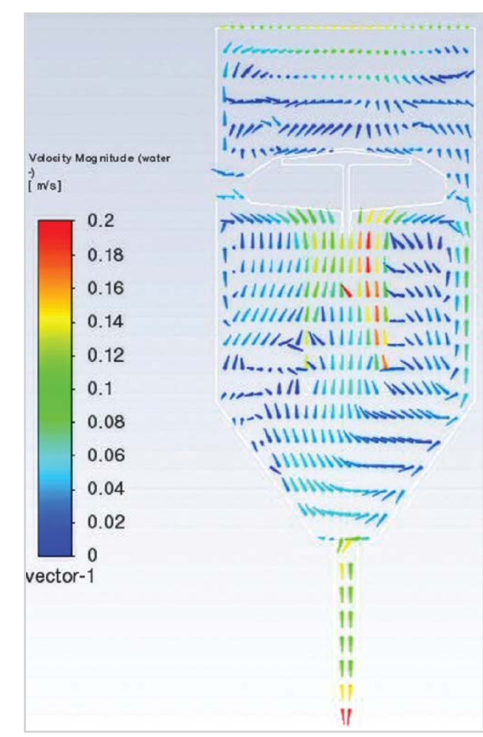

FIGURE 3 / CFD Output for Velocity Vectors

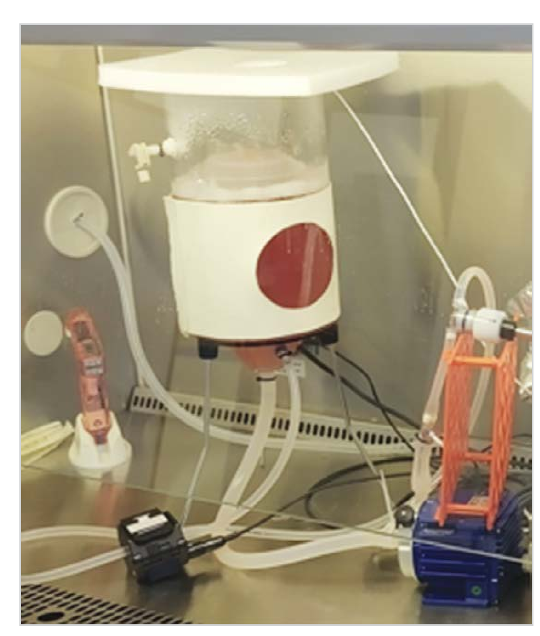

FIGURE 4 / Diffuser Functional Test Setup

FIGURE 5 / First Sterile Batch Run with CHO Cells

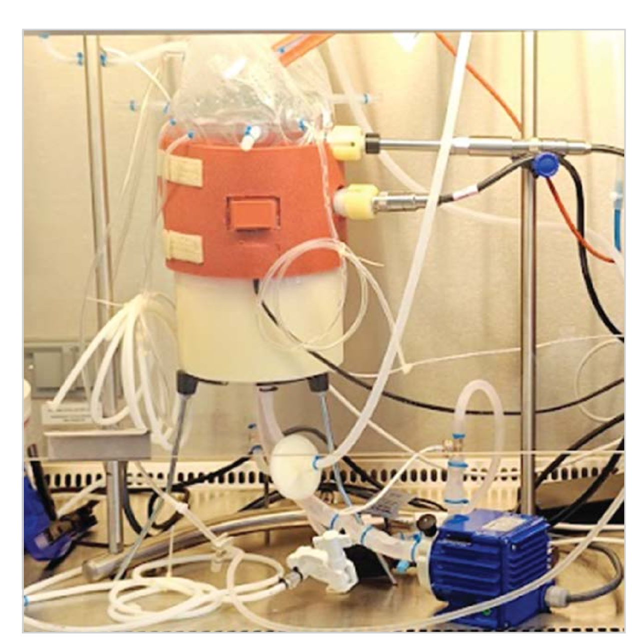

FIGURE 6
Sterile Batch Run with CHO Cells and First Vessel with Film and Port Plates

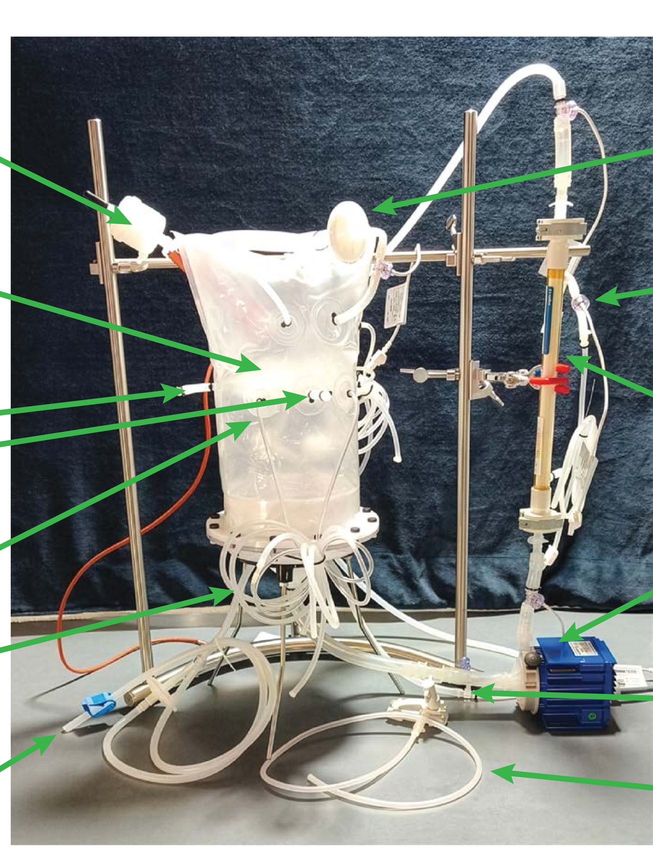

FIGURE 7
A Novel Single-Use Perfusion Bioreactor Without a Mixing Impeller: Front View

FIGURE 8
A Novel Single-Use Perfusion Bioreactor Without a Mixing Impeller: Rear View

1 Samantha Wang, Scott Godfrey, Janani Ravikrishnan, Henry Lin, Jens Vogel, Jon Coffman, Boehringer Ingelheim Fremont CA, "Shear contributions to cell culture performance and product recovery in ATF and TFF perfusion systems," *Journal of Biotechnology* 246 (2017) 52-60.