Title: Establishing and Scaling-up a Microalgae Culture for Biofuel Production

Approvals:

Preparer:_____Rhykka Connelly _____ Date _____04Jul13_____
_____Patricia Phelps_____Date _____04Jul13_____
Reviewer:_____Sonia Wallman_____ Date _____04Jul13_____

1. **Purpose:**
   1.1. To inoculate a photobioreactor with microalgae
2. **Scope:**
   2.1. Applies to the production of lipids from microalgal (Chlorella, Nannochloropsis, etc.) cells.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. water bath SOP
   4.2. spectrophotometer SOP
   4.3. pH meter SOP
   4.4. microscope SOP
5. **Definitions:** N/A
6. **Precautions:** Microalgae are live and should be treated with 10% bleach prior to safely discarding.
7. **Materials:**
   7.1. 10mL vials of *Chlorella vulgaris* obtained from UTEX #265 or Carolina Biological #152075 (or bio-prospected)
   7.2. Bristol medium premixed powder (GroFizz cat #110)
   7.3. filtered water
   7.4. 500 ml photobioreactor kit (GroFizz cat# 102)
   7.5. 2L photobioreactor kit (GroFizz cat# 100)
   7.6. light source (GroFizz LED platform cat# 104)
   7.7. 250ml glass bottle (Carolina cat# 716221)
   7.8. 15ml centrifuge tubes (Carolina cat# 215085)
   7.9. serological pipettor (Carolina cat# 736877)
   7.10. 10ml serological pipette tips (Carolina cat# 736125)
   7.11. p1000 pipettor (Carolina cat# 214659)
   7.12. p1000 pipette tips (Carolina cat# 214717)
   7.13. spectrophotometer (Carolina cat# 653302)
   7.14. spectrophotometer cuvettes (Carolina cat# 653311)
   7.15. stir plate (Carolina cat# 701012)
   7.16. stir bar (Carolina cat# 701091)
   7.17. balance (Carolina cat# 702010)
   7.18. centrifuge for 15ml centrifuge tubes
   7.19. aluminum weighing dishes (Carolina cat# 702338)
   7.20. oven (Carolina cat# 701291)
   7.21. micro-scale pH meter (pH7 and pH4 commercially prepared buffers) (Carolina cat# 186009)
   7.22. microscope with 1000x magnification (Carolina cat# 595522)
   7.23. microscope slides and cover slips (Carolina cat# 631920)

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8. Procedure:

8.1. **Solution and Media Preparation**

8.1.1.1. **Establish starting (500ml) microalgal culture:**

8.1.1.2. Gather the following items and place on a clean lab bench area**:**

Bristol medium pre-mixed powder
500ml filtered, or chlorine-free, water
500mL Erlenmeyer flask
pH meter
magnetic stir bar and magnetic stir plate
assembled 500ml photobioreactor kit
light source

8.1.1.3. Add 300ml of filtered water to a clean 500mL Erlenmeyer flask.

8.1.1.4. Add stir bar and place on stir plate. Stir at medium speed.

8.1.1.5. Add packet of Bristol Medium.

8.1.1.6. pH the medium

8.1.1.7. if necessary, titrate the medium to pH 7.5 – 8.0.

8.1.1.8. Bring final volume in the flask to 500 ml with filtered water.

8.1.1.9. Stir to dissolve the ingredients using a magnetic stir bar and stir plate.

8.1.1.10. Transfer ~250ml medium to assembled 500ml photobioreactor (PBR).

8.1.1.11. Add Chlorella aliquot to medium in PBR. The glass body of the PBR should be sterilized by autoclave or a 10% bleach solution to eliminate live organisms. The lid should be sterilized with a 10% bleach solution. Rinse thoroughly with filtered water to remove all bleach residue.

8.1.1.12. Bring volume to ~450ml with remainder of prepared medium. Volume should be approximately 10 – 20mm from the top of the reactor.

8.1.1.13. Tighten lid of PBR, initiate air flow.

8.1.2. **Baseline culture measurements:** pH, dry cell weight (DCW), pigment concentration (Chlorophyll a), microscopic observation

8.1.2.1. Gather the following on a clean lab bench area:

15ml centrifuge tubes
serological pipettor
serological pipet tips (10ml)
aluminum weighing dishes
p1000 pipettor
p1000 pipet tips
filtered water

8.1.2.2. **Dry Cell Weight Ananlysis -** Record the weight of an aluminum weighing dish.

8.1.2.3.Transfer 10ml of well-mixed culture to a 15ml centrifuge tube.

8.1.2.4. Pellet algal cells via centrifugation at 3000rpm for 5 minutes.

8.1.2.5. Replace supernatant with 10ml of filtered water.

8.1.2.6. Centrifuge cells again at 1000 x g for 5 minutes.

8.1.2.7. Discard supernatant.

8.1.2.8. Add 1ml filtered water to pellet. Resuspend by gently pipetting up and down.

8.1.2.9. Transfer the concentrated algae to the pre-weighed aluminum dish

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8.1.2.10. Place dish in 50 - 60°C oven until a constant weight. (Weigh dish after 30 min, 1h, 1.5h)
8.1.2.11. Record weight of dried algae plus the dish. Calculate the weight of the dried algae.
8.1.2.12. Calculate the dry cell weight of the culture per liter. (multiply algal wt. x 100)
8.1.2.13. **Pigment Analysis** - Turn on Spectrophotometer.
8.1.2.14. Transfer 2ml of well-mixed culture to a spectrophotometric cuvette.
8.1.2.15. Record absorbance of culture at 435nm, 455nm, 660nm, 680nm.
8.1.2.16. **pH Analysis** - Transfer 25ml of culture to a small beaker.
8.1.2.17. Record pH of culture.
8.1.2.18. **Microscopic Analysis** - Transfer 10 ml of culture to a 15ml centrifuge tube.
8.1.2.19. Pellet the cells via centrifugation at 3000 rpm for 5 minutes.
8.1.2.20. Discard supernatant.
8.1.2.21. Resuspend the cells in 500ul media.
8.1.2.22. Transfer 7ul of well-mixed concentrated cells to a microscope slide.
8.1.2.23. Place a coverslip on the droplet.
8.1.2.24. Observe the culture at 10X.
8.1.2.25. Observe the culture at 40X. Look for Chlorella, competing microalgae or cyanobacteria, predators, other objects. Record observations.
8.1.2.26. Repeat all analyses daily or every other day until culture reaches a density of >500mg/L.
8.1.2.27. Treat cultures accordingly if out of pH range or predators or competitors are observed.

8.1.3. **Scale-up to 2L culture**

8.1.3.1. Gather the following items on clean lab bench area:
2L Photobioreactor kit
LED light kit
Bristol medium (2L)
15ml centrifuge tubes
serological pipettor
serological pipet tips (10ml)
aluminum weighing dishes
p1000 piptettor
p1000 pipet tips
filtered water

8.1.3.2. Once the density of the 500ml culture reaches > 500mg/L, prepare 2 liters of Bristol medium using prepared media packet.
8.1.3.3. Add 1L of prepared media to sterilized 2L PBR.
8.1.3.4. Transfer >400 ml of culture from the 500ml PBR to the 2L PBR.
8.1.3.5. Add media to bring the final volume within 20 – 30mm from the top of the reactor.
8.1.3.6. Reassemble PBR and start air flow.
8.1.3.7. Place reactor inside the LED light kit.
8.1.3.8. Monitor DCW, pH, pigment accumulation, and culture composition as described above (8.1.2.25) until the DCW density reaches > 1 g/L.

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8.1.3.9. At a DCW > 1g/L, the culture may be split, harvested for lipids, or maintained. Culture densities may reach ~ 3g/L.

9. History:

Name	Date	Amendment
R. Connelly	2011	Initial Release
R. Connelly T. Phelps S. Wallman	2013	Put into 2013 SOP format

TIME POINT (min)	**Ab (435nm)**	**Ab (455nm)**	**Ab (660nm)**	**Ab (680nm)**	**DCW (g/L)**	**pH**	**Microscopic observations**
T_{day1}							
T_{day2}							
T_{day3}							
T_{day4}							
T_{day5}							
T_{day6}							
T_{day7}							