Micro-Oxymax Layman's Guide to Measurement Principles

Introduction: The Micro-Oxymax Respirometer (Figure 1) measures the production and/or consumption of many gases simultaneously. Data is typically reported as a percentage concentration of each individual gas with respect to the given sample. These percentages are used to calculate other data such as rates and cumulative gas production / consumption. However, the measurement of these simple percentages becomes surprisingly complex when working with such small volumes and concentration changes. The Micro-Oxymax boasts a maximum sensitivity of 0.2µL / hour rate calculation. Automatically, one begins to think about the accuracy of the gas sensors and their ability to accurately measure such small changes in concentration. Though the sensors do play an important role, what really makes the system this sensitive is the "*Closed Loop Measurement Method.*" This can be further enhanced by adding certain options that are specific for the experiment. The following article will describe, in simple terms, how the Closed Loop Measurement Method works, describe the various techniques used by the different gas sensors employed in the Micro-Oxymax System, and break down the optional components for tailoring the system for specific experiments.
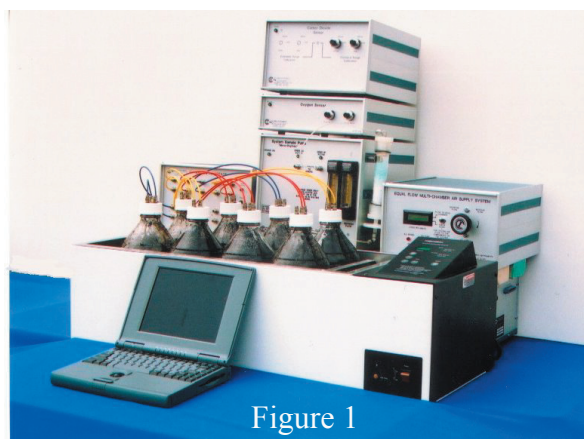
Figure 1

The Closed Loop Measurement Method is such a key principle of measurement that we've patented it in the United States, Europe, and Japan. For reference, the patent numbers are 4947339 (US), 0372429 (EUR), and 2117492 (JAP). The 16 page document is full of mathematical equations and descriptions of measurement procedures that can be difficult for the everyday user to fully understand. In short, the Closed Loop Measurement Method is simply a control mechanism to isolate a sample's head space gas, thoroughly mix it, pass it through gas sensors, and return it to the sample chamber (Figure 2).

Simple enough, but the patent also describes the method by which we calculate concentration percentages. Granted, the gas sensors provide us with an immediate value. However, the value we get from the sensors is not exactly representative of the concentration value within the sample chamber. Simply put, the values given by the gas sensors must be back-calculated to get the corresponding concentration of gas inside of the sample chamber. In order to back-calculate from the concentration of gas in the sensors to the concentration in the Sample Chamber, the volumes of each must be

Figure 2

measured before hand. But measuring these volumes in the traditional manner (length * height * width) is very difficult, especially considering that the volume of the sample must be accounted for in the Sample Chamber; this would be impossible to measure by hand. The Micro-Oxymax utilizes a pressure sensor; by pressurizing various volumes and comparing the pressure readings of known volumes versus unknown volumes, we can
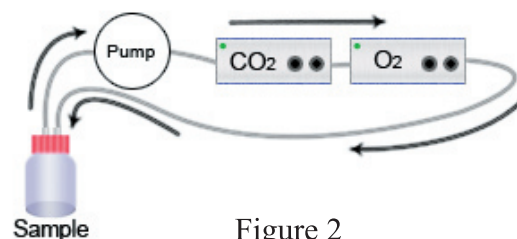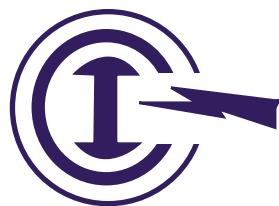
use Boyle's Law ($P_1V_1 = P_2V_2$) to calculate the volumes automatically. But in the effort to avoid all of the math in the patent, its easiest to follow through the 10 basic steps of the 1st measurement cycle. This includes measuring barometric pressure, sensor volume, sample chamber volume, reference air gas concentrations, purge air gas concentrations, sample head space gas concentrations, and calculations of sensor drift.

Figure 3

Before we get into each individual step, its helpful to know what components lie inside of the system and what they do. Refer to Figure 3 at the right for the flow diagram.

Reference Chamber: This is exactly 1L in volume and made of stainless steel. It has two main functions. 1) It is a fixed volume that we use to calculate sensor and sample head space volume using various pressures. 2) It stores a known concentration of air that we use to calculate sensor drift after each measurement cycle.

Analog / Digital Converter: This takes the analog signals from the gas sensors and pressure sensor and converts them to a digital signal. This is needed for 2 reasons. 1) The PC requires digital input. 2) The valves that control air flow through the system receive a digital signal (open or closed).

Manifold and Valves 1 - 8: These simply direct the air flow for each stage of the measurement process.

Pressure Sensor: This measures the amount of pressure passing through the pressure regulator. This is primarily used during the sensor and chamber volume measurements. Recorded pressures are used to calculate volumes using equations derived from Boyle's Law.

Pressure Regulator: This keeps the pressure constant as the gas enters the sensors. Any changes in pressure inside of the sensors would result in erroneous readings.

Pump: Simple enough, the Pump pumps air into, out of, and through the system for all the various functions. Its the quiet humming sound heard when the system is up and running.

Outside of the system are the sample chamber and the gas sensors, which are located in their own separate cabinets. The principles of operation of the sensors will be discussed later. The Sample chamber shown in these diagrams is connected directly to the "System Sample Pump." Most Systems have multiple samples with a multiplexer lying between the System Sample Pump and the Sample Chambers, called an "Expansion Interface." The Expansion Interface plays a passive role during these processes. It is comprised of a system of manifolds and valves that act as an intermediary between each individual Sample Chamber and the System Sample Pump and Gas sensors. In other words, the Analog / Digital Converter and Controller tell the Expansion Interface which sample it needs to measure and the Expansion Interface opens the necessary valves and closes the rest. Since its role is passive, it is left out of the diagrams to more closely relate the System to the sample.
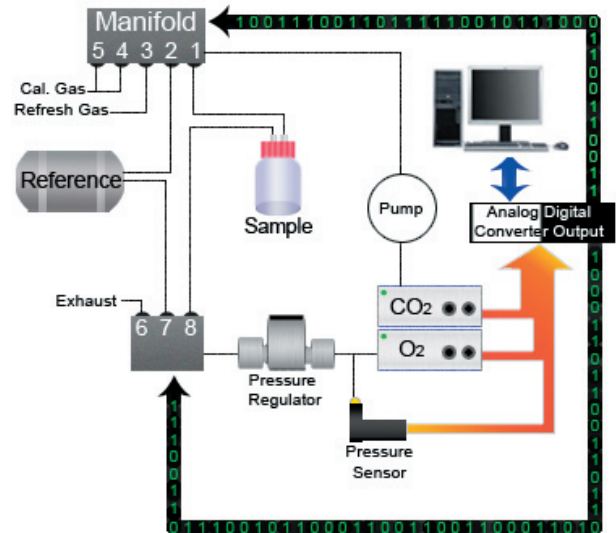
10 Steps of the First Measurement Cycle:

Step 1: The first step is to measure the Barometric Pressure in the room. We open the Reference Chamber, Sample Chamber, and Sensors to the outside environment. The pressure inside the system equalizes with the barometric pressure in the room (Figure 4). The pressure inside the system is recorded. The Sample Chamber and Reference Chamber are opened to the outside since they comprise the bulk of the volume in the closed system.
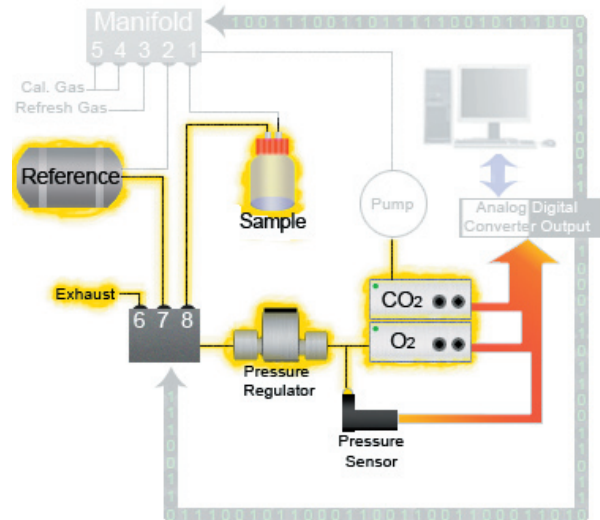
Step 1

Figure 4

Step 2: Measuring the sensor volume is next. The sensor volume is important to know so that the volume can be accounted for and subtracted when the sample is measured. When the gas analyzers report their values, it includes gas concentrations inside the Sample Chamber plus all of the tubing and the sensors themselves. Since concentration is a percentage of a volume, the sensor and plumbing volumes must be subtracted to yield accurate measurements for the sample.

Measuring the sensor volume is a 3 step process. First, fresh air is used to pressurize the sensors to a value about 50 mmHg above the barometric pressure (Figure 5). Once this is achieved, the valve allowing the flow of fresh air (aka. Refresh Gas) is shut of and everything pauses momentarily to allow the pressure to settle. Typically, the pressure will still rise a little bit after the initial pressurization. Once settled, the pressure is recorded. Since this is the second pressure we've recorded, we'll call this P_2 for labeling sake.

Next, the pressurized sensors are allowed to equalize with the Reference Chamber (Figure 6). Once this pressure settles, it is recorded. Typically this pressure is only slightly higher than barometric pressure. The original 50+ mmHg pressure difference within the small sensor volume is greatly reduced when equalized with the 1000 ml reference chamber that is at barometric pressure (from Step 1). We'll call this Pressure P_3.

The final step for measuring the Sensor Volume is the calculation. If we compare: A) the pressurized sensors's pressure value (P_2), subtract the barometric pressure and then
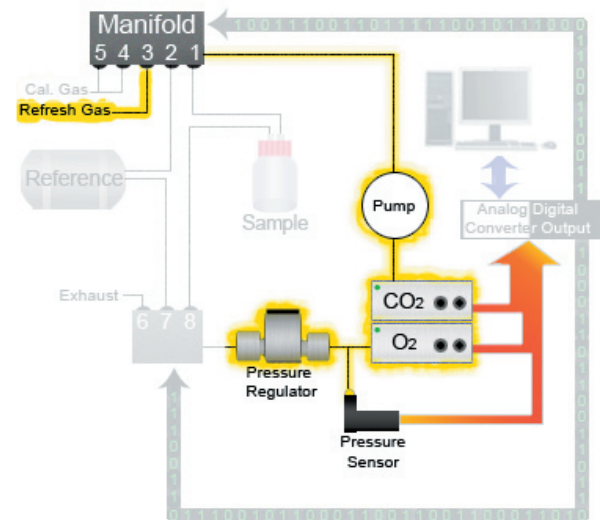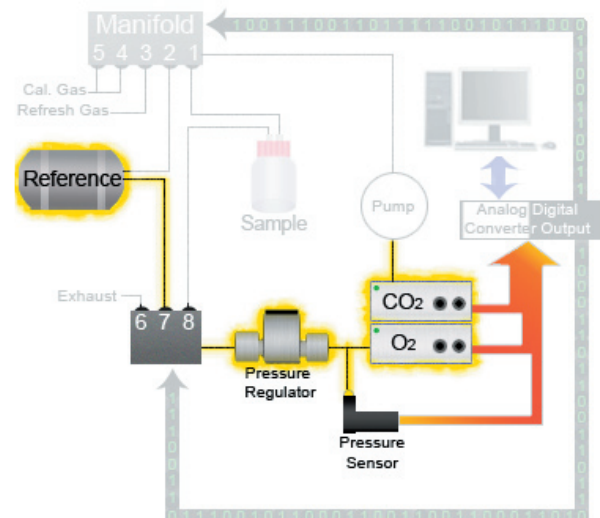
Step 2

Figure 5

Figure 6

compare it proportionally to B) the equalized pressure with respect to the Reference Chamber (P_3) minus the barometric pressure, *and* use the Reference Chamber volume as a known volume, you can solve for the sensor volume using Boyle's Law. Confused? We subtract barometric pressure from the two pressures we record in this step because the reference chamber is already at barometric pressure. Ideally what we want (in order to use Boyle's Law) is to have a single pressure at Volume 1, then take the same mass of air and distribute it to a Volume 2 which gives a different pressure. We happen to know Volume 2 (its 1 L), and we measure both pressures before and after the volume change, so we're solving for Volume 1. By subtracting the barometric pressure, effectively what we're doing is subtracting the mass of air that was already in the Reference Chamber when we mixed them, it cannot be included for the calculation of sensor volume. The actual formula we use, derived from Boyle's Law, is as follows:

$$V_S = \frac{V_R}{\left(\dfrac{(P_2 - P_A)}{(P_3 - P_A)} \right) - 1}$$

...where:

V_S = Sensor Volume
$V_{R'}$ = Reference Chamber Volume
$P_{A'}$ = Barometric Pressure
1 = a remainder from the cancellation of units

Step 3: Now we measure the Sample Chamber Volume. All we do here is basically repeat Steps 1 and 2. First, we equalize the pressure between the Reference Chamber, Sample Chamber, Sensors and room air until they reach barometric pressure. We close all the valves and then begin pressurizing the system with fresh air again. Except this time, we start with the Reference Chamber and Sensors and bring them to a pressure that is about 50 mmHg above barometric pressure (Figure 7). We allow the pressure to settle and record our fourth pressure (P_4). Once this pressure is recorded, we then allow the pressure to equalize with the Sample Chamber (Figure 8). Once settled, we get our fifth pressure (P_5).

Now we're ready to calculate our Sample Chamber Volume. This calculation is only slightly different than the calculation for sensor volume. The difference is that we use the volume of the Reference Chamber *plus* the Sensor Volume for our known volume (we pressurize both the Reference Chamber and Sensors in Step 3, versus the Sensors only in Step 2). Again, using Boyle's Law, we calculate volume with the following derived formula:

$$V_T = \frac{V_R + V_S}{\left(\dfrac{(P_4 - P_A)}{(P_5 - P_A)} \right) - 1}$$

...where:

$V_{T'}$ = Sample Chamber Volume
V_S = Sensor Volume
$V_{R'}$ = Reference Chamber Volume
$P_{A'}$ = Barometric Pressure
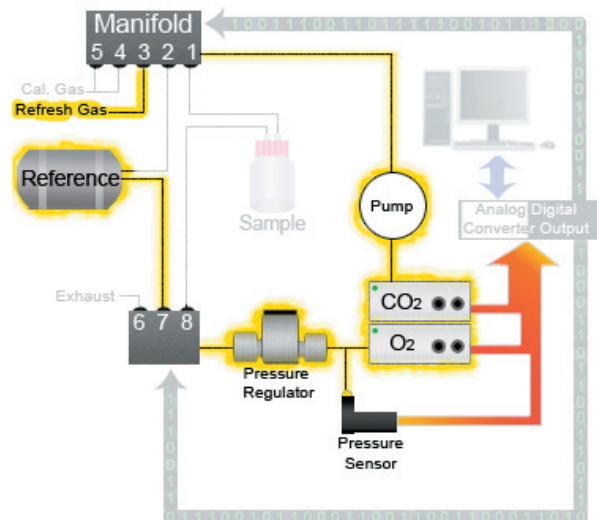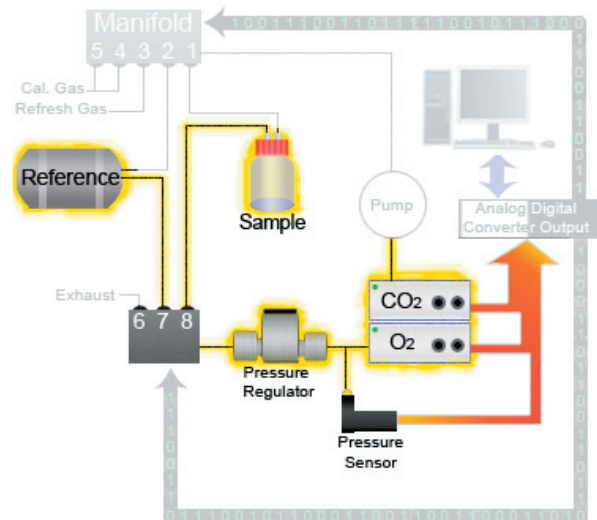1 = a remainder from the cancellation of units

Step 3

Figure 7

Figure 8

Step 4: Now that we have the volumes measured, we adjust them to STP (Standard Temperature and Pressure), and we refresh the Sample Chambers with fresh air (Figure 9). A custom mixed gas can be used instead of ambient air if desired. This can be in the form of a pre-mixed cylinder or a dynamic gas mixer (like our Pegas 4000MF). For multiple chamber systems, the Sample Chambers are refreshed individually so that the sample air between chambers is not mixed. At this time, the reference chamber is flushed with the same air (fresh air / mixed gas).
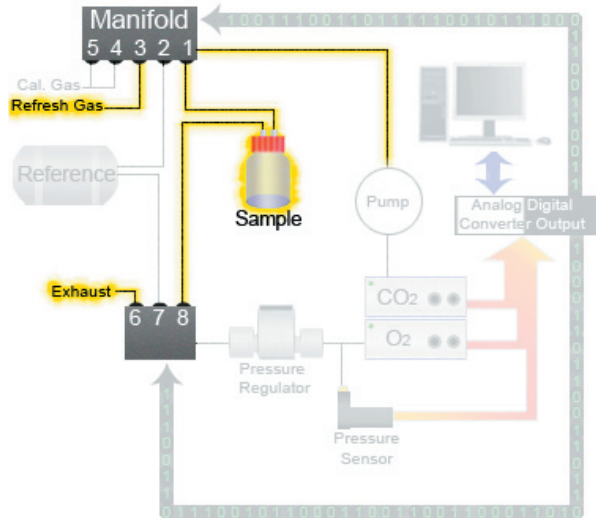
Step 4

Figure 9

Step 5: Next we measure the concentration of the gases inside of the Reference Chamber (Figure 10). The Reference Chamber is still full of ambient air / mixed gas at this point (from Step 4) so that we have a start point for a reference air measurement. The reason we measure the concentration of the gases inside of the Reference Chamber to account for sensor drift (discussed later in step 10).
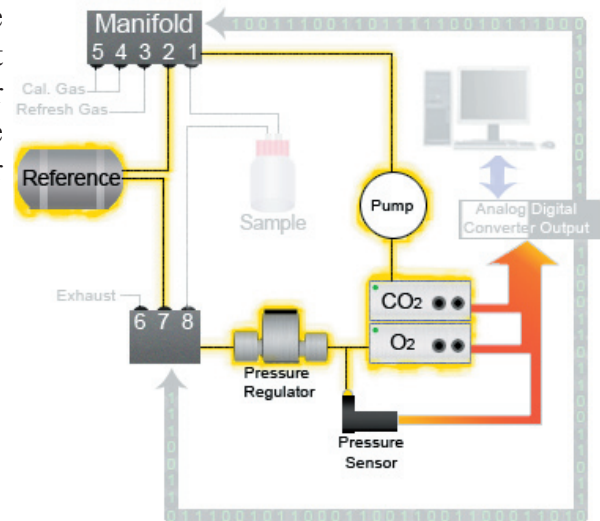
Step 5

Figure 10

Step 6: Now the purge gas concentrations are measured (Figure 11). This step is rather important. In between the measurement of each sample, the sensors are purged with ambient air to remove the residual gas left in the sensors from the previous sample. But when we measure the next sample, the small amount of gas inside the sensors is mixed with the Sample Chamber gas. So the purge gas is measured by the gas sensors so we know what we've mixed in. We later subtract it from the sample's measurement.
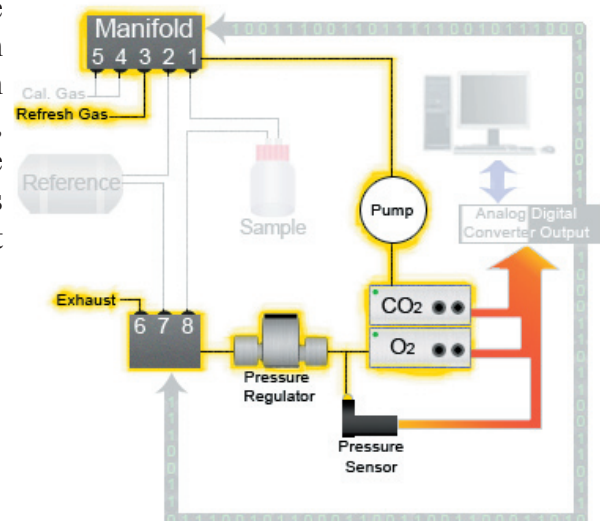
Step 6

Figure 11

Step 7: We can finally measure our sample! The System circulates the gases from the head space of the Sample Chamber through the sensors then re-circulates them back into the Sample Chamber for a factory set time period of three minutes, and takes a reading from the sensors (Figure 12).
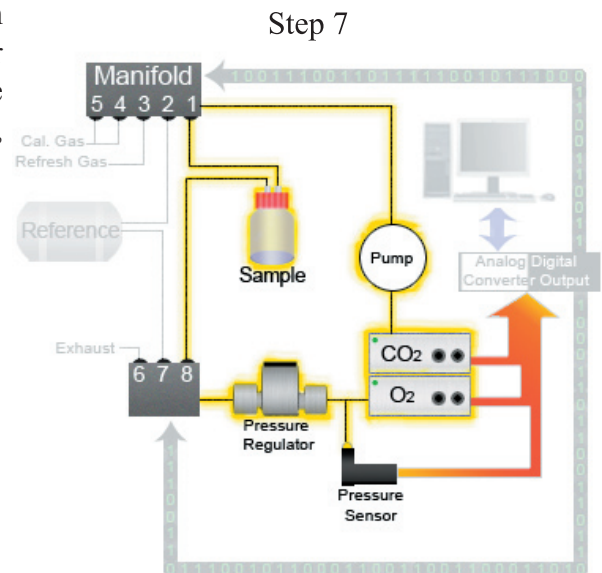
Figure 12

Step 8

Step 8: After we measure the head space of the first sample, the gas sensors are purged to remove any residual sample gas (Figure 13). This is necessary in order to prevent the previous sample's readings to interfere with the next, and to prevent them from mixing back into the next sample's head space.
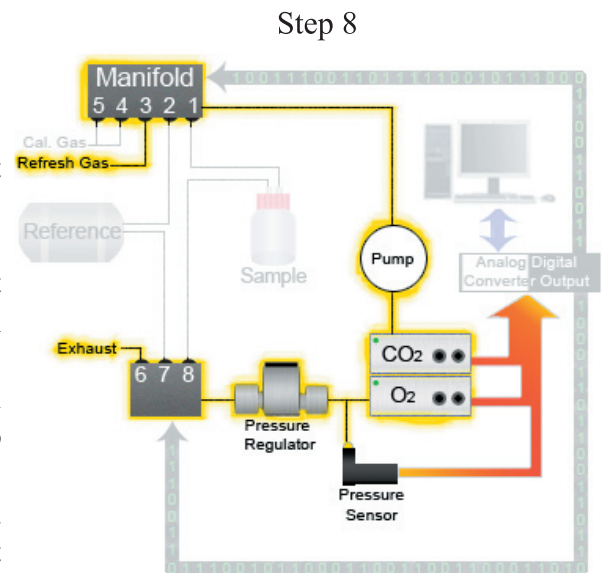
Step 9: After the sensors are purged, we move onto the next sample. We repeat Steps 7 and 8 until all samples have been measured. This gives the first column of data which shows the starting concentrations of each gas, but no rates have been calculated yet. A second data point is needed for this. If "*Auto Interval*" was selected at the start of the experiment, we immediately go back to the first sample and repeat Steps 7 and 8 again. If the "Auto Interval" was *not* selected and a time put in its place, the the System waits for that time before sampling the first chamber again.

Figure 13

Once two samples have been taking, we compare the difference in gas concentrations, multiply by the volume of head space, and divide by the time of the sample interval (Figure 14).
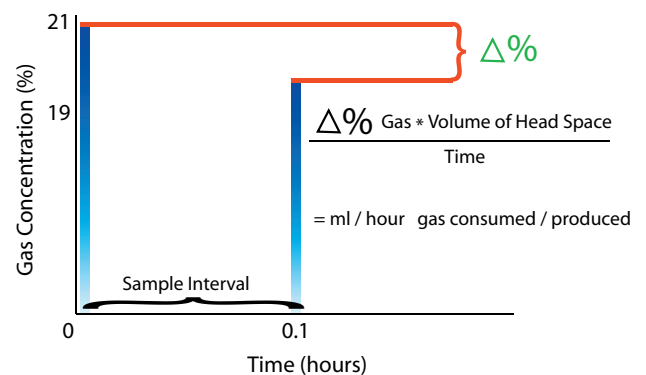

$$\triangle\% \quad \frac{Gas * Volume\ of\ Head\ Space}{Time}$$

$$= ml\ /\ hour\quad gas\ consumed\ /\ produced$$

Figure 14

Step 10: Lastly, we measure the Reference Chamber air again (Figure 15). We compare these numbers to those recorded in Step 5. The difference in the two values (if any) is termed "Sensor Drift." Over the course of a long experiment, the sensors begin to wander off from their calibration setting. Since calibrating in the middle of an experiment is impossible, we measure a known sample of air at the end of each measurement cycle, and see how it compares to itself over time. The values reported for the samples are adjusted to account for drift.

Sensor drift is very slight. From cycle to cycle its not noticeable. But over the span of several days, slight movements in reported values add up and can make a difference by the end of the experiment. Some sensor technologies are more susceptible to drift than others. Electrochemical sensors are the most susceptible. Others, such as Infra Red and Paramagnetic

Step 10

Figure 15

sensors, experience virtually no drift. This is due to the nature of the measurement principles, which we will discuss now.

Gas Sensor Technologies: The Micro-Oxymax uses several types of gas sensor technologies to measure the concentrations of different gases. These include Paramagnetism, Electrochemical Fuel Cells, and Non-Dispersive Infrared detection. Following is a brief description of each and their principle of operation.

Paramagnetism: (Figure 16) This is a physical property of Oxygen that is shared by very few other gases. It exhibits a rather unique behavior within a magnetic field and how it interacts with other objects in that magnetic field. The Paramagnetic Oxygen Sensor consists of a "dumbbell" suspended in a magnetic field by a very fine wire. Sample gas enters the sensor at a very low flow, under 200 ml/minute. As the sample gas passes around the dumbbell, the paramagnetic properties of Oxygen pushes the dumbbell causing it to rotate, which in turn twists the wire suspending it. The rotation of the dumbbell is directly proportional to the concentration of Oxygen present, the more Oxygen, the stronger the rotation. As the dumbbell rotates and the wire is twisted, a small mirror bounces a light source to a detector which tracks the rotation of the dumbbell. A small current is passed through the wire, which brings the dumbbell back to its original position. The more Oxygen present, the more current needed to bring the dumbbell back into position. This current is the signal generated from the sensor.
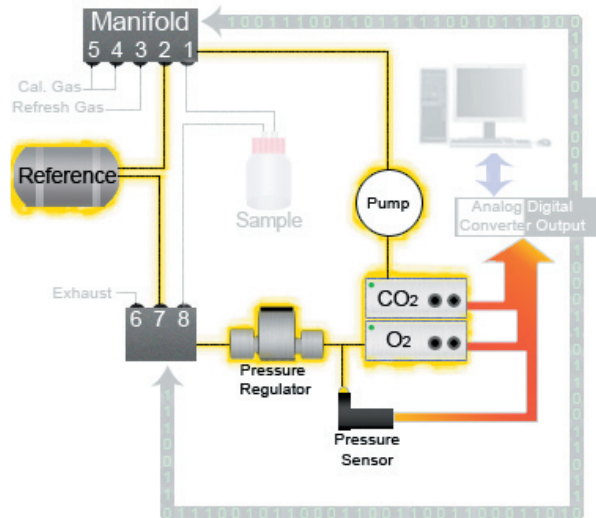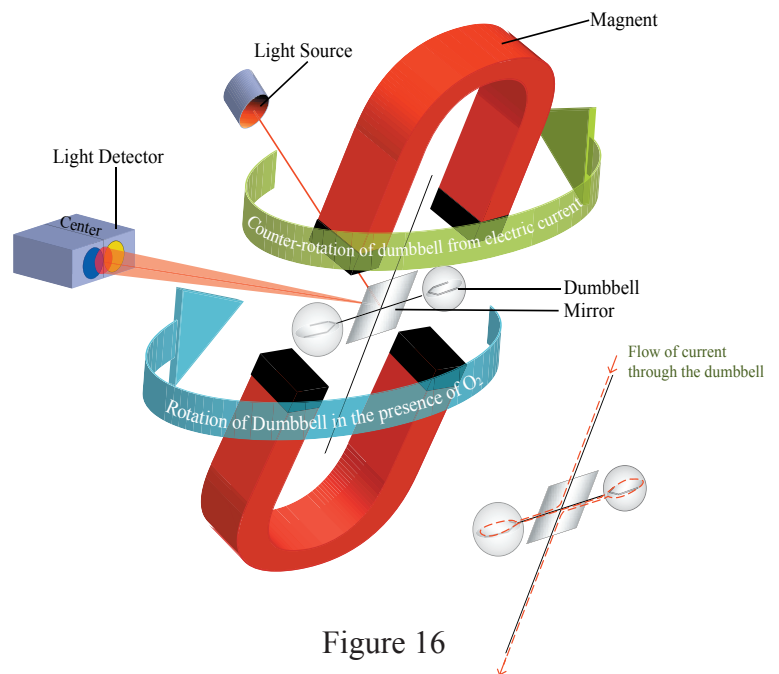
Figure 16

Electrochemical Fuel Cell: (Figure 17) Like the name implies, this sensor is indeed a battery. And just like a battery, it has a finite life span. The gradual "death" of the cell results in sensor drift (which we account for at the end of each measurement cycle). We use it for the detection of Oxygen, Hydrogen, Hydrogen Sulfide, Nitrous Oxide, Carbon Monoxide (2000 ppm), and Sulfur Dioxide. Electrochemical Cells work by chemically reacting to the respective gas present in the sample. The target gas (Oxygen, for example) passes by the cell resulting in a chemical reaction which produces an electric current proportional to the concentration of oxygen in the sample. Electrochemical Fuel Cells consume some of the target gas during the measurement. Therefore, the amount consumed is taken into consideration when making calculations and subsequent measurements.
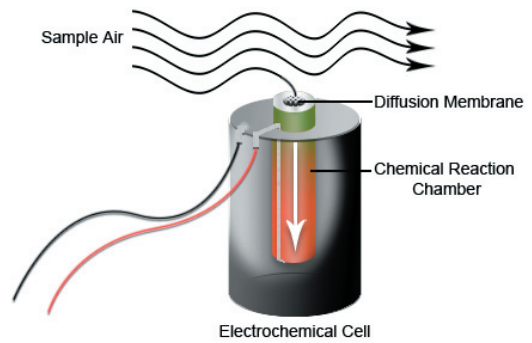

Figure 17

Non-Dispersive Infrared Detection: (Figure 18) This method of measurement is made by simple spectroscopy; emitting a known amount of a specific wavelength of infra-red light through a sample and recording the amount absorbed by the sample. We use this method for the detection of Carbon Dioxide, Methane, and Carbon Monoxide (10%). Each of these compounds absorb a very narrow wavelength range of infra-red light. Each respective sensor emits the corresponding wavelength of IR light, it passes through the sample gas, and a detector records how much of the IR light makes it through. This value is inversely proportional to the concentration of gas in the sample.
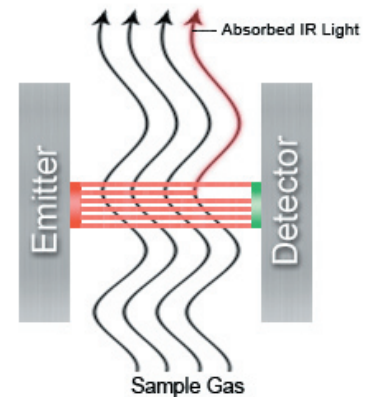

Figure 18

Optional Accessories: Now that we understand the basics of how the Micro-Oxymax works, we can look at some of the options that allow the system to be customized. The Micro-Oxymax is a versitle instrument that can measure respiration of any kind of sample; soil, water, sludge, compost, polymers, anaerobic cultures, etc. But sometimes the nature of the sample requires additional precautions to ensure a good measurement.

Open Circuit Option: For highly active samples, such as activated sludge, compost, or even small animals, the Open Circuit Option (Figure 19) provides the high flow of air needed to keep the sample viable. In a Closed Loop System, these samples could suffocate before the System has a chance to refresh the Sample Chamber. With the Open Circuit Option, fresh air is constantly supplied to the Sample Chamber. Head space gas is sampled by "sniffing" from the flow of gas through the chamber and passing it through the gas analyzers. But in order to back calculate the gas consumption / production rates, the flow of gas through the chamber must be known. Therefore we use a precise flow controller inside and a specially
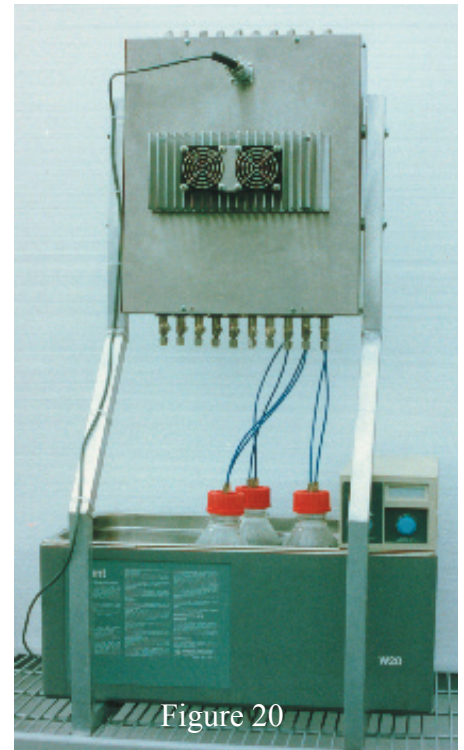

Figure 19

designed manifold to evenly and accurately deliver the exact same flow of fresh air to each chamber. This effectively turns the System into an Indirect Calorimeter, which can be used to measure not only highly active microbial samples, but also animals such reptiles, fish, amphibians, and small mammals.

Condensing Air Dryer: For aqueous or very wet samples, a condensing air dryer (Figure 20) dramitaclly increases the performance of the Micro-Oxymax. This lies between the sample and the standard gas dryer(s) on the System Sample Pump. This chills the sample gas down to about 4°C (dependant upon ambient temperature) in order to condense the water vapor in the sample gas. This has two advantages: 1) The Condensing Air Dryer prevents samples from drying out by returning its own evaporated water back to the Sample Chamber in the form of condensed water 2) This prolongs the life span of expensive chemical desiccant used in the dryer columns. For aqueous samples, this is really a must. The high evaporation rate of water would increase the head space volume in the Sample Chamber resulting in erroneous calculations for concentration and consumption / production rates (we base these calculations on the Sample Chamber volume measured at the start of the experiment).

Figure 20

Anaerobic Setup: For anaerobic samples, we use 2 chambers for every sample (Figure 21). We have the Sample Chamber with a septum in the lid. We insert a syringe into the septum; the tubing then leads to a check valve which allows the flow of air in one direction only. Sample air slowly trickles out of the Sample Chamber, through the check valve, and into a second vessel called the Measurement Chamber. We measure evolved gases from the anaerobic sample as they spill over into the measurement chamber. This setup has three main advantages: 1) The sample is more easily prepared and connected to the system with a simple syringe. This avoids the brief introduction of outside air into the Sample Chamber when connecting the tubes. 2) With the check valve, it is impossible for outside air to enter the Sample Chamber. 3) Chamber "Refresh" can be performed with ambient air rather than expensive bottled gas.

Figure 21
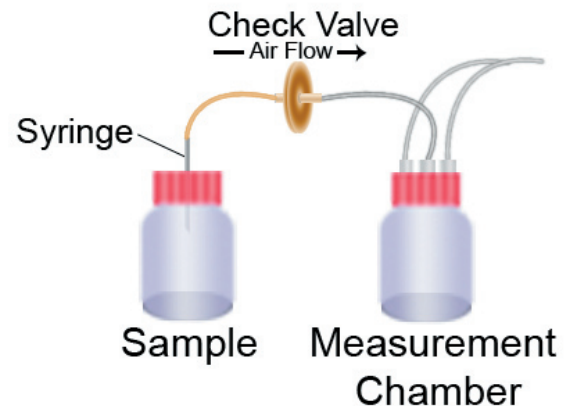
Summary: In short, the Closed Loop Measurement Method can be summurized as: Atmospheric air is introduced into reference and sample chambers, whereupon alternating circulation of the gases in the chambers through gas sensors, a pressure sensor, and a pressure regulator before the gases are returned to their respective chambers. The calculation of gas consumption / production rate by a sample is controlled by a microprocessor which receives signals from the sensors and controls the circulation of gases. Sensor drift is compensated for in the calculation through the use of multiple reference chamber readings and through volume determinations for the reference and sample chambers and the sensors. The gas sensors employed use dependable measurment principles that are applied stragiacally to accomplish the desired work. The system, being built on a semi-custom basis, can be modified to handle almost any nature of sample imagined for respirometric measurments.