Sustainable Resources Inc. NMSBA Closeout Report

Sandia National Laboratories

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Statement of Work: Sandia National Laboratories will computationally evaluate several raceway pond design modifications for improved growth of *Haematococcus pluvialis.* Sandia National Laboratories will use the model to optimize design and growth conditions such as temperature, light, and CO_2 to make design and condition modification recommendations to the Requestor.

Results

Model

To determine the effect of mixing on the growth of *H. pluvialis* two studies were conducted: one with excess CO_2 and various amounts of mixing and one with limited CO_2 and various amounts of mixing. The algae growth model calculates growth based on a standard exponential growth equation with the growth rate reduced by non-optimal conditions in temperature, light, nutrients, etc. [1] Key parameters governing the reduction of the growth rate such as optimal light (I_{opt}) and temperature ($T_{opt,1}$, $T_{opt,2}$), sub-optimal temperature coefficients ($K_{T,1}$, $K_{T,2}$), and light extinction coefficient (k_B) were extracted from literature [2-5] and are given in Table 1. Standard values were used for the remainder of the parameters.

Table 1: Final dry cell weight measurements from each culture.

Parameter	Value
I_{opt}	17 W/m^2
$T_{opt,1}$	296.15 K
$T_{opt,2}$	297.15 K
$K_{T,1}$	0.015 K^{-2}
$K_{T,2}$	0.02 K^{-2}
k_B	0.04788 m^2/g-Biomass

The environmental conditions (light and temperature) are assumed to be similar to data taken in the greenhouse at Sandia, NM in 2011 and is shown in Figure 1. The 19 day data set is used twice to get a total of 38 days of growth and allow for the *H. pluvialis* to grow to a sufficient density for noticeable light limitation in CO_2 replete study, while only 19 days are used for the CO_2 limited study. A standard initial concentration of 54 g/m^3 is applied for all cases. In all cases temperature was assumed to be uniform throughout the pond.

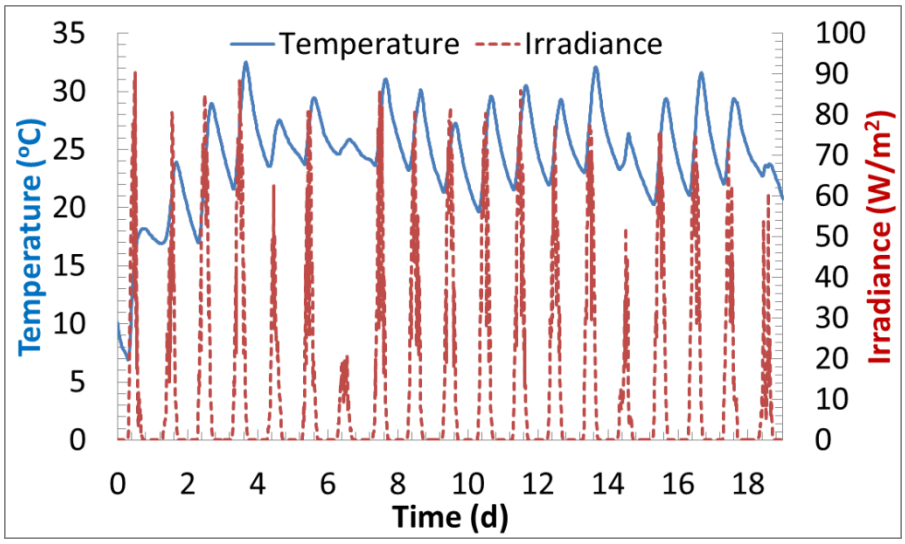

Figure 1: Assumed greenhouse environmental conditions.

For the CO_2 replete study, four mixing frequencies were looked at: no mixing and full mixing every 20 m, 10 m and 5m. A velocity of 0.2 m/s and k-epsilon turbulence was assumed for the cases with mixing and no velocity or turbulence was applied to the unmixed cases. For this study the significant variable is the light. Thus, the concentration needed to get high enough for light limitation to occur and mixing to have an effect. Figure 2 shows the predicted growth in the given conditions. It is evident from this prediction that light has little effect on the growth and only becomes an issue at high concentrations. Additionally, mixing provides only a minor improvement in the overall productivity. The predicted instantaneous and overall productivities for the four cases are shown in Figures 3 and 4, respectively.

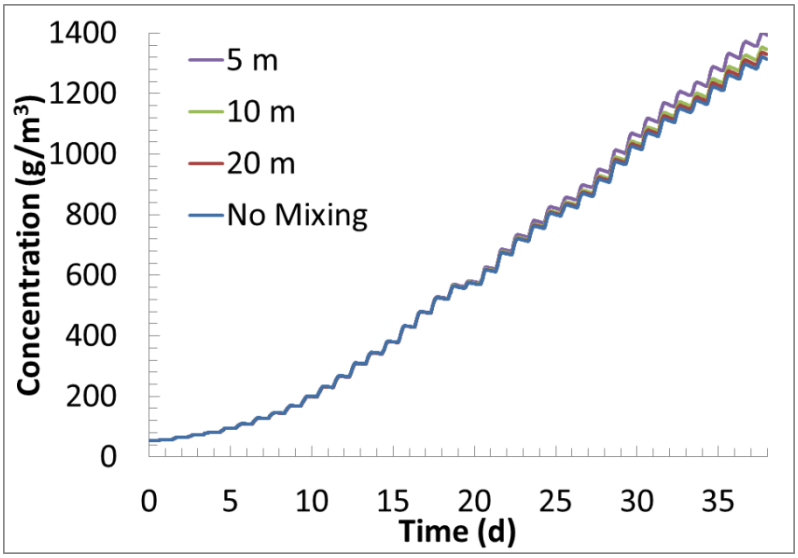

Figure 2: Predicted growth of *H. pluvialis* over time in assumed greenhouse conditions with excess nutrients and CO_2 for various mixing time scales.

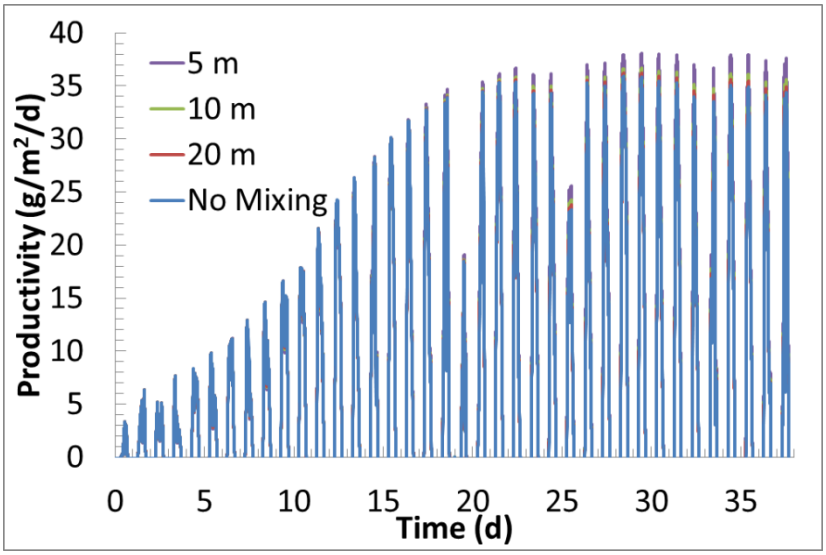

Figure 3: Predicted instantaneous productivity of *H. pluvialis* over time in assumed greenhouse conditions with excess nutrients and CO_2.

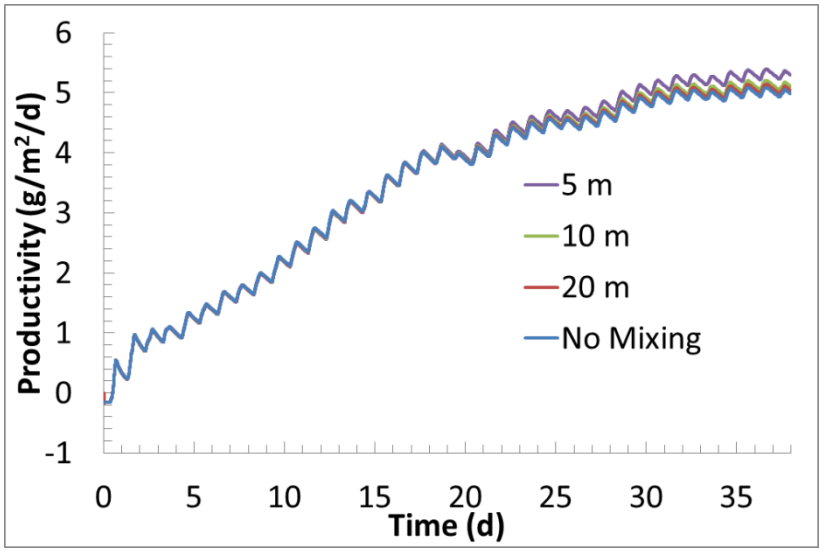

Figure 4: Predicted overall productivity of *H. pluvialis* over time in assumed greenhouse conditions with excess nutrients and CO_2.

For the CO_2 limited study, two mixing cases were looked at: no mixing and full mixing every 20 m. For both cases two CO_2 levels were looked at. First, an initially limiting CO_2 concentration of 0.1 g/m^3 is applied to the pond with a significantly higher CO_2 concentration of 10 g/m^3 applied at the surface of the pond and allowed to diffuse into the pond. Second, a higher initially limiting CO_2 concentration of 0.01 g/m^3 is applied to the pond with a higher CO_2 concentration of 1 g/m^3 applied at the surface. These values were selected for illustrative purposes only. The solubility of CO_2, reaeration, conversion into bicarbonate, and release of organic carbon from dead algae are not accounted for. Figure 5 shows the resulting predicted growth over time for each of the CO_2 limited cases as well as the replete case.

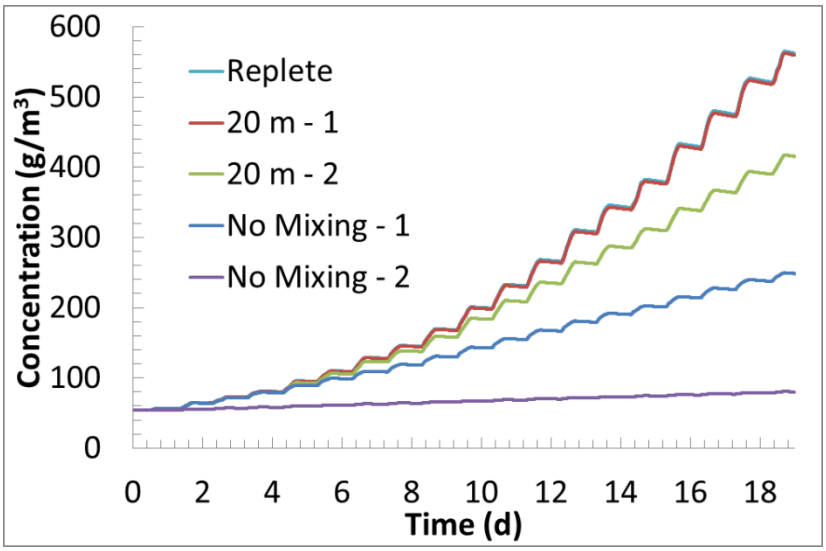

Figure 5: Predicted growth of *H. pluvialis* over time in assumed greenhouse conditions with limited CO_2 for various mixing time scales and CO_2 concentrations.

The mixing greatly increases the diffusive uptake of the CO_2 and brings CO_2 deeper into the pond. This in turn has a significant impact on the growth of the algae both allowing more CO_2 to get into the pond and spreading the CO_2 within the pond. Figure 6 shows the average predicted CO_2 concentration over time within the ponds for each of the cases. Figure 7 shows the average growth limitation factor in the pond due to the CO_2 limitation. It is noteworthy that the average limitation factor of the unmixed case 1 drops below that of the mixed case 2 during the day, even though the average CO_2 concentration in the unmixed case 1 is always higher than the average concentration in the mixed case 2. This is because of the nonlinear CO_2 concentration profile with depth and the nonlinear relationship between the limitation factor and CO_2 the concentration. In the unmixed case, most of the CO_2 is at the top of the pond, particularly during the day when the rate of algae growth is faster than the rate of CO_2 diffusion.

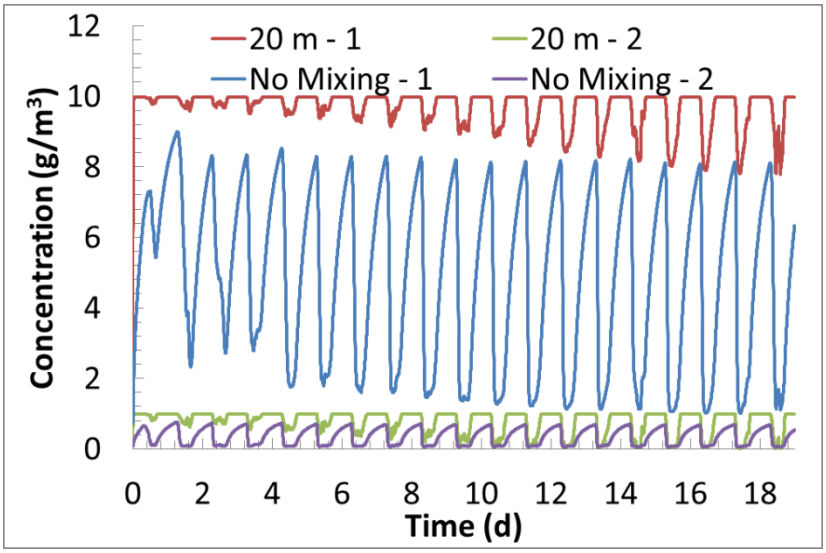

Figure 6: Predicted CO_2 concentration over time in assumed greenhouse conditions with limited CO_2 for various mixing time scales and CO_2 concentrations.

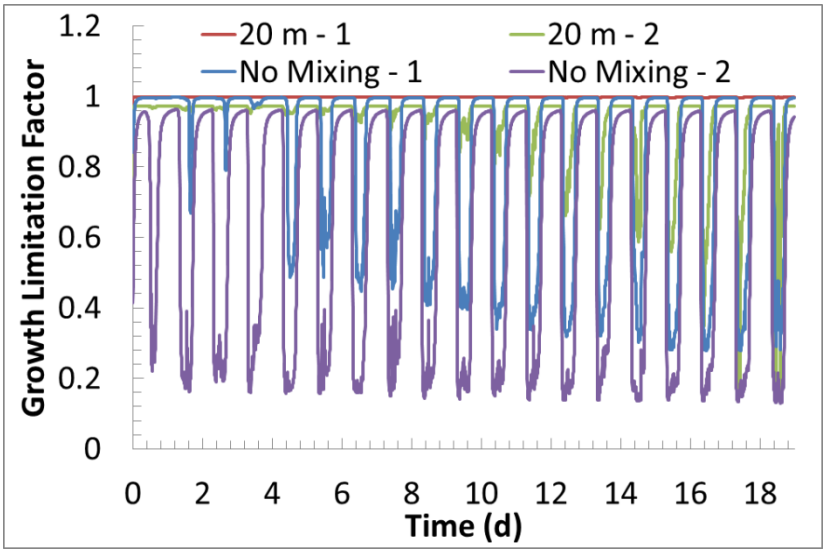

Figure 7: Predicted growth limitation factor due to CO_2 concentration over time in assumed greenhouse conditions with limited CO_2 for various mixing time scales and CO_2 concentrations.

The resulting productivities for unmixed cases were 1.54 g/m^2/d for case 1 and 0.21 g/m^2/d for case 2 and for the full mixing every 20 m were ### g/m^2/d for case 1 and 2.86 g/m^2/d for case 2. Again, a significant improvement in the productivity is seen due to mixing for both CO_2 concentrations studied.

Experimental

To determine whether the CO_2 parameters in the computational model accurately reflect the growth characteristics of *H. pluvialis*, laboratory experiments were conducted with *H. pluvialis* in which conditions of CO_2 availability were varied. Four conditions were tested: (1) stationary culture (no mixing or bubbling), (2) mixing with a stir bar, (3) bubbling with air, and (4) bubbling with air and mixing with a stir bar. These conditions led to significant changes:

- The level of CO_2 availability in the culture affects macroscale physics of *H. pluvialis* growth.

As shown in Figure 8, the macroscale physics of *H. pluvialis* cultures changes significantly with CO_2 availability. With no mixing or bubbling (i.e. stationary), the *H. pluvialis* cells are primarily in a thin layer at the surface of the culture (Figure 8A), presumably due to the higher CO_2 concentration near the surface of the culture. With mixing, there is still a layer of cells near the surface of the culture, but there are also cell aggregates (clumps) mixed throughout the depth of the culture (Figure 8B). With air bubbling and no mixing, most of the *H. pluvialis* cells remain at the bottom of the culture (Figure 8C). Bubbling the culture with air will increase the concentration of CO_2 throughout the culture, and therefore, the cells no longer need to remain at the surface. Combining mixing and air bubbling leads to the most uniform distribution of cells throughout the culture (Figure 8D).

- The level of CO_2 availability in the culture affects the cell structure of *H. pluvialis* at the microscopic scale.

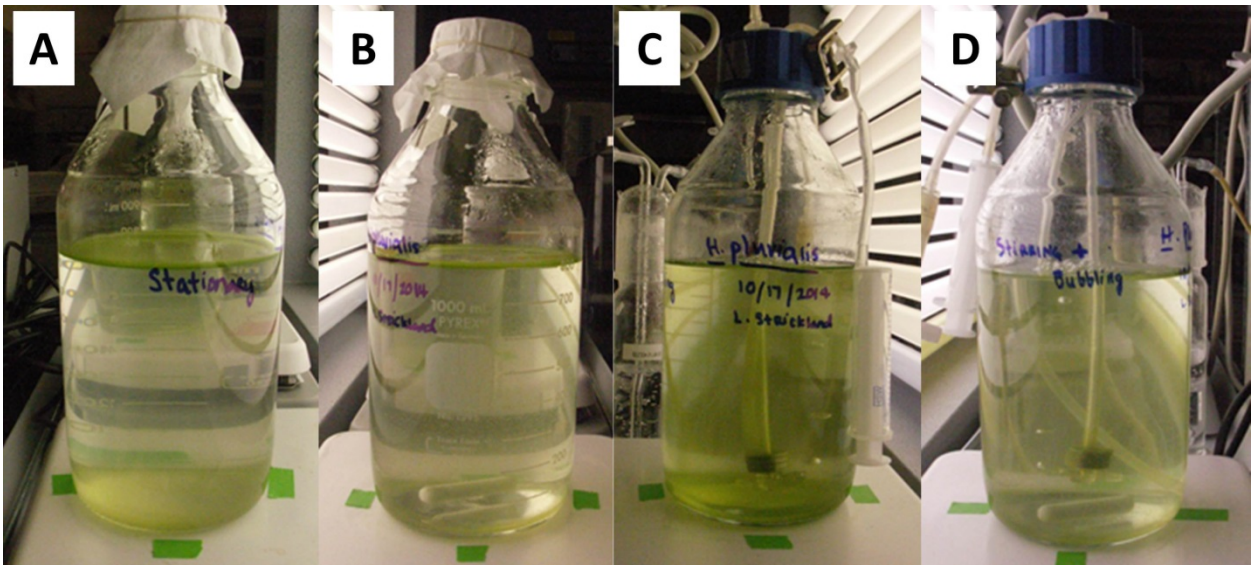

Figure 8: Cultures of *H. pluvialis* under conditions of varying CO_2 availability: Stationary (A), Mixing (B), Bubbling (C), and Bubbling and Mixing (D).

As shown in Figure 9, cellular morphology of *H. pluvialis* changes considerably under different conditions of mixing and CO_2 availability. The number of flagellated, vegetative cells increases with increasing CO_2 availability (Stationary < Mixing < Bubbling), while palmelloid cells are found mostly in the mixing culture, which may contribute to the cell aggregation observed at the macroscale (Figure 8B). While some astaxanthin-containing cysts are observed in the stationary and bubbling conditions, no conclusive trends were observed.

- While optical density, an indicator of cell growth, was found to increase with increasing CO_2 availability, the final dry cell weights of the cultures did not vary significantly.

As shown in Figure 10, the optical density of the cultures were found to increase with increasing CO_2 availability (Stationary < Mixing < Bubbling = Bubbling + Mixing). Interestingly, there is very little change between the stationary and mixing conditions; the most significant increase is associated with bubbling the culture. The addition of mixing to the bubbling was not found to provide any significant advantage. Despite this increase in optical density, no significant increase was observed in the dry cell weight measurements (Table 2). This may be due to the fact that the culture density was rather low, leading to low overall weights. Additional experimentation is necessary to confirm the effect of bubbling on *H. pluvialis* biomass production.

- The final pH of the cultures were significantly lower than the reported optimum for growth of *H. pluvialis*.

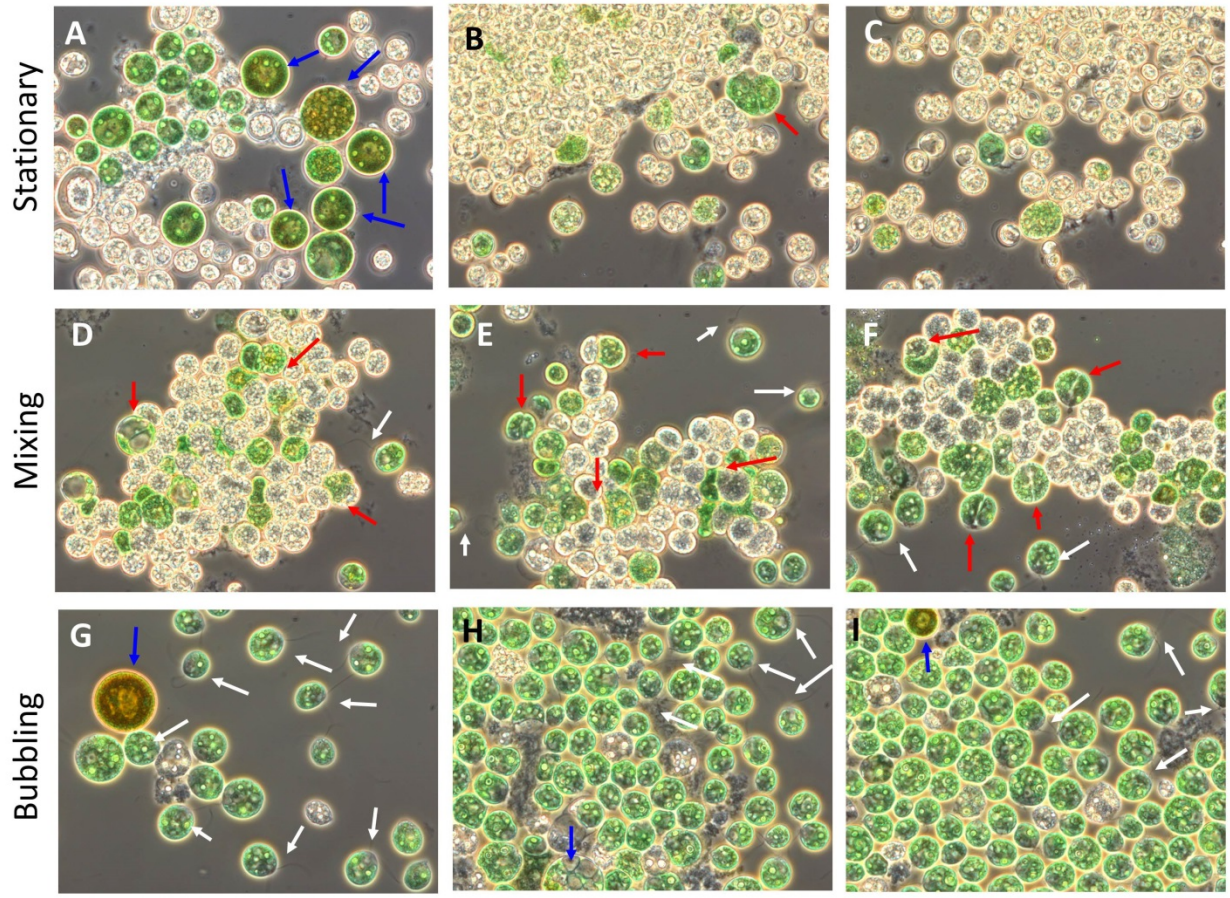

Figure 9: Light microscopy images of *H. pluvialis* cultures under varying CO_2 availability after 12 days of cultivation: Stationary (A-C), Mixing (D-F), and Bubbling (G-I). White arrows = flagellated, vegetative cells; Red arrows = palmelloid cells; Blue arrows = cysts containing astaxanthin.

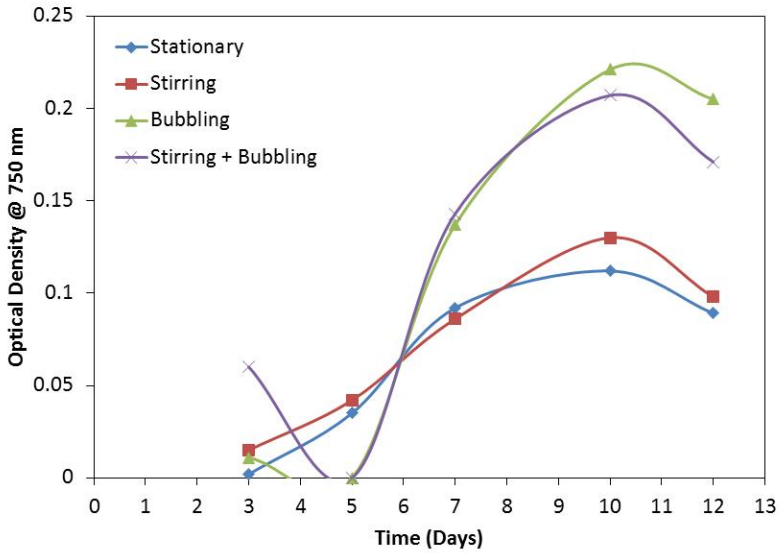

Figure 10: Optical density of *H. pluvialis* cultures at 750 nm over 12 days of cultivation under cool white fluorescent lights at 270 µmol photons m^{-2} s^{-1}.

Table 2: Final dry cell weight measurements from each culture.

Culture Condition	Dry Cell Weight (g)	
	Experiment 1	Experiment 2
Stationary	0.0469	0.0451
Mixing	0.0395	0.0443
Bubbling	0.0421	0.0491
Bubbling + Mixing	-	0.0441

As shown in Table 3, the final pH of the cultures was significantly lower than the pH reported for optimal growth of *H. pluvialis* (optimal pH = 6.0). The lower pH is likely due to the metabolic effects of *H. pluvialis* growth. This may have a significant impact on overall growth rates of the culture. Additional experimentation is recommended.

Table 3: Final pH measurements of cultures with a starting pH of 6.0

Culture Condition	pH	
	Experiment 1	Experiment 2
Stationary	4.23	4.36
Mixing	4.28	4.43
Bubbling	4.46	4.59
Bubbling + Mixing	-	5.13

Recommendations

- The model shows that mixing does little to improve growth in CO_2 replete conditions, but has the potential to greatly improve growth in CO_2 limiting conditions with CO_2 applied in localized areas.
- To fully understand the effect of mixing the buoyancy of the algae and its dependence on CO_2 concentration should be included in the model.
- Air bubbling may improve growth of *H. pluvialis*. Additional experimentation and modeling is required to confirm.
- Online pH adjustment may also improve growth of *H. pluvialis*. Additional experimentation and modeling is required to confirm.

References

1. **Gharagozloo, P. E., Drewry, J. L., Collins, A. M., Dempster, T. A., Choi, C. Y., and James, S. C. (2014) *Journal of Applied Phycology* 26, 2303-2314**
2. **Borowitzka, M. A., Huisman, J. M., and Osborn, A. (1991) *Journal of Applied Phycology* 3, 295-304**
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4. **Katsuda, T., Lababpour, A., Shimahara, K., and Katoh, S. (2004) *Enzyme and Microbial Technology* 35, 81-86**
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