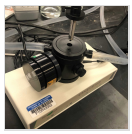

Aug 02, 2022

Oxygen Evolution Measurement: Light Curve for Algae

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ABSTRACT

Thanks to a lovely combination of trial and error I present to you an optimized* protocol for using an oxygen electrode to create a light curve for *Ostrecoccus tauri*. The light curve can be used to determine what light intensity is ideal for photosynthesis in *O. tauri*, which can then inform the light conditions for a subsequent bicarbonate curve. An *O. tauri* bicarb curve could potentially indicate (when compared to other algal bicarb curves) whether or not this species has a carbon-concentrating mechanism.

*This protocol could be further optimized by making a growth curve for O. tauri first.

*The protocol is written for O. tauri and can be adapted to other algal species. When adapting it will be necessary to reassess optimal cell density for measurements, and consider the choice of buffer for washing and incubating cells. ASW is used in this situation but will not be suitable for fresh water species

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EXTERNAL LINK

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KEYWORDS

Oxygen electrode, light curve, Ostreococcus tauri, Hansatech, Oxygraph+, Photosynthesis

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MATERIALS TEXT

1. Hansatech Oxygen Electrode Kit, which should include:
- OXYG1+ - Oxygraph+ electrode control unit
- DW1 - Oxygen electrode chamber
- S1 - Oxygen electrode disc
- S1/SMB - SMB-SMB Electrode cable
- A2 - Electrode disc membrane applicator tool
- A3 - Top plate key & alignment jig
- S2/P - Pack of 5 magnetic followers
- S4 - 30m reel of PTFE membrane
- S7A - Spare O-rings set for DW1/AD
- S6B Assorted Spare 'O' rings for DW2/2
- S16 - Electrode cleaning kit
- Rapid electrode disc polish
- USB2.0 A to B - 2m USB cable with A-plug to B-plug
- DW1MAN - User Manual
- 980266T - 12V Power Supply
- 980257 - DW Accessory Kit
- Auxiliary red light
- Port cover blanks

1. Ultra-fine cigarette paper (Rizla T. Thin Blue)
2. Cotton buds (small enough to fit in the well of the disc)
3. Sharp scissors
4. Forceps
5. Gloves
6. Paintbrush (small, the kind you'd use for painting precise lines)
7. dH_2O
8. Kimwipes
9. 1 mL Pasteur pipettes
10. Laptop with Oxygraph+ program O2View installed
11. Electrolyte solution (prepare from KCl anhydrous salt and de-ionized water)
12. ASW
13. Water Jacket
14. Circulating water bath

BEFORE STARTING

Have a checklist - (1) water jacket, (2) stirrer, (3) lid as a reminder to do these steps each time

Prepare 0.5M NaHCO3 Solution

1 Prepare 0.5 M $NaHCO_3$ Solution

1.1 Dispense **10 mL** of dH_2O into a 50 mL centrifuge tube

1.2 Weigh out **0.420 g** of sodium bicarbonate

1.3 Add **0.420 g** of sodium bicarbonate to the dH_2O

1.4 Close lid and invert until everything is dissolved

Prepare the electrolyte

2 Prepare the electrolyte

2.1 Weigh out **17.5 g** of anhydrous KCl salt

2.2 Pour the salt into a **500 mL** glass bottle

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2.3 Measure **100 mL** of dH_2O

2.4 Pour the water into the same bottle as salt

2.5 Close the lid and swirl the bottle with mild aggression until salt is dissolved

Clean and Prepare the electrode

3 Clean and Prepare the electrode

3.1 Start the water jacket going at 25 ^oC so it's ready by the time your electrode is prepared

3.2 Dampen a cotton bud with dH_2O and use it to pick up a thin layer of Hansatech Rapid Electrode Disc Polish to clean the electrode

3.3 Circle the inside of the well with the polish

3.4 Polish the platinum electrode as well.

> Try not to polish the epoxy resin surrounding the electrode as it can alter the shape of the dome, thus affecting accuracy.

3.5 Use a clean, dH_2O-dampened cotton bud to remove the residual polish. A kimwipe can be used to dry the electrode after this step, too.

3.6 Use a disposable Pasteur pipette to add a drop of electrolyte to the top of the platinum electrode.

3.7 Add 3-5 drops of water so that the silver band inside the well is completely covered.

3.8 Cut a 1.5-2 cm^2 piece of thin cigarette paper to the top of the electrode.

> Recommended brand: Rizla T ultra fine

3.9 Cut a slightly larger square of the provided PTFE membrane tape and place it on top of the paper.

> If the membrane tape is a little dusty, run it between your thumb and index fingers gently to try and wipe off any debris

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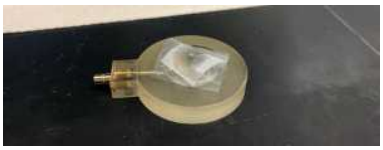

What electrode looks like after this step

3.10 Use the membrane applicator to secure the small O-ring over the membrane and cigarette paper at the base of the electrode

Membrane applicator with o-ring

3.11 Place the large O-ring in the indentation outside the well

Make sure this is one of the thicker large o-rings, otherwise your sample will leak out of the electrode chamber and your curve will be less curvey and more zig-zaggy.

3.12 Tuck any extra edges of membrane into the well using forceps so it doesn't touch the larger O-ring

Fully set up electrode disc

3.13 Attach the cord that connects the control unit to the electrode, then fit the disc into the bottom of the electrode chamber (pictures in steps 4.1-4.2).

3.14 Open O2View program on laptop

3.15 Add port cover blanks to 3 of the open ports and insert the red light to the 4th open port.

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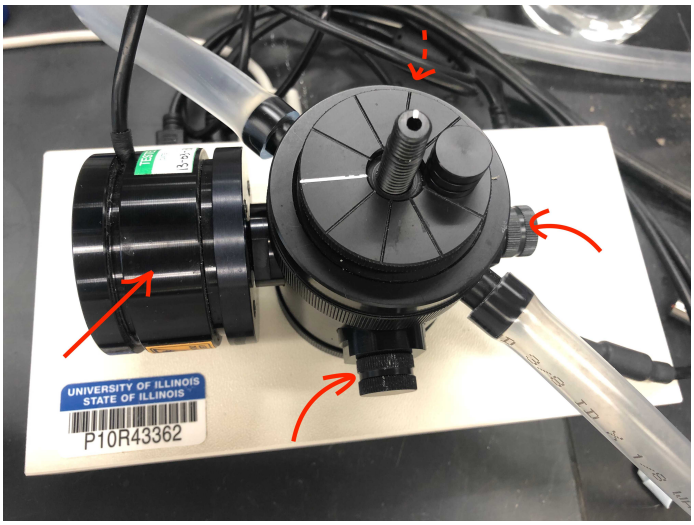


3.16 Insert the light sensor to the top opening of the electrode chamber, ensuring the circular patch is facing the light source (pictures in steps 4.1-4.2).

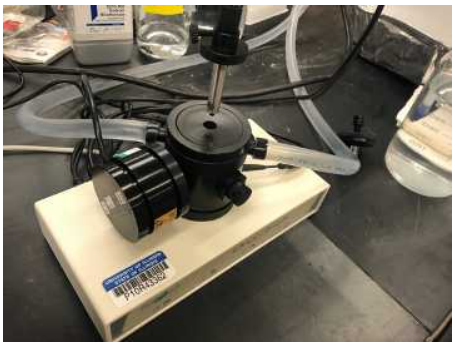
Light Calibration

4 Light Calibration

Needed for light curve, and the light curve needs to be done before the bicarbonate curve

4.1 Ensure both the light and light sensor are inserted to their appropriate ports, and that the light sensor is actually facing the light

Electrode chamber before light sensor and electrode disc are in place



Electrode chamber with electrode disc fastened in place and light sensor being inserted to the chamber.

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4.2 In the O2View program, in the upper menu bar, click Calibrate > calibrate light (automatic)

4.3 This should take a few minutes but it will do it itself.

Electrode Calibration

5 Electrode calibration

5.1 Add **1 mL** of ASW to the electrode chamber

There should also be one of the small (tiny) magnetic stirrers already be inside

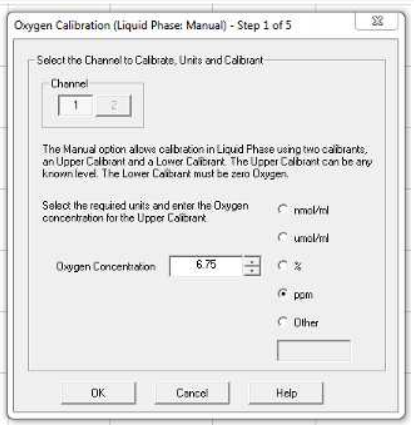
5.2 Insert plunger (hollow lid-like contraption with glass on one end. Glass end goes into the chamber)

5.3 Calibrate > Liquid phase calibration (manual)

5.4 Follow the prompts that pop up on the screen

5.5 Use their default 25 µmol/mL oxygen concentration setting.

Previously entered 6.75 ppm based on this chart and our use of ASW for these samples, still a little unsure about which is the most accurate.



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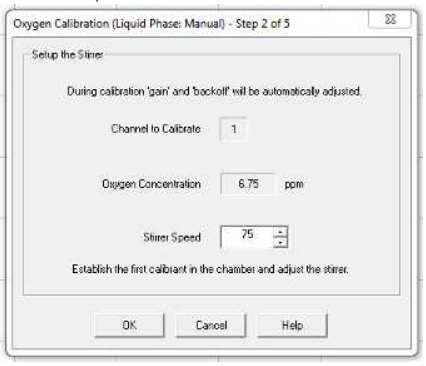
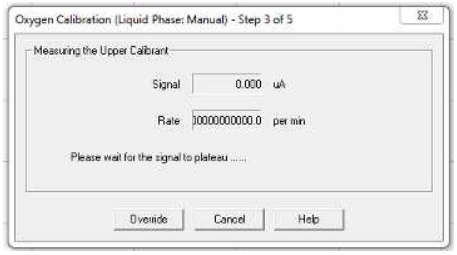
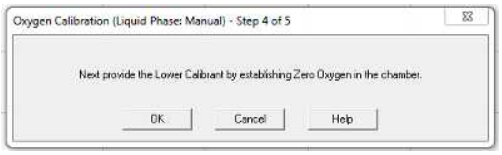
5.6 Enter stirrer speed of 75



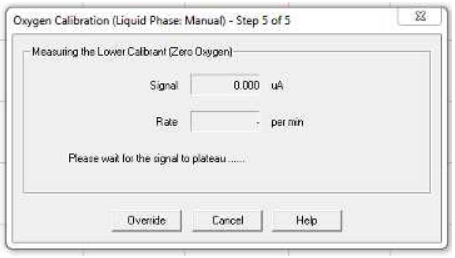
5.7 Continue to follow prompts:





Don't click "OK" on prompt 4 of 5 until you have your zeroing chemical ready. Nitrogen gas was the preferred method of zeroing the oxygen due to its availability and lessened risk of affecting any subsequent measurements (unlike Sodium Dithionite, which can also be used). The nitrogen tank is intimidating and can take a few tries to get used to.

5.8 Nitrogen gas was introduced to the chamber from a large tank at the end of the bench through a tube with a 1000 µL pipette tip attached. The pipette tip goes directly into the ASW in the electrode chamber, preferably without jamming it into the electrode disc. Leave it in until the signal has plateaued and the calibration is saved.



Prepare Light Curve Culture Samples

6 Preparing Light Curve Culture Samples

It's suggested to prepare each sample right before performing a light curve on it, rather than preparing them all together and having some sit out for longer.

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6.1 In a flow hood, pour **25 mL** of a healthy culture into a 50 mL centrifuge tube

"Healthy" is entirely subjective but generally suggests a more vibrant yellow-green

6.2 Prepare a balance with another tube of **25 mL** of dH_2O

6.3 Centrifuge for **00:05:00** at **5000 g** and 20°C

5m

6.4 Discard the supernatant by micropipetting

6.5 Resuspend in **10 mL** of ASW by gently disturbing the pellet with a small paintbrush

Too large of a paintbrush will absorb all your cells and media. It can be done, but it is alarming.

If you only have a large paintbrush and it does eat your cells, you can try adding another 1.5mL directly onto the brush to sort of wash the cells off. It might not look convincing, but it should allow you to have enough left for a test.

6.6 Balance with dH_2O again in centrifuge for **00:05:00** , **5000 g** , 20 °C

5m

6.7 Discard the supernatant

6.8 Add **1 mL** ASW to the pellet

6.9 Swirl the tube gently

6.10 Use the paintbrush again to resuspend. Press the paintbrush against the inside of the tube when pulling it out to try and save as much of the sample as possible.

PFD Table Set up for Light Curve

7 Photon Flux Density Table Set up for Light Cruve

7.1 Remove ASW from chamber

7.2 Make sure the stirrer is still going (can turn off while removing ASW)

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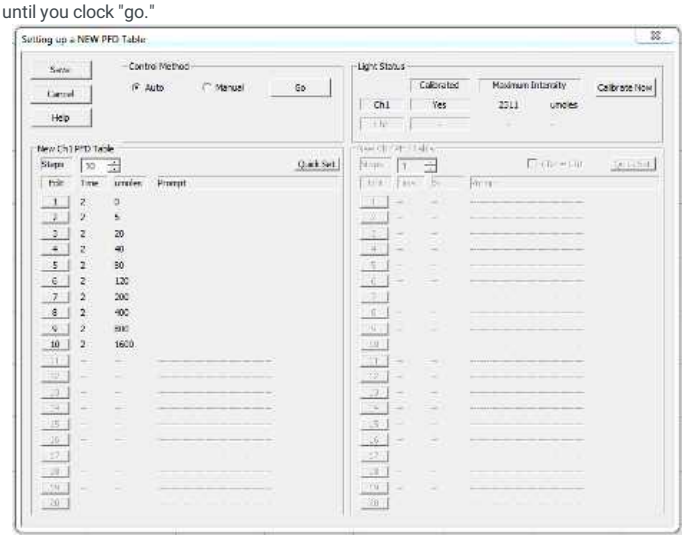
7.3 In the top menu bar, click Hardware > New PFD table

7.4 Select auto

7.5 Adjust so the following intensities of light (umoles) are selected for 1 minute each: 0, 5, 20, 40, 80, 120, 200, 400, 800, 1600. Click save if you need to close the window, otherwise leave the window up until you clock "go."



This screenshot is from before time was adjusted to 1 min. 2 mins tends to result in a flatline because it allows too much oxygen to build up in the chamber.

7.6 Add **900 µL** of culture

7.7 Add **100 µL** of $NaHCO_3$ solution

7.8 Place the plunger into the chamber

7.9 Click "Go" on PFD window

7.10 When program is done, turn off stirrer

7.11 View QY Data tab and save the file

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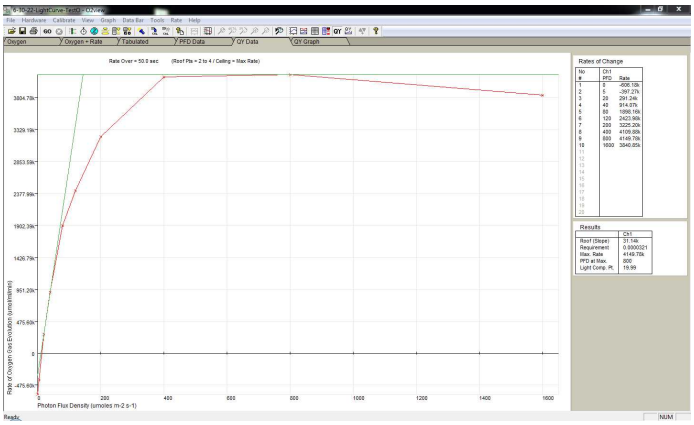

Graph pictured above shows 400-800umols of light as being the best for *O. tauri* photosynthesis.

Clean Up

8 Clean Up

8.1 Remove the sample from the electrode chamber using a pasteur pipette

8.2 Make sure everything is turned off (stirrer, light)

8.3 Take out and put away the optical port blanks

8.4 Take out the light

8.5 Unscrew the bottom of the electrode chamber, careful not to let the electrode and magnetic follower fall

8.6 Pull out the electrode disk, catch the magnetic follower

8.7 Rinse the inside of the electrode chamber with dH_2O

8.8 Take the O-rings off the electrode and rinse them with dH_2O

8.9 Take off the membrane and paper from the electrode and dispose of them in the bio-waste bin

8.10 Rinse the electrode disc with dH_2O

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8.11 Rinse the magnetic flea with dH_2O

8.12 Make sure everything is gently and thoroughly dried, use a kimwipe for the electrode disc.

8.13 Repeat the same polish process as done at the beginning on the electrode/electrode disc. It's important to clean the electrode disc thoroughly right after you're done using it to prevent any tarnish buildup, which can happen after one day of use. The electrode disc should also be stored with a dehydration packet.

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