The *OPN Oxygen Electrode Chamber*: An Inexpensive, Open-Source Oxygen Electrode System for Measuring Respiration

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**Introduction**

In the past twenty years additive manufacturing technology has rapidly evolved and continues to expand its utility in new branches of science and science technology. With decreasing costs, 3D printing has become readily available and accessible. Laboratory technology is often expensive and inaccessible for less economically advantaged schools systems globally. As such, it is a promising arena for 3D printing technology to make scientific laboratory equipment accessible to all students. Oxygen electrode systems are an important piece of laboratory equipment, useful for measurements of respiration and metabolism. These systems are typically expensive and comprise a significant cost to school laboratories.

A number of oxygen electrode systems are commercially available for sale, such as the **HACH® LDO Model 2** (and similar models from vendors such as **Clark®** and **Oakton®**). These systems are expensive with costs ranging from $1000.00 to $5000.00, not including shipping or tax.

Our lab designed an inexpensive laboratory oxygen electrode system in order to increase access to this important piece of equipment. This manual describes how to construct and calibrate the system using 3D printed components and affordable and readily accessible parts. Included in this manual is a model experiment that can be used to familiarize yourself with the designed set-up.

Finally, in keeping with the names given to other instruments and pieces of equipment developed in the OPN Lab, our oxygen electrode set-up is called the **OPN Oxygen Electrode System**. This name is given as the plans and parts are intended to be open-sourced and publically available for all to use. We hope that the **OPN Oxygen Electrode System** will increase availability of access to scientific laboratory equipment.
Constructing the OPN Oxygen Electrode System

The OPN Lab’s Oxygen Electrode System consists of multiple parts. These components were constructed using 3D printed pieces and items readily available online and in most science laboratories. For readers without access to 3D printing technology, there are a number of online vendors (Shapeways®, makexyz®) that will print and ship any STL files for a fee based on the size and volume of the part. http://3dprintingpricecheck.com/ estimates a cost of $3.50 to print the required STL files for the OPN 3D Printer using makexyz 3D printing service (value calculated in February 2018). In the long term, purchasing a mainline commercial 3D printer ($500 or less) and the related filament ($25 or less) will be a more economical option, especially if printing multiple parts over time.

Components of the OPN Oxygen Electrode System

To make and configure the OPN Oxygen Electrode System, readers will need the following materials:

A. Chamber Component
   a. Standard 3D printer and related filament (ABS filament was used; PLA will suffice)
   b. CAD and STL files for the OPN Oxygen Electrode Chamber (available at https://pages.stolaf.edu/opn-lab/)
   c. Adhesive Velcro strips
   d. 5mm magnetic stir bar
   e. Epoxy glue (5min)

B. Stir Plate Component
   a. Magnetic stir plate (The OPN Stir Plate was used for this setup)
      i. https://pages.stolaf.edu/opn-lab/
   b. Adhesive Velcro strips

C. Oxygen Electrode Component
   a. Milwaukee MW 600 Dissolved Oxygen Probe
   b. 250ml Erlenmeyer flasks
   c. 10ml pipette
   d. 1M KCl
   e. H2O
**Constructing the OPN Oxygen Electrode System**

**STEP 1:**
- Print the STL files for the *OPN O₂ Electrode Chamber* (Available on The OPN Lab website) with 100% infill (Fig. 1).

![Figure 1: The image shows the two components of the O₂ electrode chamber, the chamber base (left) and chamber cap (right).](image)

**STEP 2:**
- Fill the chamber base piece with water to test for any points of leakage.
- Use epoxy glue to seal any cracks in the chamber base (See Troubleshooting if leaking persists)

**STEP 3:**
- Peel off adhesive from a one-inch strip of Velcro.
- Attach Velcro strip to the underside of the chamber base (Fig. 2).
- Peel off adhesive from a one-inch strip of Velcro.
- Attach Velcro strip to the surface of a stir-plate (Fig. 3).
Figure 2: The image shows a one-inch strip of Velcro affixed to the underside of the chamber base.

Figure 3: The image shows a one-inch strip of Velcro affixed to the surface of a stir plate (OPN Stir Plate)
**STEP 4:**

- Attach the chamber base to the stir plate using the Velcro adhesive.
- Place 5mm magnetic stir bar in the chamber.
  - Make sure the stir plate speed is set such that the stir bar is not bouncing inside the chamber.
- Cap chamber with the chamber top printed piece (Fig. 4).

**Figure 4:** The image shows the *OPN O₂ Electrode Chamber* securely attached to a magnetic stir plate.
Troubleshooting the Chamber

Leakage

To prevent any water leakage from the chamber, our lab printed the chamber base piece with 100% infill using ABS filament.

The chamber cap piece does not need as high of an infill density as it is not designed to hold water. Still, we suggest testing each print with water prior to any use of the equipment. Any cracks should be sealed with an epoxy-glue sealant as instructed in Step 2.

Incompatible Pieces

Depending on the 3D printer, filament, temperature, and speed of printing, users may find that the chamber cap piece does not fit cleanly on the chamber base piece. This may be the result of plastic warping, rushed printing, or random error.

To prevent this complication, we recommend making sure that pieces are printed at recommended temperatures for the used filament and that printer speed is kept low. This will help produce a smooth chamber base piece that fits nicely into the chamber cap piece. We also recommend using sandpaper to smooth out the inner edges of the chamber cap if the pieces continue to fit poorly. Be sure only to sand the inside of the chamber cap and not the outside of the chamber base as this can create a porous exterior surface which can contribute to fluid leakage.
Setting Up the *OPN Oxygen Electrode System*

**STEP 1:**
- Read the Manual for the Milwaukee MW 600 Dissolved Oxygen Probe
- Remove the protective cap from the Milwaukee Oxygen Probe.
- Fill membrane cap with 1M KCl solution.
- Screw membrane cap onto the probe.
- Place the oxygen probe in a beaker of H$_2$O and turn on the device. Let sit for 10 minutes.

**STEP 2:**
- Prepare the magnetic stir plate by attaching the *OPN Oxygen Electrode Chamber* and placing the 5mm magnetic stir bar in the chamber.
- Add 8ml of H$_2$O to the chamber and aerate using a 10ml disposable pipette (Fig. 5).
- Insert the oxygen probe into the chamber. Turn on the stir plate.
- Wait till the oxygen meter on the probe is stable.
- Remove the probe. You are now ready to begin taking your control measurements.

**STEP 3:**
- Aerate the solution using the disposable pipette.
- Insert the oxygen probe into the chamber. Turn on the stir plate. These steps should be done quickly.
- Begin taking measurements after 30 seconds. Take measurements every 10 seconds for 3 minutes.
  - This should serve as a control measurement for an experiment.
- Following measurement, remove sample from the chamber using a pipette.
Figure 5: This image shows the how one would aerate a solution prior to taking measurements.
Figure 6: This image shows the Milwaukee Dissolved Oxygen Probe inserted into the $\textit{OPN O}_2$ Electrode Chamber.
**Yeast Experiment**

We designed an experiment for users to try as a means of becoming comfortable with the system. For this experiment, one will need the following materials:

- OPN Oxygen Electrode System
- 50ml Erlenmeyer flasks
- 0.4g Baker’s Yeast
- 10ml pipette
- 1M KCl
- H₂O

**STEP 1:**

- Add 0.4g of Baker’s Yeast to 100ml of water in a 250ml Erlenmeyer flask. Stir gently.
- Mix solution and let sit for one hour (until C₀₂ bubbles form)

**STEP 2:**

- Remove the protective cap from the Milwaukee Oxygen Probe
- Fill membrane cap with electrode solution (1M KCl)
- Screw cap onto the probe.
- Place the oxygen probe in a beaker of water and turn on the device. Let sit for 10 minutes to equilibriate.

**STEP 3:**

- Calibrate the *OPN Oxygen Electrode System* following Steps 2 and 3 on page 9.
- Rinse the probe with water.

**STEP 4:**

- Add 8ml of yeast solution to the chamber and aerate using a 10ml pipette.
- Insert the oxygen probe into the chamber. Turn on the stir plate. These steps should be done quickly.
- Begin taking measurements after 30 seconds. Take measurements every 10 seconds for 3 minutes.
- Following measurement, remove sample from the chamber using a pipette. Rinse the probe cap.
Results

We compared the dissolved oxygen (ppm) rates of respiration for water, solutions of varying yeast concentrations, and solutions which underwent radiation interventions (microwave samples). Measurements were taken every 10 seconds for three minutes. All samples were a total of 8ml. Yeast concentrations are labeled as the ml of prepared yeast in a particular 8ml sample. You can see from the graph that the rates of respiration are proportional to the amount of yeast in each sample. We hope this experiment serves as a foundation for readers to design and develop their own experiments using the equipment.
Hazards

There are a number of safety hazards associated with 3D printing. In particular, the 3D printing bed can become very hot when in use. Readers should exercise caution when removing any prints from the printer bed. Also, the 3D printing process can release harmful nanoparticles into the air, which can cause several serious health issues, including asthma attacks, respiratory arrest, strokes, and even cardiac arrest. Readers should keep their 3D printer in a well ventilated area and wear proper protective equipment when operating the printer (e.g., masks or respirators and goggles or safety glasses to protect their respiratory systems and eyes from these nanoparticles). We encourage readers to review the safety information pertaining to their 3D printer, and research to learn more about 3D printing safety.

Disclosures

I declare that I have no conflicts of interest related to any product, brand, company, website, or other item discussed in this manual. As well as with other open-source instruments and equipment developed by this lab, we encourage readers to improve upon the designs and methods set forth in this manual by using other materials and equipment. We urge them to bring their own insights and inspirations to the project.

References