Title: Microalgae Lipid Extraction and Separation by Thin Layer Chromatography for Biofuel Production

Approvals:

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

1. **Purpose:**
   1.1. To extract and analyze lipids from microalgae for biofuels
2. **Scope:**
   2.1. Applies to the extraction 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. microalgae scale-up SOP
5. **Definitions:** N/A
6. **Precautions:** Microalgae are live and should be treated with 10% bleach prior to safely discarding. Solvents are used to extract lipids; perform solvent extraction steps under a hood, do not inhale.
7. **Materials:**
   7.1. 10mL of *Chlorella vulgaris* obtained from UTEX #265 or Carolina Biological #152075 (or bio-prospected)
   7.2. 2L photobioreactor kit (GroFizz cat# 100)
   7.3. light source (GroFizz LED platform cat# 104)
   7.4. Rapid Lipid Extraction Kit (GroFizz cat# 202)
   7.5. methanol (Carolina cat# 861281)
   7.6. chloroform (Carolina cat# 853980)
   7.7. hexane (Carolina cat# 867180)
   7.8. ether (Carolina cat# 861348)
   7.9. acetic acid (Carolina cat# 841290)
   7.10. glass jars for TLC
   7.11. iodine crystals (Carolina cat# 868982)
   7.12. 10ml serological pipette tips (Carolina cat# 736125)
   7.13. p10 pipettor (Carolina cat# 214653)
   7.14. p10 pipette tips (Carolina cat# 214717)
   7.15. glass vials (Carolina cat# 715064)
   7.16. stir plate (Carolina cat# 701012)
   7.17. stir bar (Carolina cat# 701091)
   7.18. balance (Carolina cat# 702010)
   7.19. chemical fume hood
8. **Procedure:**
   8.1. **Extraction of lipids from *Chlorella* and Calculation of Lipid Percentage**
      8.1.1.1. Gather the following items and place on a clean lab bench area**:**
      microalgae culture
      Rapid Lipid Extraction kit

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methanol
chloroform
hexanes
ether
acetic acid
small beaker (25ml)

8.1.1.2. Perform a dry cell weight analysis as described in the culture scale-up SOP.
8.1.1.3. Pre-weigh a glass vial from the kit.
8.1.1.4. Using the kit syringe, collect 10ml of well-mixed microalgal culture.
8.1.1.5. Screw filter disk onto syringe.
8.1.1.6. Depress syringe over a small beaker, collecting the microalgae onto the filter disk. The flow through fluid should be clear.
8.1.1.7. Discard the flow through fluid.
8.1.1.8. Remove the filter disk.\
8.1.1.9. Pour ~10 – 20ml of methanol into a small beaker.
8.1.1.10. Take up 3 ml of methanol into the syringe.
8.1.1.11. Replace filter disk.
8.1.1.12. Slowly depress plunger over the pre-weighed glass vial.
8.1.1.13. Repeat steps 9.1.1.7 and 9.1.1.8.
8.1.1.14. Remove filter disk.
8.1.1.15. Under a ventilated chemical hood, pour ~10 – 20ml of chloroform into a small beaker.
8.1.1.16. Take up 3 ml of chloroform.
8.1.1.17. Replace filter disk.
8.1.1.18. Slowly depress the plunger over the same glass vial, pooling the fractions.
8.1.1.19. Evaporate in a chemical hood overnight, or on a hot plate at 60°C under a ventilated fume hood.
8.1.1.20. Remove the glass vial from heat, if necessary, and cool to room temperature.
8.1.1.21. Once the solvents have evaporated and the vial is room temperature, re-weigh the vial.
8.1.1.22. Calculate the gravimetric weight of the extracted lipids as a percentage of the dry cell weight of the culture.

weight of the vial + extract _____
weight of the vial _____
gravimetric weight of the extract _____/10ml of culture

dry cell weight from 10 ml of culture _____

Divide the gravimetric weight of the extract by the dry cell weight, then multiply by 100 to yield the percentage lipid within the sample.

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8.1.1.23. **Analysis of extracted lipids by thin layer chromatograhy:**

8.1.1.24. Gathe**r** the following on a clean lab bench area**:**

lipid extract in glass vial
chloroform
methanol
hexane
ether
acetic acid
glass developing jar for TLC
iodine crystals
glass visualization jar
p10 pipettor
gel loading tips
Rapid Lipid Extraction kit TLC plate pre-spotted with lipid standards ((biodiesel, triglyceride, free fatty acid, diglyceride, and monoglyceride)
digital camera

8.1.1.25. **Thin Layer Chromatography**

8.1.1.26. With gloves, remove TLC plate from sheath. Place the aluminum side down on the bench. The white silica side should face up and have 3 dots near the bottom of the plate. Use care to not disturb the plate as the silica will chip off and affect the migration of the lipids on the plate.

8.1.1.27. Resuspend your lipid extract from step 8.1.1.22 in 100ul of methanol and 100ul of chloroform.

8.1.1.28. The first lane of the TLC plate has been pre-spotted with lipid standards. Using a p10 with a gel loading tip, take up 5ul of your resuspended lipid extract into the pipet tip. Apply a small amount of the extract onto the second dot on the plate. Allow the solvent to completely evaporate before adding additional extract to the same spot. Continue until all 5ul of the extract are applied to the same spot.

8.1.1.29. Using the same technique, spot 10ul of lipid extract onto the 3rd dot.

8.1.1.30. Mix 80ml hexanes, 20ml ether, and 1ml acetic acid together under the chemical fume hood. This is the mobile phase.

8.1.1.31. Add the mobile phase to the glass developing jar. The mixture should cover the bottom of the jar, but not exceed the height of the spots on the TLC plate.

8.1.1.32. With gloves, transfer the spotted TLC plate to the jar, leaning the plate against the wall of the jar. Make sure that the liquid does not immerse the spotted samples.

8.1.1.33. Place the cover on the jar.

8.1.1.34. The solvents will carry the lipids up the plate by capillary action.

8.1.1.35. Stop the development once the solvent front is within 1cm of the top of the plate. Do not allow the solvent front to reach the top of the plate.

8.1.1.36. Take the plate out of the developing jar and allow to air dry in a chemical fume hood.

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8.1.1.37. Add a approximately 1 g of iodine crystals to the visualization jar.
8.1.1.38. Transfer the dried plate to the visualization jar. Yellow bands will appear over time.
8.1.1.39. Once the bands are visible, take the plate out of the jar.
8.1.1.40. Using a cell phone or digital camera, take a digital picture of the plate. The yellow bands will disappear with time.
8.1.1.41. With a pencil, draw lines around the yellow bands. If the bands are fading or are difficult to see, the plate can be re-exposed to the iodine crystals to deepen the color density of the bands.
8.1.1.42. Pixel density can be measured using a software program called Image J.

9. History:

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