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A comparison of methods for measuring CO_2 and O_2

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Introduction

Understanding the dynamics of respiratory gasses gives critical insight into the metabolic processes of a biological system. An efficient and effective means of measuring respiration is essential to understanding aspects of the biosphere. Many methods have been developed for measuring changes in CO_2 and O_2, both as integrated systems and as individual components. Many experiments use an alkali trap with subsequent titration as an inexpensive method for CO_2 measurement. *Haney et al.* (2008) compared the titration method against infrared gas analysis (IRGA) and found them to be highly correlated, with r^2=0.95. The use of IRGA provides the potential for automation in a system due to the electronic output. *Bowling et al.* (2001) described a system using a pneumatically driven piston to inject sample air, which gave consistency and high accuracy (a coefficient of variance of 0.05%). This and similar systems afford accurate measurements by incorporating complex mechanics, but can be expensive and time intensive to maintain. An emphasis was placed on simplicity and cost effectiveness, while still allowing for some degree of automation and instantaneous measurement results.

When measuring respiration, the majority of systems used focus on CO_2 as the measured gas, generally due to the differences in proportional gas changes relative to background concentrations. *Blonquist et al.* tested the validity of using an oxygen sensor to measure the respiration of soil and found it to be possible given corrections for temperature, pressure and humidity. The study also found oxygen measurements to be less affected by solubility in water and therefore advantageous in aquatic or semiaquatic environments. While assessing the biodegradability of hydrocarbons in soil, *Miles and Doucette* (2001) found that a measurement of respiration by oxygen depletion was comparable to measurements by known chemical depletion and collection of marked $^{14}CO_2$.

While the respiration rate can be measured by either CO_2 or O_2, the measurement of both gives further understanding to the underlying biological processes. Respiratory quotient (RQ), the ratio of CO_2 produced to O_2 consumed, is often used to determine the type of substrate being consumed and presence of anaerobic conditions. While some make the assumption that the RQ value is approximately 1 under aerobic conditions and

therefore interchange respiration rates as determined by CO_2 evolution or O_2 depletion, *Dilly* (2003) found that RQ is rarely steady and is often well above or below 1 for microbial populations. Therefore respiration rates cannot be assumed comparable when measured by different respiratory gasses and an incorporation of both may be necessary to allow for proper understanding and comparison of metabolic processes.

Four types of sensors were tested: syringe IRGA injection, an oxygen probe, and two types of CO_2 probes. Each was combined with a data acquisition system, to create a measurement system capable of recording the gas concentration. To allow for better assessment of respiratory processes, each system was tested to compare its strengths and limitations in a closed system application.

Materials and Methods

Syringe Injection

The core of the measurement system is a LI-COR LI-6251 IRGA. For data acquisition a Campbell Scientific CR10T datalogger was used in conjunction with the LoggerNet software package, including RTMC pro (also by Campbell Scientific). During operation, a pump pulls room air through a fine filter to prevent dust from entering the system. This air then passes through 2 vertical columns filled with soda lime to remove all CO_2, creating a clean, CO_2 free air stream. This air stream is regulated by a rotameter to a velocity 500 *cc/min* to ensure that all CO_2 is removed and for consistency between measurements. A 1-5 ml sample is injected via syringe into the airstream, which then passes through the IRGA measurement chamber, outputting a voltage.

The system is calibrated each time it is used by injecting a known reference gas, from which the datalogger calculates a correction multiplier automatically in software. This procedure is a quick and simple way to correct for any pressure or temperature effects on the IRGA. The voltage output from the IRGA is measured by the datalogger as a millivolt reading. The peak of this reading is stored by the datalogger and converted to ppm CO_2 using the calibration multiplier. This value is then retrieved by the computer and graphically displayed.

To assess the repeatability of the instrument, 13 samples of reference gas were analyzed over 10 minutes and their outputs were compared to the known CO_2 concentration.

Oxygen Probe

Four Oxygen sensors (Apogee Instruments Model-SO) were mounted to read the oxygen concentration of a 1 L Mason jar. The data was acquired using a Campbell Scientific CR1000 datalogger, along with a thermocouple and a pressure sensor (Apogee Instruments SB-100). Each jar had a long tube to allow equilibration of pressure with the external environment. The sensors were calibrated to changes in temperature and pressure using an empty jar filled with a known reference gas. The stability of the measurement was observed for 6 day period, and an estimate of the small O_2 consumption rate of the sensors was estimated.

CO_2 Probe

Two probes were tested, a CARBOCAP GMM220 and a CARBOCAP GMP343, both by Vaisala Instruments. Each works as an infrared gas analyzer with the sample air diffusing into the measurement chamber, no active mechanical air sampling is used. The GMP343 and GMM220 measurements were recorded by a Campbell Scientific CR1000 datalogger. The temperature of the environment, as measured by at thermocouple, was recorded, along with the temperature of the GMP343, as measured by an incorporated thermistor. Each sensor was mounted to a 1 L Mason jar with a long tube to allow for pressure equilibration. To measure the stability of the sensor, the sensors were each mounted in empty 1 L Mason jars and place in a temperature controlled chamber with temperatures oscillating between 5 and 40º C. The sensors were then corrected for the effects of temperature and pressure.

Results

Syringe Injection

The measurement of 13 reference gas samples, known to be 400 ppm CO_2, gave a mean reading of 399.8 ppm CO_2 with a standard deviation of 4.9.

Oxygen Probe

The output O_2 was corrected for pressure and temperature in accordance with the manufacture's specifications, which produced a rather flat line in comparison to the uncorrected signal, as seen in Figure 1. In an independent study, Adams (2010 unpublished) did a similar experiment with 4 Apogee sensors, the results of which are shown in Figure 2.

A calculation of apparent O_2 consumption rate, via regression, give a near zero value. The apparent respiration rate, along with those of the Adams (2010 unpublished) study are shown in Table 1.
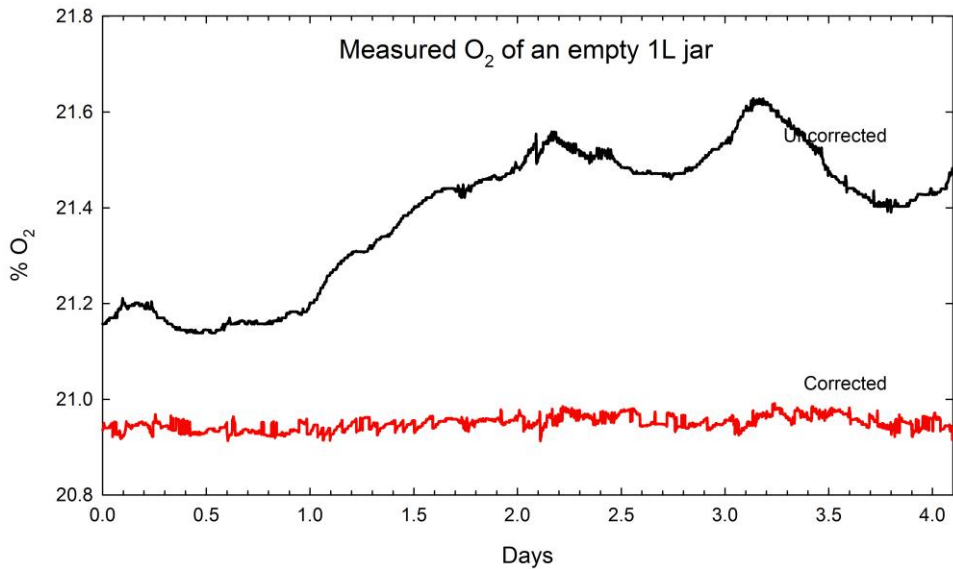

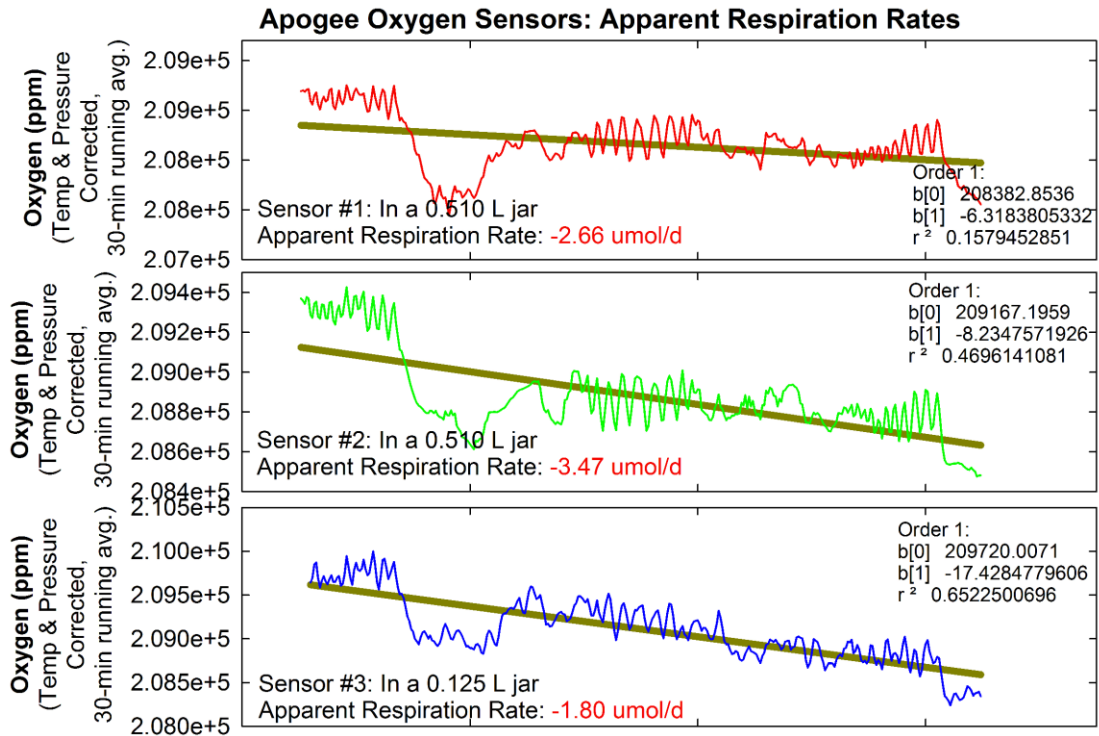Figure 1: Measurement of O_2 in an empty 1 liter jar.

Figure 2: Measurement of O_2 from Adams data

Sensor	Adams #1	Adams #2	Adams #3	Nelson
Apparent O_2 consumption rate (μmol:$O_2 day$)	-2.66	-3.47	-1.80	0.0002

Table 1: Apparent O_2 depletion rates of oxygen sensors in an empty jar

CO_2 Probe

The standard correction provided for the GMP343 by Vaisala was shown to be influenced by temperature. A new calibration equation was build using the Ideal Gas Law, the results of which are seen in Figure 3. Similarly, the GMM220 needed calibration to remove variations due to temperature and to convert the sensor output to ppm CO_2. *Johnson et al.* (2010) also found an additional correction to the GMM220 sensor necessary while using the sensor to measure concentrations in aquatic environments. The result of the calibration function created is shown in Figure 4. Both sensors show a rhythmic cycling which corresponds with the change in temperature. This variation was considered to be artificially induced by the relatively rapid change in temperature (7.5° C per hour).
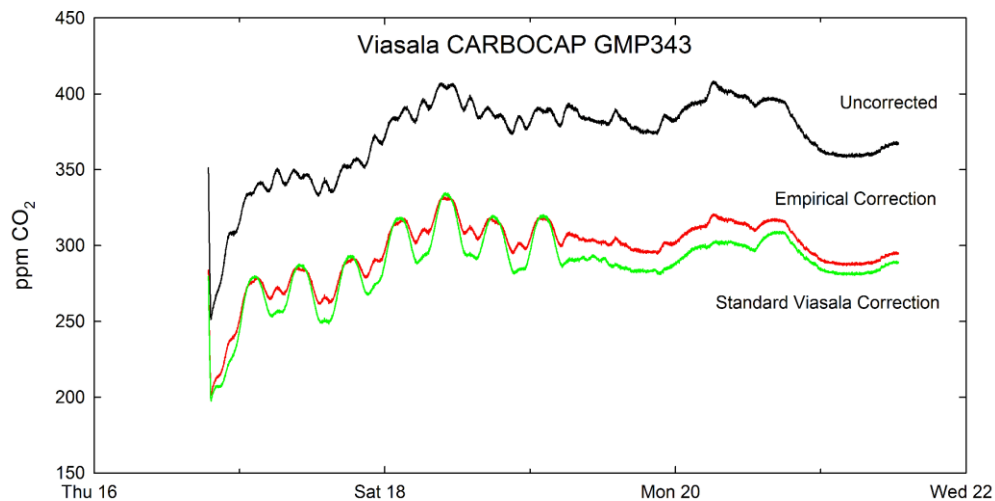

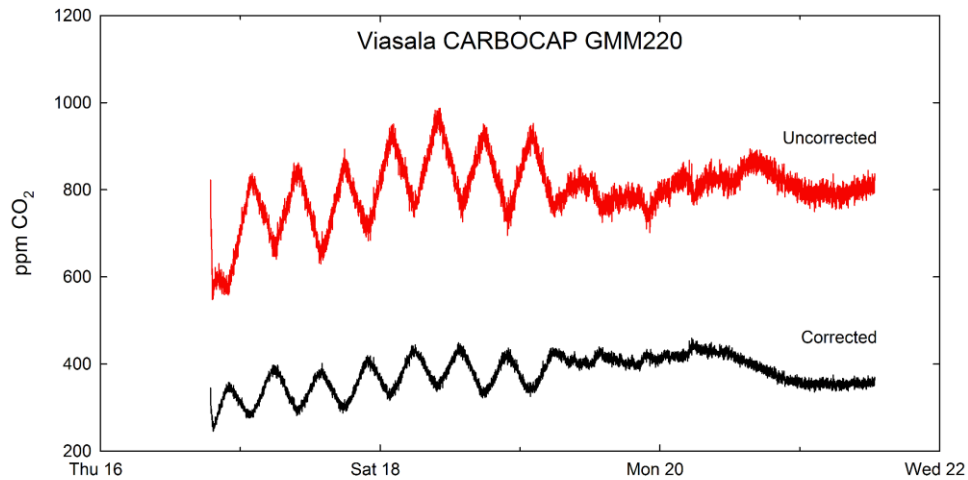Figure 3: Comparison of the corrections on a GMP343 sensor.

Figure 4: Comparison between GMM220 output and the corrected output.

Discussion

Syringe injection

This system allows for the rapid measurement (less than 1 minute per sample) with a coefficient of variation of 1.2% when taken near the time of calibration. The low sample time gives the capability of measuring a large number of samples quickly, from a variety of treatments, with one sensor. Though the system can be relatively expensive (>2000 USD for an IRGA alone), it is capable of measuring samples from many different experiments at once, so the price per sample is quite low. Measurement of concentrations very far past the reference gas gives increasing errors and can go off scale around 2000 ppm.

The system, as described here, cannot take continuous measurements. This leads to the possibility of missing micro trends or extremes exhibited by the experiment. There is also the possibility of error induced by measurement of extremes when the sample is taken.

Oxygen Probe

The difference between the Adams (2010 unpublished) system and system tested in this study was the small pressure equilibration tube, which the Adams study lacked and instead attempted completely sealed jars. This difference can be seen in the erratic nature of the Adams data, which cannot accurately correct for pressure changes in the jar because the jar interior pressure may vary from atmospheric. Though pressure equilibration provided a less erratic line, it failed to measure any O_2 consumption, which, based on manufacturer specifications, should be approximately 2 ($\mu mol{:}O_2 day$)

The advantage of an oxygen probe sensor is the ability to do a continuous measurement for relatively cheap (approximately 250 USD per probe). Therefore, a reasonable number of replicate systems can continuously monitored run at one time. Because the background level of oxygen in the atmosphere is 20.95%, a change comparable to a doubling of atmospheric CO_2 (from 400 to 800 ppm) constitutes a change from 20.95 to 20.99% O_2. The error in these sensors makes the measurement of small changes difficult. Therefore it is recommended for use where large changes are expected to take place. Due to the greater range of measurement the sensor can detect changes for long periods of time without going off scale.

CO_2 Probe

The rhythmic cycling with temperature is considered to be an artifact of the rapid changes seen in an artificial environment, and is not considered to be a major factor in natural environments were rapid temperature fluctuations are not common. The effect was not considered detrimental to the sensor performance, but care should be taken if the sensor is deployed in environments with rapid temperature changes.

The CO_2 probe system provides a continuous measurement that also detects small changes. The company specifications for the GMP343 and the GMM220 are (3 ppm + 1% of reading) and (30 ppm + 2% of reading) respectively. This precision allows for the detection of micro trends and extremes that the other systems may miss. The system is also more expensive than the oxygen probes, so running replicate experiments at the same time becomes very expensive (especially when using the more expensive GMP343). The GPM343 shows less signal noise and greater accuracy when compared to the GMM220.

Conclusions

Each system has apparent advantages and disadvantages. For experiments with many treatments and/or replicates, the syringe injection method would allow for many small microcosms to be ran at one time, but does not allow for continuous measurement. The probe type sensors allow for continuous measurement which can detect small fluctuations that have the potential to bias discrete measurements but are subject to error by changes in environmental factors. Measurements of respiration rate based on CO_2 can be calculated from small changes over a given time period, whereas respiration measurement by oxygen has a wider range and is not as susceptible to bicarbonate fluxes. A method incorporating both CO_2 and O_2 should be considered to provide greater accuracy and understanding of biological systems. Table 2 shows a comparison of the four sensors.

sensor type	coefficient of variation	continuous measurement	range
Syringe injection	1.2%	N	0-2000 ppm
Oxygen probe	6.9%	Y	0-100 %
CO_2 probe (GMM220)	30 ppm + 2% of reading*	Y	0-2000 ppm
CO_2 probe (GMP343)	3 ppm + 1% of reading*	Y	0-1000 ppm

Table 2: Comparison of CO_2 and O_2 sensors

*manufacturers data

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