Algae Scale-Up SOPs

These are not necessarily the *best* way to grow algae in a PBR, but this is what seems to work for us and some customers. Algae culturing is heavily dependent on the strain you are growing, and the conditions that it is acclimated to and your water conditions. Often at the seasonal transitions, water quality and chemistry will change.

General notes

- Using a 1 in 10 dilution for inoculation is conservative (ie 20L inoculum into 200L)
- We recommend growing algae with replete nutrients. We recommend 0.5 ml/L of Proline's F/2 formulation for initial scale up. This is approximately 4 x the guillard's f/2 recipe, or a 2F). For harvesting we go closer to a F4 recipe.
- Starting with a light intensity of 10% is conservative.
- 22ºC is a good temperature to aim for to grow most aquacultured strains. Culture temperatures above 25-30ºC can cause slower growth or algal crashes in many strains.
- 40 SCFH is a reasonable amount of aeration. Larger strains (Tetra, Thalassiosira weissflogii, etc.) may require more aeration to stay suspended.
- Setting small harvests for every 2 hours helps maintain better resolution on the OD sensor longer into the culture's life.
- Cleanliness is massively important throughout the cleaning and inoculation process.

IP Generalized Continuous Scale up

Day	Volume (L)	Light %	Notes
Inoculum	18 L	IP's LEDs	1 x 20 L carboy
Day 0	200	10-12	• pH set to 8.2 • Nutrients added at 0.5 ml/l of Proline, Fritz, of Kent's F/2 (100ml total)
Day 1	200	10-12	• Monitor growth rate, cell density (manually) and CO2 consumption rate to infer growth • If logarithmic growth is observed from inoculation to day 1, start continuous flow of 2.5 GPH (10 LPH) with 0.5 mL/L (5 ml/hr) of nutrients of Proline F/2 concentrate • If minor growth has occurred, but not a cell doubling, start dilution at 1 GPH (4LPH) with proportional nutrients 2 mL/hr, and turn up flow once logarithmic growth is observed • If no growth is detectable, let it rest today and start dilution on day 2.
Day 2	440	10-12	• A density drop from the dilution is normal compared to the previous day. • If there is only a straight dilution (ie no growth), slow or stop water addition for a day. Do not allow algae to sit more than 2 days without nutrient addition.
Day 3	680	12-15 13-18	• Density should be outcompeting the dilution at this point and the proportional increase can be met with extra light (ie a 20% more dense culture, Should safely handle 20% proportionally more light. This is **NOT** 20% on the system. Over the current 10% this is only 2% more light. • If growth response is clear within 3-4 hours of increased light a second increase can be attempted
Day 4	920	15-25	• Increase light conservatively (smaller increases are more conservative) • Density could be up to ⅓ of the inoculum density at this point if everything is growing exponentially
Day 5	1160	18-33	• Continue to increase light relative to density
Day 6	1200 +200	23-45	• Continue to increase light relative to density, but treat 65% as a conservative maximum intensity knowing that intensity above that may cause photoinhibition.
Day 7	1200+200	25-50	

IP Generalized Semi-Continuous Scale up

Day	Volume (L)	Light (%)	Notes
Inoculum	18 L	IP's LEDs	• 1 x 20 L carboy
Day 0	200	10	• pH set to 8.2 • Nutrients added at 0.5 ml/l of Proline, Fritz, of Kent's F/2 (100ml total)
Day 1	200	10-12	• Rest day • Monitor growth rate, cell density (manually) and CO2 consumption rate to infer growth • Increase light proportional to density increase
Day 2	400	10-12	• Add 200L of water with 100 mL of nutrients
Day 3	400	12-18	• Rest day • Increase light proportional to density increase
Day 4	600	12-20	• Add 200L of water with 100 mL of nutrients
Day 5	960	15-30	• Rest day • Increase light proportional to density increase (limit increase to 5% at a time)
Day 6	1200	20-50	• Add 200L of water with 100 mL of nutrients
Day 7	1200	25-65	• Rest day • Increase light proportional to density increase at ~ 5% each increment.
Day 8	1200 + 200	25-65	• Continue on with 200 L taken out every second day and increase light to push density higher.

NH Iso PBR 1250L

Day	Volume (L)	Light (%)	Notes
Inoculum	2x1 gal ~ 8 L	T5HO 6,400	2 x Apple cider jugs
Day 0	120 (water just above dome level)	15-20	pH set to 8.2 Nutrients added at 1.35 mL/L of housemade L1 media (~= 4x [Guillard's F/2] ~= 0.7 ml/l of Proline, Fritz, of Kent's F/2
Day 1	120	15-20	Rest day start exponential growth monitor co2 consumption rate
Day 2	270	20-30	Add 150 L with the same concentration of nutrients. Add 5-10% light based on growth seen
Day 3	420	30-40	Add another 150 L, with nutrients Increase light % as per growth Start continuous water addition at 2 GPH (~8 LPH), with nutrients dosed hourly at 1.35ml/L of water entering 10.8 ml/hr of their nutrients (~= 5.6 ml/hr of Proline etc) Increase light if CO2 consumption drop
Day 4	600	30+	
Day 5	780		
Day 6	960		
Day 7	1140	75%	Max out at 75% light Once full & harvesting turn flow up to 3.5 GPH and increase nutrients to 1.45 ml/l of flow (0.75 ml/L equivalent to Proline)

Very Conservative General Process PBR 1250L (CCE)

Day	Volume (L)	Light (%)	Notes
Inoculum	1 x 10 L	Fluorescent	
Day 0	120	10	pH set to 8.2 500 mL of nutrients (roughly equivalent to 4F)
Day 1-5	120	10	Potential rest days Wait until the algae becomes a *brown ale* colour Add 250 ml -500 ml nutrients every 2-3 days to ensure algae doesn't starve of nutrients
+1	260	10	Once brown ale colour, start continuous flow rate to 2.5 GPH ~10 LPH
+1	500	10	
+1	740	10	
+1	980	10	
+1	1200	10	Harvesting now, increase light 1%/day