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# An Automated Multi-Chamber System for Quantifying Biological Oxygen Demand

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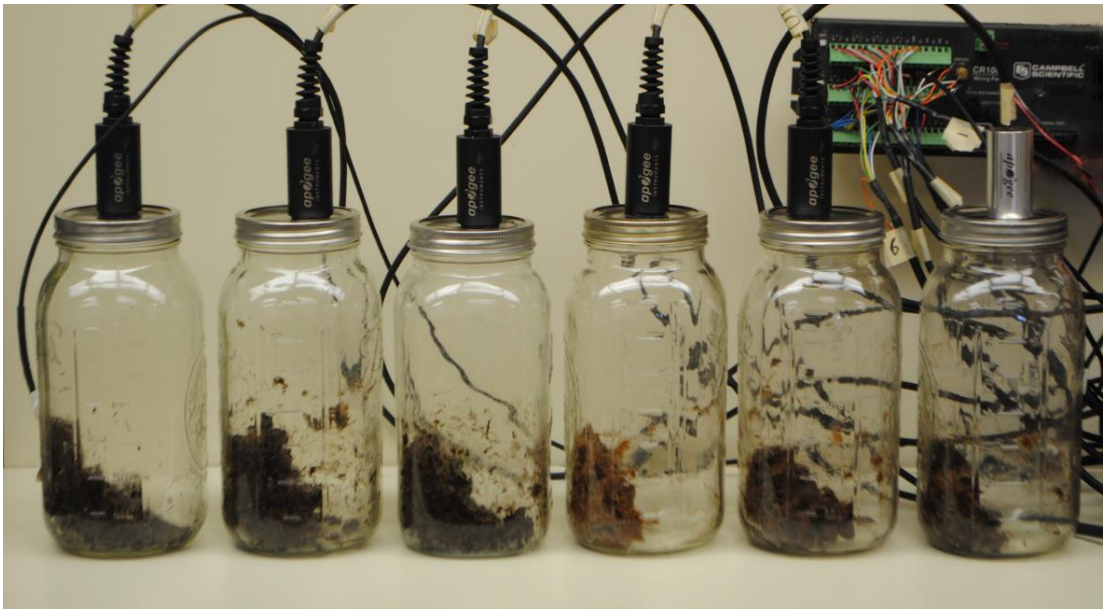


*Figure 1: Six galvanic-cell oxygen probes (Apogee Instruments) attached to the lid of 2 L canning jars. A datalogger (Campbell Scientific) is in the background. Fully hydrated media is placed in an upright position to allow drainage and increase aeration. The jars are placed in an insulated box to stabilize temperature. A CRBasic program to measure and calculate rates is included in Appendix C.*

## *Abstract*

Quantifying respiratory fluxes is important for understanding global carbon cycles, microbial population growth, postharvest quality and compost stability. Here we review methods for measuring respiratory fluxes and describe a simple, closed system that measures the depletion of oxygen to determine these fluxes. The system uses a galvanic cell oxygen electrode coupled with a data acquisition system capable of microvolt resolution. Measurements are automated and graphed in real-time. We discuss best practices for using the system.

## *Introduction*

The equation for respiration for a mole of hexose is:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 2870\ kJ \quad (eq.1)$$

To quantify the respiration rate, any of the five variables of *eq. 1* can be measured; either the depletion of inputs or production of outputs. However, measuring $O_2$ depletion and $CO_2$ production are the two most common methods. Field et al. (1991)

and Sestak et al. (1971) reviewed techniques for measuring photosynthesis. Techniques of measuring respiration of compost are briefly discussed in Gomez et al. (2006). We provide a short review of techniques for measuring respiration in Appendix A.

Here we describe the design, physio-chemical considerations, and best practices of an automated multi-chamber system that quantifies respiratory fluxes based on the depletion of oxygen.

# *Design principles and system assembly*

This system uses galvanic-cell oxygen probes (Apogee Instruments, Logan UT; model SO-110), which measure the partial pressure of $O_2$. These probes are attached to a standard canning jar lid that can be fitted to any size jar to create a sealed chamber. The probes are wired to a datalogger (Campbell Scientific, Logan UT, CR1000 or CR1000x), which can provide real time monitoring of the depletion of oxygen from which the respiration rate can be calculated (*Fig 1*).

This system requires a custom nut that must be 3D printed or purchased from Apogee Instruments. Procedures for assembling the system are described in Appendix B. A CRBasic program to measure and calculate rates is included in Appendix C.

In a closed system, as oxygen is consumed via respiration, the partial pressure of oxygen decreases. The following ratio can be used to calculate the conversion of partial pressure to moles of oxygen.

$$\text{Partial Pressure } O_2\,(\text{kPa}) = \frac{\text{mol } O_2}{\text{mol air}} \times \text{Total Air Pressure (kPa)}$$

$$\frac{\text{Partial Pressure } O_2\,(\text{kPa})}{\text{Total Air Pressure (kPa)}} = \frac{\text{mol } O_2}{\text{mol air}}$$

*(eq. 2)*

Using the conversion above respiration rate can be calculated with equation 3 at the bottom of this page. Equation 3 can have an additional term liters of media per gram of dry mass of media. At sea level, there are 0.041 moles of air per liter at 25 C. The moles of air per unit volume can be calculated from the ideal gas law if the barometric pressure and temperature are known (R = 8.314 kPa $\cdot$L $\cdot$ mol$^{-1}$ $\cdot$ K$^{-1}$).

$$P \cdot V = n \cdot R \cdot T$$

$$\frac{n}{V} = \frac{P}{R \cdot T}$$

*(eq. 4)*

*(eq. 3)*

$$\frac{\text{mol } O_2 \text{ consumed}}{L_{media} \cdot \text{hr}} = \left( \frac{\Delta \text{Partial Pressure } O_2\,(\text{kPa})}{\text{Barometric Pressure (kPa)} \cdot (t_2 - t_1)} = \frac{\Delta \text{mol } O_2}{\text{mol air} \cdot (t_2 - t_1)} \right) \times \underbrace{\frac{\text{x mol air}}{\text{L}}}_{\substack{\text{Calculated using the} \\ \underline{\text{Ideal Gas Law}}}} \times \underbrace{\frac{\text{x L}}{\text{Jar}}}_{\substack{\text{Depends on} \\ \underline{\text{chosen jar size}}}} \times \underbrace{\frac{\text{Jar}}{\text{x } L_{media}}}_{\substack{\text{Volume of} \\ \underline{\text{added media}}}}$$

# *Best practices*

Due to the inhibitory effects of low oxygen and high $CO_2$ on respiration (discussed in Appendix A) it is necessary to periodically purge the system. Determining the purging point is difficult, and we recommend purging when the jar gets down to about 60% of the maximum amount, or about 12% $O_2$. A good approach is to crack the lid and pump in room air, but cracking the lid without a pump works as well, although it is slightly slower at bringing the system back to 20.95% $O_2$. A larger jar provides a longer time interval to measure respiration before having to purge the jar with fresh air, but it is less sensitive to small changes in respiration. Therefore, a larger jar is recommended for applications with faster respiration and smaller jars for slower applications.

Non-biological changes in the partial pressure of oxygen can be caused by fluctuations of temperature and barometric pressure, but changes in barometric pressure do not affect this system because it is sealed. However, temperature changes will cause changes in the partial pressure of oxygen in the closed jars. An insulated box (e.g. picnic cooler), packing peanuts, and the controlled heaters in the oxygen probes reduce temperature fluctuations to less than ±0.5 C. This corresponds to a 0.3% error in the measurement of respiration, but temperature can be measured and corrected in software.

This system has excellent digital resolution and makes continuous measurements. There are two essential components of the system: a data-logger and the sensors. The CSI model CR1000 data-logger used in this system has a 13-bit resolution, and the sensor has a signal of about 55 mV in ambient air. The best voltage range with this logger is thus 0-250 mV. Taking the average range of the sensor signal yields 55 mV per 20.95% oxygen or 2.6 mV per 1% oxygen. The calculation of the digital resolution is:

$$\frac{\text{Voltage Range (mV)}}{\text{Bit resolution}} = \text{Digital Resolution (mV)}$$

*(eq. 5)*

An example calculation, using the parameters of the specific sensors and loggers used in this system, is as follows:

$$\frac{250\text{ mV}}{2^{13}} = 0.03\text{ mV}$$

*(eq. 6)*

The digital resolution of the percent oxygen then is:

$$0.03\text{ mV} \times \frac{\%\ O_2}{2.6\text{ mV}} = 0.012\%\ O_2$$

*(eq. 7)*

The resolution can be improved by using the newer CSI model CR1000x datalogger, which has 24 bit resolution. The data can also be smoothed by using a running average. However, 0.012% $O_2$ is adequate accuracy. For comparison, this would be a measurement of a 120 ppm change in $CO_2$. Galvanic cell oxygen probes consume oxygen. However, over 120 days of running

the sensors in empty jars, the linear decrease in the partial pressure of oxygen showed a consumption rate of about 0.1 (±0.03) µmol $O_2$ per day (*Fig. 2*). This is insignificant for most applications.

An empty control jar can correct for all the above listed errors, but the errors are small and we did not find that the use of a control jar was necessary.

A more detailed account of best practices can be found in the manual for the galvanic cell-oxygen probes used in this system. This is available on the Apogee Instruments website at https://www.apogeeinstruments.com/content/SO-100-200-manual.pdf.

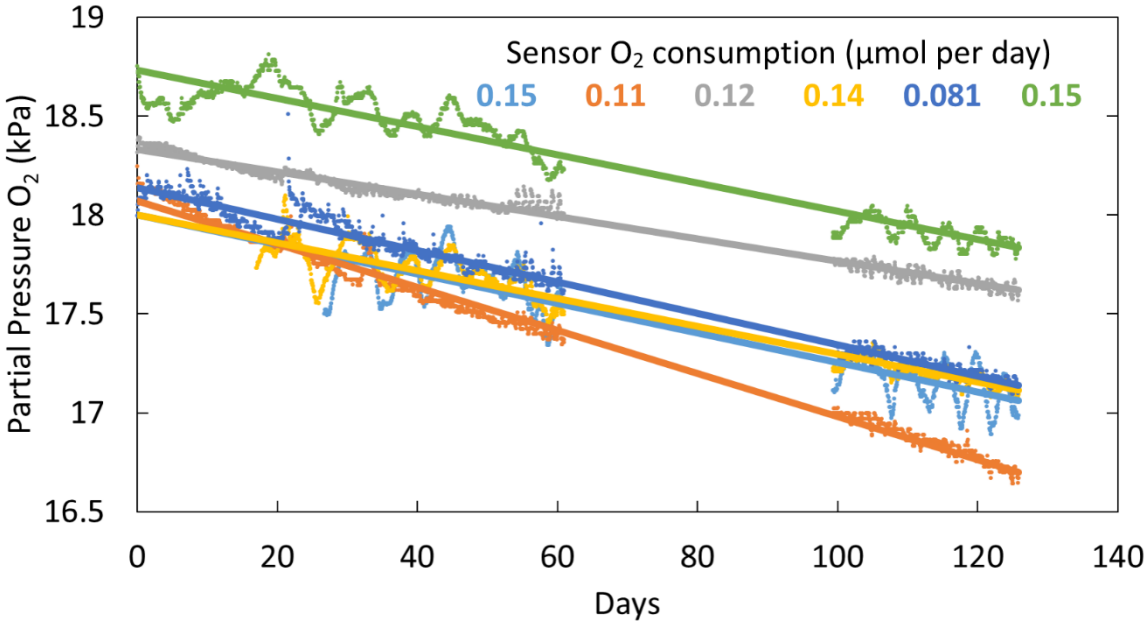


*Figure 2: Oxygen consumption of six sensors in empty jars. The jars were either 125 or 500 mL volume. The oxygen consumption rate of each sensor is shown. These O2 consumption rates are insignificant for most types of studies.*

# Appendix A

A review of techniques for measuring respiration

As mentioned above, any of the five components of eq. 1 can be used to measure respiratory fluxes.

### *Water production*

While it is possible to measure respiration via water production, it is difficult and rarely attempted (Sestak et al. 1971; Saltviet, 2016). The amount of water produced in respiration is small compared to the large background of water in a cell. Heat production in respiration is an additional problem that causes significant water loss. To measure biochemical water movement it is necessary to use water containing an isotope of hydrogen.

### *Mass loss*

Dry mass loss caused by the oxidation of carbohydrates to $CO_2$ can be used as a measurement of respiration. Theoretically, a flux of 100 mmol of $CO_2$ would correspond to 3 g loss of hexose. Water fluxes make it difficult to measure the change in mass. Moreover, this method can be inaccurate when respiring compounds with different C:H:O ratios than glucose. More reduced compounds like lipids are have a much higher C:O ratio than the less reduced sugars. Similarly to water, isotopic labelling of carbon compounds can be used, but it is an expensive technique.

### *Heat production*

The conversion of Glucose into ATP is about 30-35% efficient. Glycolysis and the electron transport chain yield 30-32 ATP (30.5 kJ $mol^{-1}$) from one mole of glucose (2870 kJ $mol^{-1}$). The remaining 65-70% of the energy is lost as heat. Precise measurements of heat production require a calorimeter, but it can be adequately measured in some applications like compost stability with a thermometer. However, since this method measures all exothermic reactions within a system, it is not a true measurement of respiration. Additionally, processes like moisture content and porosity can also contribute to heating (Gomez et al. 2006).

### *$CO_2$ measurements*

$CO_2$ production and $O_2$ consumption are the most common methods of measuring respiration. For a review of the history and methods for measuring $CO_2$ see Yiqi & Zhao (2010). There are several methods for quantifying $CO_2$ production.

*Alkali Absorption Method*: This method uses $CO_2/CO_3^{2-}$ chemical principles to measure the release of $CO_2$. It uses a strong base (NaOH or KOH solutions) or Soda Lime to trap gaseous $CO_2$ as solid carbonate ($CO_3^{2-}$). While either can be used, Minderman & Vulto (1973) recommend using Soda Lime because it measured about double the $CO_2$ flux as a 2 N KOH solution, commenting that there was no reason soda lime would have caused a doubling in flux. The fixed carbonate is measured by either a change in mass, a titration, or electrical conductivity. This method can be reliable but often underestimates respiration (Kucera & Kirkham, 1971; Bekku et al., 1997; Yiqi & Zhou, 2010).

*Infrared Absorption Method*: Infrared gas analyzers (IRGA) measure the absorption of infrared radiation by $CO_2$. This method can be implemented in a flow-through system where pre- and post- chamber air is measured (Van Iersel & Bugbee, 2000), or it can be implemented in a closed system that measures non-steady-state $CO_2$ build-up; however, this method can be problematic.

One critical problem with measuring respiration by $CO_2$ evolution is that $CO_2$ gas is highly soluble in water and significant errors caused by trapped bicarbonate ($HCO_3^-$) begin to form at a pH above about 4.3 (*Fig. 3*). Further errors can occur above a pH of 8.3 when significant amounts of carbonate ($CO_3^{2-}$) begin to form (*Fig. 1*).

Equations 8 determine the amount of dissolved carbon.

$$K = \frac{[H_2CO_3\,(aq)]}{[CO_2\,(aq)]} = 1.3 \times 10^{-3}$$

$$K_H = \frac{P_{CO2}}{[CO_2\,(aq)]} = 29.4$$

$$K_{a1} = \frac{[HCO_3^-][H^+]}{[H_2CO_3]} = 4.47 \times 10^{-7}$$

$$K_{a2} = \frac{[CO_3^{2-}][H^+]}{[HCO_3^-]} = 4.69 \times 10^{-11}$$

*(eq. 8)*

The effect of pH on the equilibrium percentages of the three mineral carbon species is shown for a closed system in Figure 3. The pH will fluctuate in a system with metabolic activity. Changes in the

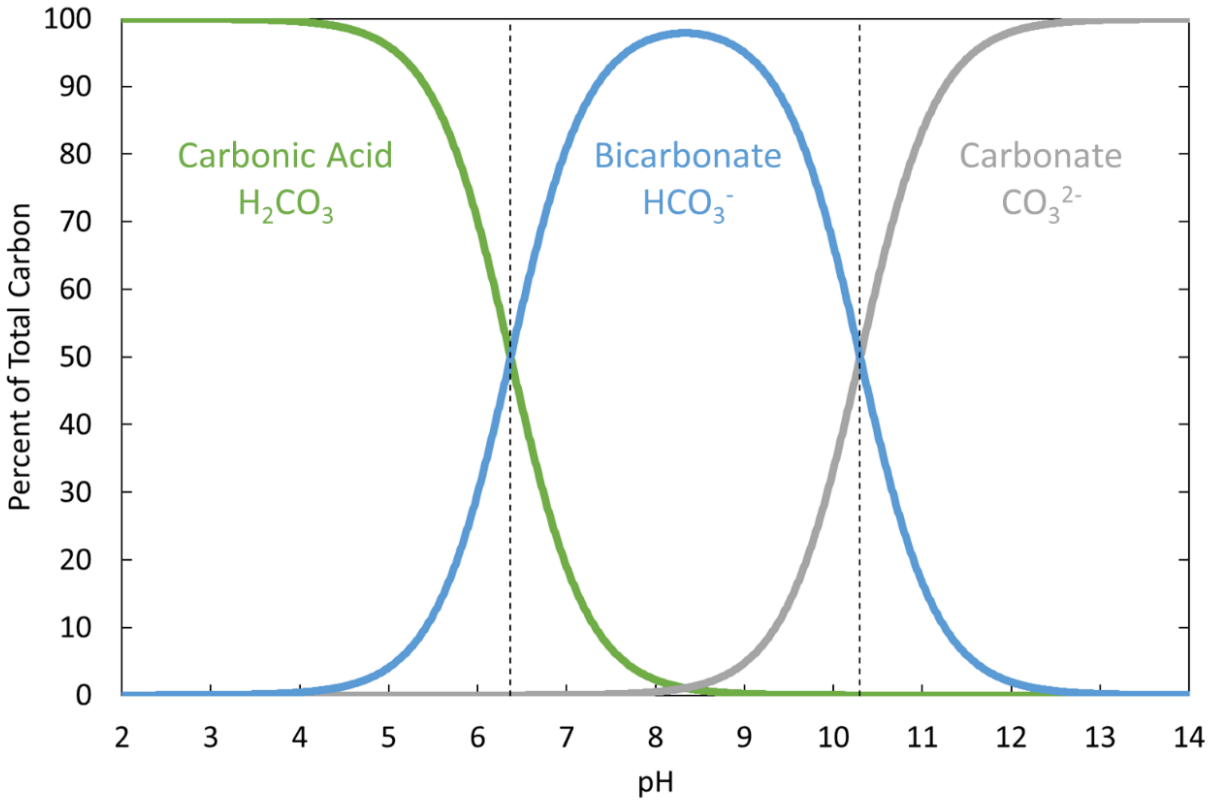


*Figure 3: Bicarbonate equilibrium in a closed system*

partial pressure of $CO_2$, uptake of anions and cations, and excrete organic acids can all change the pH of the environment. This constantly changing pH makes it nearly impossible to accurately measure the flux of $CO_2$ in systems with large amounts of water. Using eqs. 8, $CO_2$ fluxes can be corrected, but this is difficult.

One simple method to control these fluxes is to keep the pH of the system below 4.3 to prevent any bicarbonate from forming, although this may lead to biological anomalies. Software programs, such as AQUA, use inputs of pH, $Ca^{2+}$ and $Mg^{2+}$ concentrations to provide a more-complete calculation to correct respiration (Sierra & Renault, 1995). Lampert (1984) pointed out that correction factors must be made when measuring $CO_2$ in closed aquatic systems, and recommends monitoring pH a resolution of a thousandth of a pH unit. Because of pH issues, as well as complexation with cations, Lampert concludes that oxygen is the preferred method for measuring respiration in aquatic systems.

Because rising $CO_2$ concentrations decreases pH, pH can be used as an indirect measurement of respiration (Verduin, 1951). Although, because of advancements in $CO_2$ analyzers and oxygen sensors, this technique has rarely been used in practice.

Finally, IRGAs are designed to measure $CO_2$ concentrations in the range of parts per million. Because they have such a high sensitivity for small changes in $CO_2$, these bicarbonate problems can be avoided by making short-term measurements of respiratory fluxes. Measuring $CO_2$ increase works well if the factors above are taken into consideration.

*Gas Chromatography Method*: Similar to measurement using an IRGA, a gas chromatograph can be used to measure $CO_2$ fluxes.

### *Oxygen Consumption*

The consumption of oxygen is widely used to measure respiration, and it avoids the complications of bicarbonate equilibria. $O_2$ cathodes, galvanic cells, or an $O_2$ voltaic cell with calcium-stabilized zirconium oxide can be used for measuring $O_2$ consumption. Oxygen consumption, like $CO_2$ evolution, can also be measured with gas chromatography.

*Manometric method:* This method is commonly known as the Warburg technique, named after the Nobel Prize winner in Physiology or Medicine - Otto Heinrich Warburg. A manometer is used to measure the decrease in pressure in a sealed chamber, which is caused by biological oxygen consumption at a constant $CO_2$ concentration. The concentration of gaseous $CO_2$ is maintained by trapping it in an alkali solution. The alkali-trapped $CO_2$ can simultaneously be measured providing a respiratory quotient. This technique is susceptible to substantial errors but is very inexpensive. For more information, see Sestak *et al*. (1971) and Umbreit *et al.* (1949).

Oxygen measurements are widely used to measure respiration of aquatic ecosystems and are the preferred method for measuring the stability of compost (Gomez et al. 2006).

*Types of Chambers*

Closed chambers are categorized as either dynamic or static. Dynamic chambers circulate air out of the chamber, past the sensor, and back into the chamber. Static chambers, without an internal fan, generally use the alkali absorption technique and have the potential to underestimate respiration, due to slowed diffusion caused by a decrease in the concentration gradient (Rochette et al., 1992; Norman et al., 1997; Heinemeyer & McNamara, 2011). As the $CO_2$ concentrations in the headspace increase, diffusion rates decrease (Gao & Yates, 1998).

In a closed system, the concentration of oxygen declines over time while the concentration of carbon dioxide increases. Assuming a respiratory quotient ($CO_2$ produced/$O_2$ consumed) of one, if the concentration of oxygen goes from about 21% down to 0% the concentration of carbon dioxide will increase from about 0% (0.04% for 400 ppm) up to 21%. Respiration rate is dependent on the concentrations of both gases, with high carbon dioxide and low oxygen both generally inhibiting microbial growth. In a model by Sierra & Renault (1995) using Michaelis-Menten kinetics, increasing $CO_2$ concentration promoted respiration at low concentrations (4%) but inhibited at higher concentrations. This promoting effect was not observed in artificial aggregates. The magnitude of $CO_2$ inhibition at different concentration can

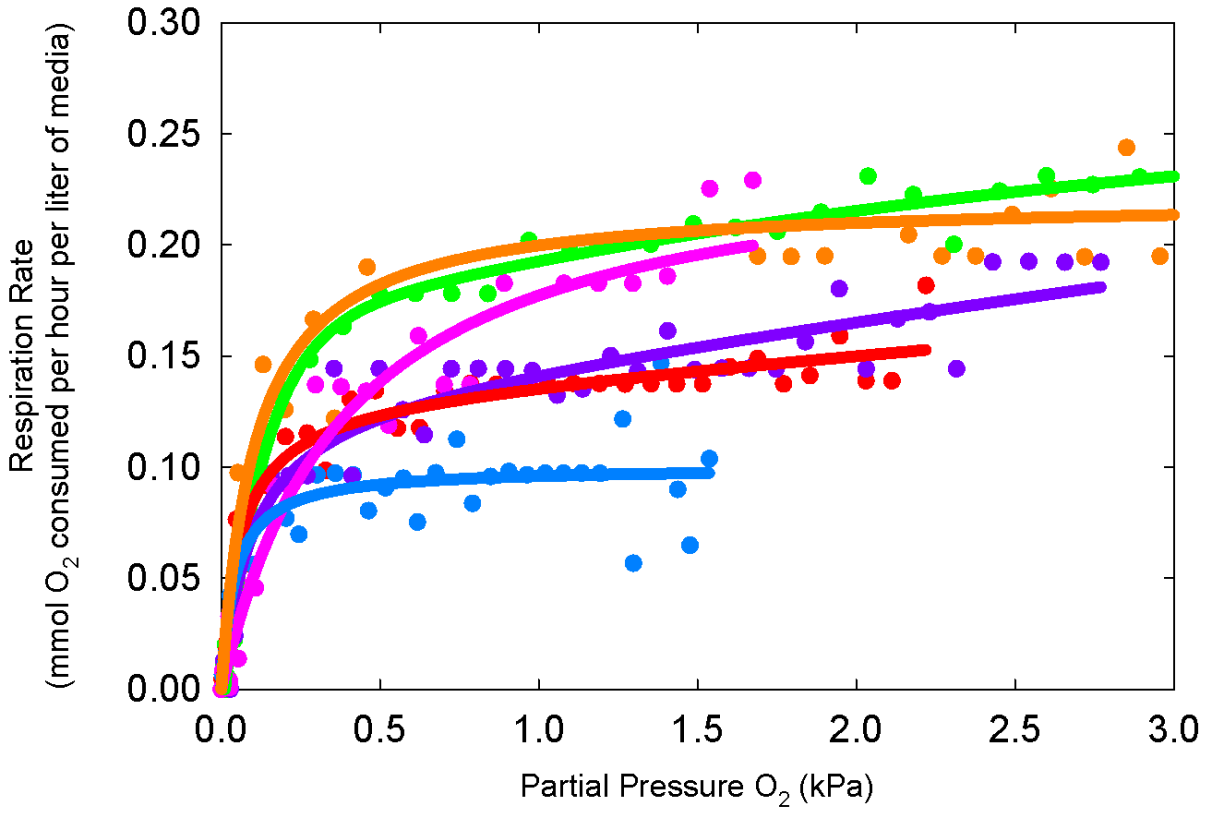


*Figure 4: Respiration rate at low oxygen concentration. Six replicate studies are shown.*

vary greatly depending on the microorganism.

They also saw a decrease in oxygen consumption as the concentration of oxygen decreased. This may be due to the decreased gradient between the air and the solution. Our data show organisms can pull the oxygen content down to less than one kPa of $O_2$ (*Fig. 4*). However, it is a best practice to avoid letting the oxygen get too low.

Chambers designed for field measurements typically do not have a bottom and are placed above soil. These chambers have their own problems and are reviewed in Davidson et al. (2002).

### *Effect of elevated $CO_2$ on respiration*

There is a large body of work concentrated on the inhibitory effects of $CO_2$ on respiration (Macfadyen, 1973; Bekku et al 1997; Daniels et al. 1985), and the potential mechanisms are reviewed in Daniels et al. (1985) and Dixon & Kell (1989).

Being aware of the pros and cons of different techniques, methods and systems for measuring respiratory fluxes can help interpret data, improve the design of respiratory systems and develop better experiments.

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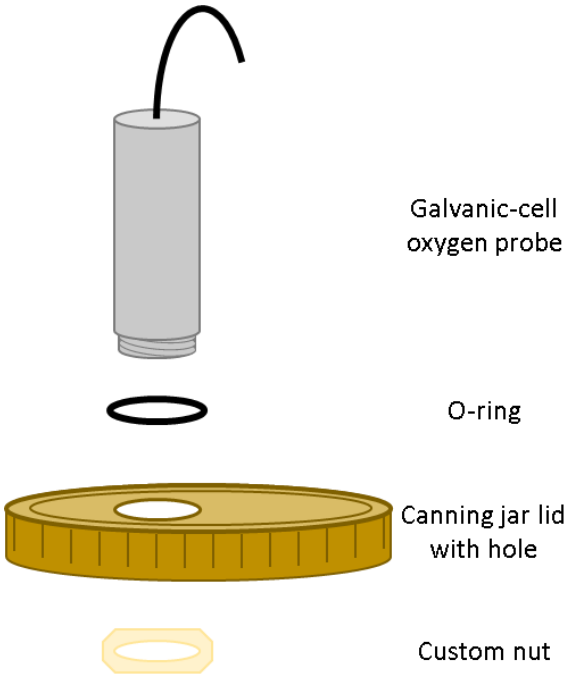
# Appendix B:

## Attachment of the sensor to a canning lid

Canning jar lids are modified to include a hole to accommodate the sensor. A 7/8" (22 mm) (e.g. from General Tools) hole punch was used to make a hole to insert the sensor. A wide-mouth lid is recommended for ease of getting media in-and-out of the jar. The oxygen probe is tightened to the lid with a custom nut (7/8 -16 UN), which must be 3D printed or purchased from Apogee Instruments. An O-ring (I.D. 3/4, O.D. 1, C.S. 1/8) is necessary to make a tight seal between the sensor and lid. After the sensor is compression sealed to the lid, it can be screwed onto to any-size canning jar.



# *Appendix C*

## *Copy of the Campbell Scientific, model CR1000, CRBasic program*

```
'CR1000 Series Datalogger


'Explanation of Constants and Variables Used in Datalogger Program
'CF = calibration factor (slope) to convert voltage signal to pressure
'Offset = offset (intercept) to convert voltage signal to pressure
'BattV = datalogger battery voltage
'PanelT = datalogger panel temperature
'Signal = mV signal output from oxygen sensor
'O2 = absolute oxygen concentration in kilopascals
'SensorTC = sensor temperature in degrees Celsius


'Declare Public Variables
Public BattV, PanelT
Public Signal(6), O2(6), O2_RA(6),
Public sensorTC(2), SensorTC_Average', O2_TC(6), BaroSignal, Pressure, O2_PC(6)
Public TC_L, TC_R
Public RespRate_1HR(6)
Public kPaDiff_1HR(6)
Dim CF(6), Offset(6)
Dim MediaVolume(6)
Dim H, J', K, L
'Const TC = 0.0292 'TC and CalTemp to correct for temperature
'Const CalTemp = 24.76
Const RespMultiplier = 203.152'This value is used to calculate respiration rate from the O2 signal
'Const CF_P = 0.0303 'CF_P and Offset_P are the slope and intercept for the pressure sensor
'Const Offset_P = 16.028
'Const PC = -0.24 'PC and CalP to correct for Pressure
'Const CalP = 86.02
```

```
Alias sensorTC(1) = SensorTC_1
Alias sensorTC(2) = SensorTC_5

Units O2() = PP kPa 'partial pressure kPa
Units O2_RA() = PP kPa
Units sensorTC() = C
Units SensorTC_Average = C
'Units O2_TC() = PP kPa
Units TC_L = C
Units TC_R = C
Units RespRate_1HR = umol/hr
'Units RespRate_2HR = umol/hr

'Define Data Tables
DataTable (Oxygen,1,-1)
  DataInterval (0,1,Min,10)
  Sample(1,PanelT,IEEE4)
  Average(6,O2(),IEEE4,False)
  Sample (6,O2_RA(),IEEE4)
  Average (2,sensorTC(),IEEE4,False)
  Sample (1,SensorTC_Average,IEEE4)
  Average (1,TC_L,IEEE4,False)
  Average (1,TC_R,IEEE4,False)
  'Average (6,O2_TC(),IEEE4,False)
  'Average (1,Pressure,IEEE4,False)
  'Average (6,O2_PC(),IEEE4,False)
EndTable

DataTable (Oxygen_ONEHOUR,1,-1 )
  DataInterval (0,1,Hr,10)
  Minimum(1,BattV,IEEE4,0,False)
  Sample (1,O2_RA(),IEEE4)
  Sample (1,RespRate_1HR,IEEE4)
```

```
EndTable

'Main Program
BeginProg

  'Values for mV to PO2 conversion
  CF(1) = 0.3990
  Offset(1)= 0.5267
  CF(2) = 0.3891
  Offset(2) = 0.4591
  CF(3) = 0.4202
  Offset(3) = 0.5294
  CF(4) = 0.4236
  Offset(4) = 0.5803
  CF(5)= 0.4188
  Offset(5) = 0.4816
  CF(6) = 0.3979
  Offset(6) = 0.3271

  'volume of media in jar
  MediaVolume(1) =150
  MediaVolume(2) =150
  MediaVolume(3) =150
  MediaVolume(4) =150
  MediaVolume(5) =150
  MediaVolume(6) =150

  Scan(1,Sec,0,0)
    Battery(BattV)
    PanelTemp(PanelT,_60Hz)


    'Measure oxygen sensors and temperature thermistors
```

```
VoltDiff (Signal(),6,mV250,1,True,0,_60Hz,1.0,0)
Therm109 (sensorTC(),2,13,Vx1,0,_60Hz,1.0,0)
Therm109 (TC_L,1,15,Vx2,0,_60Hz,1.0,0)
Therm109 (TC_R,1,16,Vx3,0,_60Hz,1.0,0)


'For Pressure Correction in a flow hrough system:
'VoltSe (BaroSignal,1,mV5000,16,1,0,_60Hz,1.0,0)
'Pressure=CF_P*BaroSignal-Offset_P

'convert a mV signal to the partial pressure of oxygen
For J = 1 To 6
  O2(J) = CF(J) * Signal(J) - Offset(J)
Next J

'Smooth data with a running average
AvgRun (O2_RA(),6,O2(),30)
SensorTC_Average = (sensorTC(1)+sensorTC(2))/2

'For Pressure Correction in a flow through system:
'For K = 1 To 6
  'O2_PC(K) = O2_RA(K) + PC*(Pressure - CalP)
'Next K

'Temperature correction
'For L = 1 To 6
  'O2_TC(L) = O2_RA(L) + TC*(SensorTC_Average - CalTemp)
'Next L

'Heater control
If SensorTC_Average <= 25 Then
  SW12 (1)
Else
```

```
  SW12 (0)
 EndIf

 'Respiration rate calculation
 For H = 1 To 6
  kPaDiff_1HR(H) = Oxygen_ONEHOUR.O2_RA(H,2)- Oxygen_ONEHOUR.O2_RA(H,1)
  RespRate_1HR(H) = -kPaDiff_1HR(H)*RespMultiplier/MediaVolume(H)
 Next H

 'Call Output Tables
 CallTable Oxygen
 CallTable Oxygen_ONEHOUR
NextScan
EndProg
```

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