Designing an Algal Co-culture System for Increased Sustainability in Cellular Agriculture

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I. Introduction

When recognizing human contributions to climate change, animal agriculture cannot be ignored. Factory livestock contributes to 9% of carbon dioxide, 30% of methane, and 65% of nitrous oxide emissions [1]. This does not account for water, land, and energy use, or the safety and ethical concerns of factory farming. There is currently an effort to develop the field of cellular agriculture to improve our meat production practices. This field focuses on growing animal tissue *in vitro* by applying tissue engineering principles. One model predicts this could reduce greenhouse gas emissions by 96%, land usage by 99%, water usage by 90%, and energy usage by 40% when compared to traditional beef production [2]. There is potential for this field to revolutionize our food production industry while improving our relationship with the environment. However, one of the most prohibitive factors in scale-up is cell culture media. The cost of media is high and volatile due to fluctuations in the price of fetal bovine serum, which makes consistent large-scale operations difficult [3]. While efforts are being made to move away from serum-based media with supplementation of growth factors and hormones, the quantities required for scale-up still warrant new cost reduction strategies. The team is proposing a co-culture system that takes inspiration from the carbon and nitrogen cycles that replenish nutrients in the environment. Media needs to be replaced over time because of the accumulation of toxic byproducts like lactic acid and ammonium [4]. This co-culture system could metabolize such compounds, thereby extending the media lifetime. The success of this system would decrease the cost and environmental impact of large-scale cellular agriculture.

II. Design Process

The team identified a set of objectives to guide the design process and fulfill the need. The system must be scalable, promote primary bovine satellite cell (PBSC) proliferation, allow harvesting of PBSCs, and reduce the cost associated with cell culture media. The team also determined cell culture constraints to make the system feasible. The PBSCs must remain viable and retain their stem-phenotype, and the recycling cells must remain viable and metabolically functional.

The design was split into two aspects. The first is the co-culture system, which comprises the cell types, culture medium, temperature, and other culture conditions. Multiple photosynthetic cell types were explored, including microalgae, cyanobacteria, and plant species. After determining and ranking the needs, a custom value analysis matrix was used to choose a design. The microalga *Chlamydomonas reinhardtii* was chosen as the recycling cell for the co-culture system. The second aspect of the design is the scale-up, which is the proposed bioreactor design for large-scale production using our co-culture system. Conceptual designs were made for multiple bioreactor systems, and the top designs were compared using the same value analysis matrix. A hollow-fiber bioreactor design was chosen.

III. Final Design

The final co-culture design uses *C. reinhardtii* to uptake metabolic byproducts of PBSC culture and replenish the media with oxygen. The system is closed to gas exchange, so all additional oxygen is supplied by *C. reinhardtii*. The co-culture is seeded at a 1:200 ratio of PBSCs to *C. reinhardtii* in DMEM supplemented with 10% FBS, 1x P/S, and growth factors. The cells are grown at 30°C with a 12-hour light cycle.

The final scale-up design is a hollow fiber bioreactor. This is a system that pumps media through an array of small capillaries with semipermeable membranes. The PBSCs grow on the outside of each fiber, while *C. reinhardtii* suspended in DMEM flow through the lumen, as seen in Figure 1.

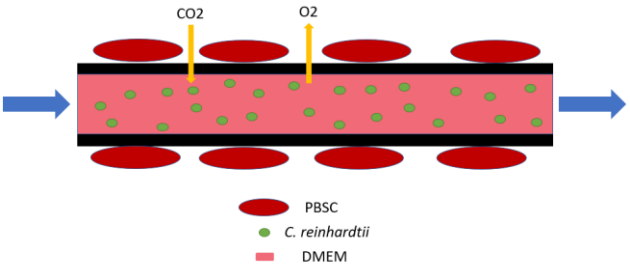

Figure 1: Cross-Section of a Single Hollow Fiber.

Solute diffusion through the membrane is represented by dissolved oxygen and carbon dioxide exchange. A conceptual diagram of the bioreactor system is shown below in Figure 2, where the blue arrows indicate the direction of fluid flow. A light source is placed upstream of the hollow fiber chamber as an energy source for photosynthesis.

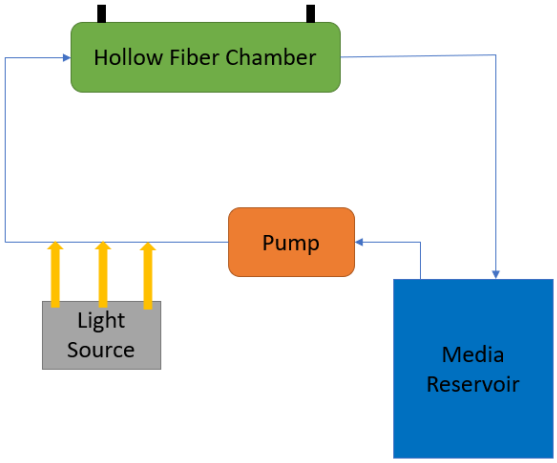

Figure 2: Conceptual Diagram of Hollow Fiber Bioreactor.

IV. Results & Discussion

The various experiments led to several important findings regarding the PBSC and *C. reinhardtii* co-culture. Experiments using trans-wells with varying algae to PBSC ratios demonstrated that the 1:200 ratio of PBSC to *C. reinhardtii* resulted in the highest PBSC proliferation rate in three days of coculture. This finding allowed the team to determine an ideal ratio of PBSC to *C. reinhardtii* in the final design.

The team used sensors that measured dissolved oxygen (DO) concentration and pH of the PBSC monoculture and the co-culture of PBSCs and *C. reinhardtii*.

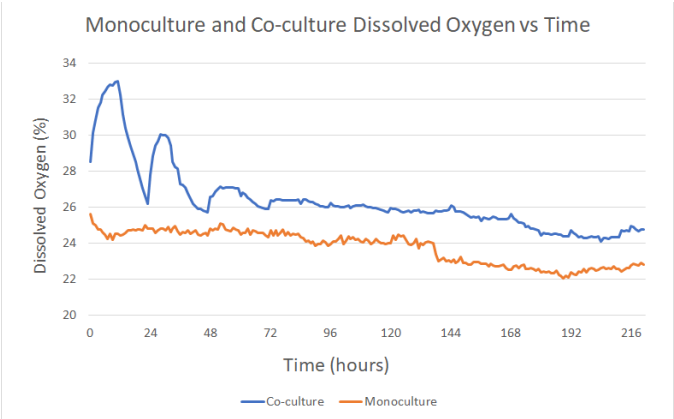

Figure 3: Comparison of dissolved oxygen concentration between the co-culture and monoculture.

Figure 3 illustrates the DO concentration of the co-culture system (blue) and the monoculture system (orange) in DMEM for 10 days at 30°C under hypoxic conditions. As seen in the figure, the co-culture exhibited increased DO concentrations when compared with the monoculture's DO during the first three 12-hour light cycles. This increase was sustained throughout the 10-day incubation period. This suggests that the presence of *C. reinhardtii* in the culture system has led to an increase in the percentage of dissolved oxygen in the system through photosynthesis.

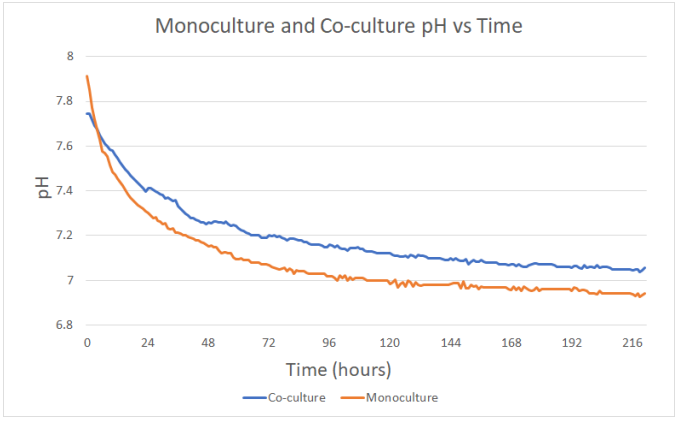

Figure 4: Comparison of pH between the co-culture and monoculture.

The data obtained from Figure 4 demonstrates that the coculture was able to dampen the decrease in pH over the 10-day culture period. There are multiple possible explanations for this observed phenomenon. Our team hypothesized that the gradual decrease in pH could be attributed to the uptake of CO_2 through photosynthesis. This could also suggest a decreased production of lactic acid due to higher oxygen concentrations and a decrease in anaerobic respiration.

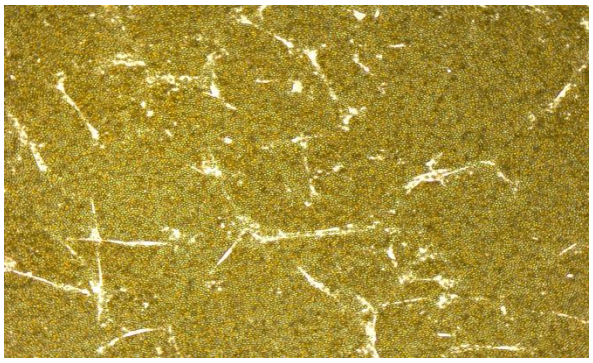

Figure 5. PBSC and *C. reinhardtii* co-culture after 72h of incubation in DMEM at 30°C, 20x magnification.

Figure 5 illustrates the co-culture condition of PBSCs with *C. reinhardtii* after 72 hours. As seen above, the PBSCs and the *C. reinhardtii* were able to remain viable in co-culture. Overall, the data from this project suggest that *C. reinhardtii* co-cultures should be further explored as a strategy for media cost reduction in cellular agriculture.

References

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