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STEM Technology Applied to Biology Labs: Quantifying Cellular Respiration with Sensors and Arduino Microcontroller Technology

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Re-programmable microcontrollers and sensors are an effective way to integrate technology into STEM courses by having students record direct measurements of CO_2 from aerobic or anaerobic cellular respiration. An ideal introduction to instrumentation, this sensing system lacks some known drawbacks of the "black-box" data loggers and expensive pre-fabricated sensors. The Arduino microcontroller connected to a digital component CO_2 sensor enables students to directly measure CO_2 output (ppm) for under $200 (computer needed). A healthy open-source community supports this type of DIY approach to science, including demonstrations of skills needed, like basic programming and basic electronics.

Keywords: respiration, STEM, technology-based learning, sensor, biology, yeast, peas

Introduction

Students will measure rates of cellular respiration and/or fermentation in yeast and germinating peas, and they are given choices of how they might perturb the system by adding additional sugar to the yeast culture. Many cellular respiration lab activities are already available to instructors. What makes our activity different? In this major workshop, we will show participants how students can follow carbon dioxide levels using sensors interfaced with Arduino, or programmable microcontroller technology. This laboratory exercise was developed after years of having students use respirometers made of test tubes and pipets to follow carbon dioxide levels by the displacement of water in the pipette. Faculty (and students) were frustrated with challenges with setting up of the respirometer, the use of caustic chemicals (KOH), and the difficulty relating the results to actual atmospheric carbon dioxide levels in parts per million. Yet, the faculty in our biology program were also not willing to spend our program budget on more advanced automated systems (such the Vernier system). We altered the cellular respiration laboratory two years ago by having students measure carbon dioxide in parts per million using fairly inexpensive Arduino components and sensors. The fact that the microcontrollers and the sensors are visible to the students and are not hidden within a device has given students an introduction to scientific instrumentation that avoids some of the "black box" issues associated with more automated devices. Although we are demonstrating the use of this technology in an introductory lab on cellular respiration, the technology is applicable to other biology laboratory topics and to undergraduate research projects.

The objectives of this laboratory are to:

- Introduce students to the concept of cellular respiration and fermentation, especially the inputs of glucose (or other sugars) and oxygen (in aerobic cellular respiration) and the output of carbon dioxide
- Help students gain a better understanding of the parts per million units of carbon dioxide that are typically reported in scientific reports and the media
- Familiarize students with the major gas components of our atmosphere and their relative abundance
- Introduce students to scientific instrumentation in the form of sensors, microcontrollers and open source code
- Introduce students to the measurement of carbon dioxide at the Mauna Loa Observatory

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- Assist students in making connections between cellular respiration, photosynthesis, and atmosphere carbon dioxide levels
- Encourage students to creatively explore the use of inexpensive sensors and microcontroller technology to develop their own undergraduate research project.

Student Outline

At the level of the whole organism, plants and animals superficially display very different strategies for feeding themselves. On the other hand, at the cellular level, plants and animals (and many other organisms) use identical or very similar processes to extract energy from organic molecules. Aerobic cellular respiration and anaerobic fermentation are two biochemical pathways used by many cells: bacterial cells, single-celled eukaryotes, animal cells, fungal cells, and plant cells.

Metabolic Similarities: Obtaining Glucose, Making ATP, and Releasing CO_2

Aerobic cellular respiration is the process by which most cells (bacterial, protist, fungal, plant, & animal) acquire energy from food molecules in the presence of oxygen. Some cells are also able to obtain energy from food without oxygen. Yeast cells, for example, are capable of extracting energy from sugar in the absence of oxygen through a process called fermentation. Fermentation and cellular respiration are considered *catabolic* processes because they involve the breaking down of a molecule and the release of the energy from the bonds of that molecule. Fermentation and cellular respiration share the following steps:

1. A cell obtains an organic fuel such as glucose.
   How does a plant cell acquire glucose? How does a human acquire glucose? How does a bacterium acquire glucose?

2. The cell extracts energy from the bonds of the glucose (or other organic fuel), and the energy is saved in a more convenient molecule for cellular work called adenosine triphosphate (ATP).
   What biochemical pathways are used by plants to obtain ATP for cellular work? What biochemical pathways are used by animals to obtain ATP for cellular work? What biochemical pathways are used by bacteria to obtain ATP for cellular work?

3. As the glucose (or other organic fuel) is broken down, carbon dioxide is produced. The carbon dioxide is released from the cell.
   How does a plant cell release CO_2? How does a human release CO_2? How does a bacterium release CO_2?

Photosynthesis and cellular respiration are not mutually exclusive processes. Even though plants can photosynthesize, plant cells still need to break down sugars and other organic fuels just as much as animals do. Photosynthesis is a process that creates organic fuels, but in order for a plant cell to obtain sufficient ATP to survive and function, plant cells and other photosynthetic cells such as cyanobacteria and algae must also use cellular respiration to catabolize the sugars made during photosynthesis and to obtain enough ATP for cellular work.

Quantifying Cellular Respiration and Fermentation: Using Sensors and Arduino Technology to Measure CO_2 Levels

Because the release of carbon dioxide is common to both cellular respiration and fermentation, one way to quantify how much and/or how fast these organisms are catabolizing sugar is to measure how much carbon dioxide is released over time.

Respiration:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{heat}$$

Measuring the quantity of carbon dioxide is challenging because carbon dioxide is an odorless and colorless gas. Thankfully, many substances that are difficult for humans to see can be measured electronically using sensor technology. Gaining a basic understanding of sensors is useful for anyone pursuing a career in the biological sciences.

The parts per million (ppm) units that you will use to measure carbon dioxide today are the same units that are used by scientists around the world to measure the levels of carbon dioxide in our atmosphere. Scientists use parts per million (ppm) to describe concentrations by volume in the atmosphere or the air. So, if you were able to grab a volume of air that

contained 1 million molecules, and 400 of those million molecules were CO_2, then you would have a CO_2 concentration of 400 ppm in that volume of 1 million molecules. *In this same scenario, what would the % CO_2 be?*

Microcontrollers, Open Source Code, and Sensors

We will measure carbon dioxide levels using small inexpensive computers called microcontrollers, open source code, and sensors. The phrase "open source" generally refers to information that is available to anyone and can be modified and used by anyone. Our microcontrollers are programmable, meaning that we can re-write any kind of code that we desire. The code that we are using in lab allows the information to be detected by the CO_2 sensor, which is then communicated to the microcontroller. The microcontroller programmed with specific code to communicate with the CO_2 sensor converts the information from the sensor to produce a reading on the laptop that shows carbon dioxide in parts per million (ppm). Our microcontrollers were manufactured by Arduino (Italy), but other small programmable computers exist, such as the very small Raspberry Pi, produced by an educational charity called the Raspberry Pi Foundation. Many hobbyists use these inexpensive programmable microcontrollers and sensors that we are using today for a variety of projects (blinking LED lights, talking clocks, and a laser harp, to name a few). By incorporating different types of sensors, undergraduate students can use microcontrollers to inexpensively measure a variety of environmental variables in the laboratory.

The purpose of this lab is not only to expose you to cellular respiration and fermentation, but also to give you a sense (pun intended) of the ingenuity and creativity that scientists use as they bring together modern computer and sensor technology with biological concepts to answer challenging and important questions.

Questions

1. Describe the composition of the Earth's atmosphere. What are the three most abundant gases in the atmosphere?

2. What units are atmospheric carbon dioxide levels usually measured in? Explain that unit so that a teenager at a local high school could understand it.

3. The Mauna Loa Observatory (MLO) in Hawaii has state of the art technology to measure CO_2 levels in the atmosphere. Put the correct label on the Y axis below:

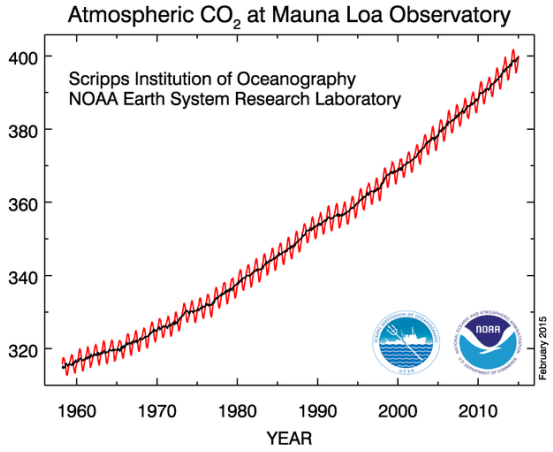

Figure taken from: http://www.esrl.noaa.gov/gmd/ccgg/trends/
For more information on the Mauna Loa Observatory:
https://www.youtube.com/watch?v=KD3-_5_Y1RA

4. Fill in the table below and answer the question below:

	Atmospheric CO_2 levels (please include units)
1960	
2015	

Are you surprised by the difference in CO_2 levels between 1960 and 2015? What explains the difference?

5. Calculate the percent of the air that is carbon dioxide using the above numbers. **Please show your work!!!** Look up the percentage of oxygen and nitrogen in the atmosphere and sketch a table showing the percentage of all three gases (carbon dioxide, oxygen, and nitrogen).

6. Notice the jagged yearly fluctuation in CO_2 levels at Mauna Loa. Over a typical year, the CO_2 in our atmosphere changes. What causes the fluctuation in CO_2 within one year? What months do you predict that the CO_2 is the highest? The lowest?

7. Write the overall net reaction for cellular respiration.

8. In order to grow, a seed must catabolize sugar. What is the source of the sugar catabolized by the peas? (Do you think the peas are photosynthesizing at this point in their life cycle?)

9. What variables do you think affect the respiration rate of seeds? Brainstorm within your group to generate a list of at least three factors that could affect seed and/or yeast respiration.

10. What does it mean when scientists say that yeast are "facultative aerobes" but humans and peas are "obligate aerobes"?

11. Many, many strains of yeast exist – for example you can buy bread yeast, wine yeast, and beer yeast. Do you think it will matter what strain of yeast we use today? Why or why not?

12. What is the difference between fermentation and aerobic cellular respiration? When yeast use fermentation what do they make in addition to CO_2? Do any human cells use fermentation? (If yes, what do they produce in addition to CO_2?)

13. We will be monitoring CO_2 with a sensor. Why is it important for students majoring in biology to know a little bit about sensors? In what ways are sensors used in the health field? In what ways are sensors used in ecological and environmental sciences? Try to be as specific as possible as you answer these questions.

Methods

Setup – Monitoring Wet and Dry Situations with Sensors

Each group of four students will monitor cellular respiration in both peas and yeast. Because the yeast are being grown in a liquid and are "wet," the yeast vials will be attached to the large ring stand so that they don't spill or contaminate the sensors. On the other hand, the peas don't pose a spillage risk and are generally "dry" and so you will be placing the peas in much closer contact with the sensors. The boxes with the sensors are set up a bit differently because of the spillage issue, and we have labeled the sensor boxes that you will use with the peas as "dry" and the sensors that you will use with the yeast as "wet."

For the peas: The peas go into a vial and will be placed directly into the "dry" sensor box.

- Put about 10 germinating peas at the bottom of a vial, open the "dry" sensor box, and cap the vial with the rubber stopper, which has a tube connecting directly to the carbon dioxide sensor.
- The vial of peas and the CO_2 sensor are housed inside the pencil box chamber, and are connected to the Arduino microcontroller, which interprets and transmits the data to the computer. Please look at the pictures and/or ask your instructor.
- Begin recording CO_2 levels in parts per million every 2 minutes for a total of 15 minutes.
- After 15 minutes of data monitoring, graph your data, making sure that you know the independent and the dependent variable.

For the yeast: This yeast solution that you will use today has very little sugar in it, just enough to allow the yeast to begin to become active. You will be using the yeast *Saccharomyces cerevisiae*. Different varieties of this yeast species are used in baking bread and in making wine and beer.

What characteristics do you think would make one strain of this yeast species good for baking bread and another strain good for making wine? Do you know anyone who makes beer or wine? From where do they obtain their yeast cultures?

Your group needs to decide before you begin what organic fuel you will add to the vial. You may choose glucose, sucrose, or sucralose. At the time indicated in the instructions below (4 minutes), you will add 1 gram of the organic fuel to the yeast solution.

Other groups will likely choose different organic fuels than your group. Do you predict that your yeast will respire more or less than other groups?

- Fill the vial with 25 mL of freshly made yeast solution and attach the vial with the yeast to a ring stand, making sure that the clamps are tight enough that the vials won't fall and spill.
- Cap the vial with the rubber stopper and make sure that the long tubing is going from the stopper on the vial into the box on the table. Keep in mind that the sensor is in the box on the table and it may take time for the carbon dioxide to fill the entire tube and for the levels inside the box to change (be sure that the pencil box is closed!).
- **Before adding the sugar**, begin recording CO_2 levels in parts per million every 2 minutes for a total of 4 minutes.
- After recording the CO_2 levels for 4 minutes, open the rubber stopper and add the sugar (1 gram) to the yeast.
- Record CO_2 levels for another 20 minutes. Graph your data, making sure that you know the independent and the dependent variable.

All groups will put their data on the graph space on the board in front of the class. There will be two graph spaces: one for peas and one for yeast. Once you have completed the data collection, wait for an opportunity to put your data on the graph space on the board. In order to best see the relationship within groups, you may connect the data points. On your yeast data lines, write the type of organic fuel that your group used. Once all the groups have graphed their data on the board, we will discuss the patterns and whether they agreed with your predictions.

What organism did you focus your attention on today?

Question - What question did your group decide to ask?

Hypothesis –Using your questions above, state the hypothesis that you and your group chose to explore today.

Procedure - What are you measuring?

What is your independent variable?

What is your dependent variable?

Data Analysis - What happened? Did you observe anything that surprised you? Please graph your data using the format explained by your instructor and attach it to this handout. What patterns do your graphs show?

Conclusion – State your conclusion and refer to patterns in your graph to support it

Materials

See Appendix A for a list and sources of components listed here and Appendix B for assembly instructions.

Six fully assembled kits are needed for a lab class of 25 students. One kit includes:

- 2 laptop computers loaded with the Arduino IDE and K30 sensing code (OS: Windows, Mac, or Linux)
- 2 Arduino programmable microcontrollers (UNO-R3)
- 2 protoshields with pre-soldered Ethernet (cat5 or cat6) connectors
- 1 K30 CO_2 sensor with pre-soldered Ethernet (cat5 or cat6) connectors (for yeast)
- 1 K30 CO_2 sensor fitted with a tube cap adapter and auto vacuum tubing, along with pre-soldered Ethernet (cat5 or cat6) connectors (for peas)
- 2 Ethernet (cat5 or cat6) cables, 1 ft long
- 2 pencil boxes (holes made for yeast and pea connections – Appendix B)
- 1 18"ring stand with medium clamp
- 2 centrifuge tubes (50-mL)
- 2 stoppers (size 6), fitted with bent class elbows
- 1 – 18" section of PVC tubing (size ¼" ID x 1/16" wall)
- 1 package of Fleischmann's ActiveDry Yeast (original) (3 packages for full lab of 25 students)
- Sugar sources for yeast (Sucrose, dextrose, sucralose, etc.)
- 15 peas (soaked for 24 hours starting 72 hours before lab, rinsed, and left with some water in dark area)

Notes for the Instructor

This is a modification of the measurement of CO_2 production from aerobic (germinating peas) and anaerobic (yeast) systems. Instead of capturing the CO_2 and measuring a response from the build-up of CO_2 in a closed system, the detection system is the K30 CO_2 digital sensor paired with an Arduino microcontroller programmed and interfaced with a laptop. The microcontroller and sensor can detect CO_2 concentrations from 0 to 10,000 ppm, which is sensitive enough to detect the slower respiration rates of germinating peas, but tolerant enough to accurately measure the high yield of CO_2 produced by the anaerobic yeast. The main components of the sensing system include a laptop loaded with the Arduino software (Arduino IDE from Arduino.cc), a USB cable, the Arduino Uno – R3 (programmable microcontroller), the K30 CO_2 sensor, and a modified pencil box. There are other structural components to support the electrical circuit that are not mentioned here, but are detailed in Appendix A (list of supplies needed), and instructions for constructing the sensing system are included in Appendix B.

The lab can be arranged in a variety of ways. Students can work in groups of four, with two students monitoring germinating peas, and the other two students monitoring yeast. This configuration is how this lab is presented here. Alternatively, groups of students could also choose which biological system to monitor using the sensing system. The configuration will depend upon how many students are in the class, and how many complete sensing systems are available for them to use.

The Methods and Setup section describe the lean yeast solution that should be made and allowed to sit for 10 min before the students perturb the system by addition of energy sources to change the respiration rates. Students can choose which kind of "sugar" in solution to add to the yeast in the centrifuge tube. Their choices will depend on what you have on hand, but we have tried sucrose, dextrose, and Hawaiian Punch. Students are given 10 mL of the lean yeast solution, and they measure and add 10 mL of the sugar of their choice and monitor the direct effect of yeast growth. Once the sugar is added to the centrifuge tube, the rubber stopper with tubing is placed on top of the tube. This tubing is a combination of glass and PVC tubing which connects the centrifuge tube that contains the yeast to the sensing chamber (modified pencil box), where the K30 CO_2 sensor is housed. After a short period of time (10-15 min, or less), carbon dioxide from respiration travels from the centrifuge tube through the tubing and to the sensing chamber. Data collection by the K30 sensor and the Arduino microcontroller are read every 2 seconds and displayed on the computer that is running the Arduino software. Unresponsive CO_2 levels could be due to expired yeast culture or other experimental effects (see the Crabtree effect).

The peas that germinated for 72 hours are added to the centrifuge tube, capped with the stopper with the tubing connecting it to the direct measurement adapter on the K30 sensor, and placed in the sensing chamber (modified pencil box). The direct measurement chamber differs from the yeast chamber only by a special fitting on top of the K30 sensor to more directly measure the CO_2 from the peas. The cellular respiration that is occurring from the germination process (sugars are mobilized and used to initiate cell growth of the radical) produces CO_2. After a short period of time (10-15 min, or less), carbon dioxide from respiration travels from the centrifuge tube a short distance to the K30 sensor by way of the direct measurement adapter on the K30 sensor. Data collection by the K30 sensor and the Arduino microcontroller is read every 2 seconds and displayed on the computer that is running the Arduino software.

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Setup and Methods

Study Organism Setup

Respiration or CO_2 output can be measured for other organisms, including mealworms (larvae and adults), waxworms, superworms, alfalfa, and *Chlorella* (an algae – use light/dark to observe an effect of photosynthesis), but our focus is on peas and yeast. The peas are started 3 days prior to the experiment, while the yeast mixture is created within 10 minutes.

Peas

Three days prior to the lab meeting (72 hours), soak dried peas (15 – 20 peas per group of 4) in distilled water for 24 hours. Rinse the peas well, and place in a shallow dish lined with paper towels that are moistened with distilled water. Put in a dark cabinet or cover with dark red fabric for the remaining 48 hours, checking at least daily that there is some water at the bottom of the shallow dish.

Yeast

Student groups should be provided with pre-measured dry ingredients for the "lean" yeast mixture (see recipe below). The dry ingredients (yeast and sugar) fit well into 1.5-mL microcentrifuge tubes, and each student group can be given a 10-mL graduated cylinder and a small 50-mL beaker of distilled water in addition to the pre-measured dry ingredients. The "lean" recipe has a small amount of sugar to start yeast growth and respiration, but not too much sugar as to cause the yeast growth and division to occur too quickly. Multiply this recipe by the number of groups you will have in your class. Students should pour the water, yeast, and sugar into a 50-mL centrifuge tube. The students should gently swirl the tube and begin taking carbon dioxide measurements every 2 minutes. After about 10 minutes (may need to be shortened or lengthened depending on the whether the carbon dioxide levels are rising quickly or slowly), students can perturb the system by adding their choice of additional sugar, a sugar substitute, or other options provided by the instructor. Instructors are strongly encouraged to test the "lean" recipe before class, as classroom conditions and the idiosyncrasies of the design of the sensor chamber can influence the speed of increase in carbon dioxide levels recorded by the sensor.

"Lean" Yeast Mixture (multiply preparation by the number of groups)

0.55 g of Fleischmann's Active Dry Yeast
1.05 g of sucrose
25 mL of warm tap water

(in our lab, it takes 45 - 50 min for a noticeable increase in [CO_2], and about 60 min to double the CO_2 output from ~446 ppm to 827 ppm)

*If you are teaching multiple sections, you may consider having a new package of yeast for each section.

Sugar Mixtures

Prepare solutions of Sucrose, Dextrose, Sucralose

Sensing System Setup

See Appendix B for a description of how to assemble the CO_2 sensing system from components.

1. Install the protoshield with the cable connector onto the Arduino, ensuring that the labeled pins on the shield line up with the same pins on the Arduino.

2. Connect the Ethernet cable to the CO_2 sensor and to the protoshield. Connect the USB cable to the Arduino.

3. Start computer and open the Arduino IDE. Open the K30 sensor code (see Appendix B). Verify and upload the code to the Arduino.

Sensing System- Data Collection -Begin Sensing

See Appendix B for additional details.

Plan your lab procedure (lecture on background, etc.) accordingly so that the yeast has had only about 10 – 15 min to start. Any longer and it will process all the sugar and likely become too slow, or will die.

1. Once the code is successfully uploaded to the Arduino, the LED light on the K30 sensor should be flashing, and the LED light on the Arduino will also be flashing. Also, there will be no orange text or messages below the code in the divided window below.

2. Open the Serial window to see the CO_2 readings from the K30 sensor. Students can take readings at any time interval, as the default code is setup to take a reading from the K30 CO_2 sensor every 2 seconds. A reading every 2 minutes has produced interesting results and keeps the students engaged in the experiment. Have the students take a background reading at this point. CO_2 concentrations can vary across small spaces, so it's important that each student group records the baseline [CO_2] for their group.

3. Apply the sensor to the live material:

A. Peas: Load the peas into the centrifuge tube and connect and place the stopper on the tube. This stopper is already connected to the tube cap adapter and the K30 sensor. Place the full unit into the pencil box with the Ethernet cable coming out of the box and leading to the Arduino.

B. Yeast: When ready (10 minutes after start), load 10 mL of yeast solution into the centrifuge tube, followed by the student's choice of energy source that has been pre-mixed (sucrose, dextrose, sucralose). Place the centrifuge tube in the clamp on the ring stand. The clamp should be at least a few inches above the sensing chamber. The stopper that has a glass elbow connected to PVC tubing that leads into the chamber (pencil box) can now be placed on the yeast tube. The pencil box chamber houses the sensor only. The Ethernet cable that connects the Arduino to the sensor comes out of the box, meeting the Arduino outside the box.

The response by the peas will take at least 15 minutes due to the need for CO_2 concentrations in the sensing system to build. CO_2 must build to change the concentration in the tube and in the pencil box housing the CO_2 sensor. Likewise, the response by the yeast will take some time, but far less depending on how much sugar was added to the lean yeast mixture. Longer connecting tubes from the test tube and larger pencil boxes will obviously extend the amount of time to see a response because the CO_2 concentrations will have to build longer to cover more distance (longer tube) and fill more volume (larger box).

Sensing System Disassembly

All items fit in the pencil box for storage.

1. Disconnect the USB from the computer, save any data that you would like to save, and shut down the computer. It is recommended that you save the K30 code in a durable location so that you have functional code to work with your equipment, should the code (or the company) disappear.

2. Disassemble the Ethernet cable from the protoshield and from the sensor.

3. If your programmable Arduino microcontrollers are used for other experiments, you can remove the protoshield from the top of the Arduino.

Useful Links

For more information on the Mauna Loa Observatory:
https://www.youtube.com/watch?v=KD3-_5_Y1RA

CO2METER.COM Documentation:
http://www.co2meters.com/Documentation/AppNotes/AN102-K30-Sensor-Arduino-I2C.pdf

ABLE2016 Workshop Materials – supporting videos on CO2 and sensor implementation
http://study4mulcahy.weebly.com/able2016.html

STEM-Sense Video:
https://youtu.be/iJk3LAGhgFg

STEM-Sense – Sensors and Biology
https://stemsense.wordpress.com/

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Appendix A
Sources of Components and Prices (Nov 2015)

ABLE: 25 students (6 groups: 25 students plus a demonstration)						
Parts List - Respiration						
Each Group Needs						
Number	Approx Pric	Part #	Description	Total	Vendor	Website
4	$1.5	PRT-00643	RJ45 8-Pin Connector	$6.0	SparkFun	www.sparkfun.co
4	$1.9	BOB-00716	SparkFun RJ45 Breakout Boards	$7.8	SparkFun	www.sparkfun.co
2	$2.0		Cat6 Computer Cable (1 foot) (AKA	$4.0	Computer	
2	$24.9	DEV-	Arduino Uno - R3 (programmable	$49.9	SparkFun	www.sparkfun.co
2	$1.5	PRT - 00116	Break Away Headers - Straight	$3.0	SparkFun	www.sparkfun.co
2	$4.9	DEV- 11665	Arduino ProtoShield - Bare PCB	$9.9	SparkFun	www.sparkfun.co
1	$0.8	WALMA RT#:	Storext Pencil Box/Case (clam shell)	$0.8	Walmart	
2			Pvc tubing (1/4" ID x 1/16" wall), 18 "		Scientif ic	
1	$0.7	73104	Bent glass elbow, 6mm OD	$0.7	Carolina Biological	
2	$0.6	71243	Rubber stoppers, single hole, size 6	$1.3	Carolina Biological	
2	$0.7	43029	Corning Centrifuge Tubes (50 mL)	$1.5	Corning	
2	$0.3	4743	Auto vacuum tubing (3/16" to 1/4"), 4 inches	$0.5	Autozone	
1	$16.2	SB14023M	Ring Stand, Rectangular Support - 4" x 6-3/4" Base, 5/16" x 18" Rod Size	$16.3	NASCO	www.enasco.com
1	$39.0	SE-0116	K30 Sensor Tube Cap Adapter	$39.0	CO2Sensors.c	
2	$85.0	SE-0018	K-30 10,000 ppm CO2 Sensor	$170.0	CO2Sensors.c	
1	$16.9	PRT-11367	Hook-up Wire - Assortment (Solid Core, 22	$16.9	SparkFun	www.sparkfun.co
1	$7.9	TOL-09325	Solder Lead Free - 100g Spool	$7.9	SparkFun	www.sparkfun.co
2	$9.9	TOL-09507	Soldering Iron - 30W (US 110 V)	$19.9	SparkFun	www.sparkfun.co
			Peas			
			Yeast			
			Sucrose			
			Sucralose			
			Dextrose			
			Total	$355.5		
			Total for 6 groups	$2,133.4		

Appendix B
Assembly of Component and Chambers

Overview: The laptop is used to upload code to the Arduino, and this set of code is referred to as a sketch. Once the sketch has been verified for errors, it is uploaded to the Arduino microcontroller with the K30 sensor already attached. The laptop receives and displays the data collected by the K30-CO2 sensor and Artuino. The basic electronics underlying this process include power (5V) and ground (GND) as well as data transmission (TX) and data receive (RX). So there are essentially two circuits: energy to power the system (5V and Ground), and a data circuit to receive data from the sensor (TX and RX). The description below outlines the setup of the sensing system.

Figure 1. Yeast sensing system.

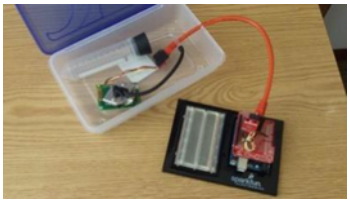

Figure 2. Pea sensing system.

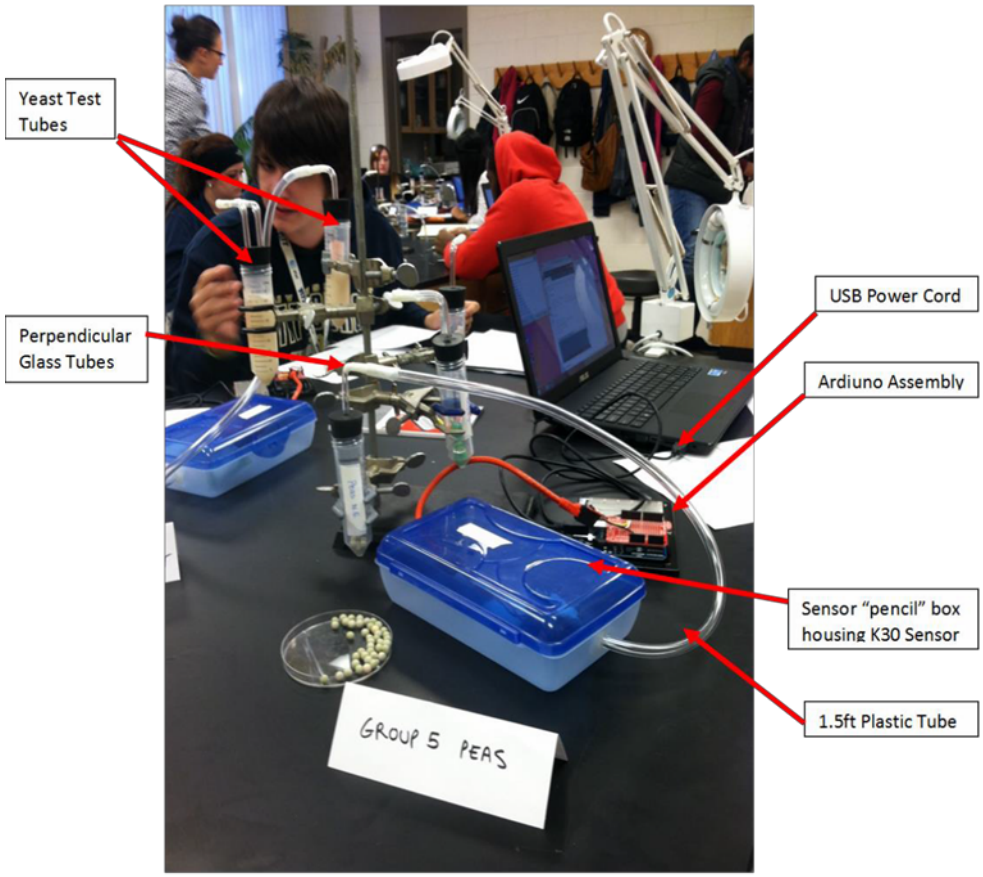

Figure 3. Cellular Respiration Laboratory, University of Pittsburgh at Bradford, Fall 2014.

Building the Arduino-K-30 Sensor System: 2 sensor systems are needed for each student group

- Heat the soldering iron, and practice soldering; watch YouTube videos to practice if you have never soldered before. To avoid damaging the K30 sensor or the Arduino, the continuity of every solder connection created should be tested using a multimeter (<$10 at Walmart). See YouTube.
- Solder the breakaway headers to the protoshield in the positions needed to meet with the Aurino's 5V, GND, TX (pin 13 on Arduino), and RX (pin 12 on Arduino)

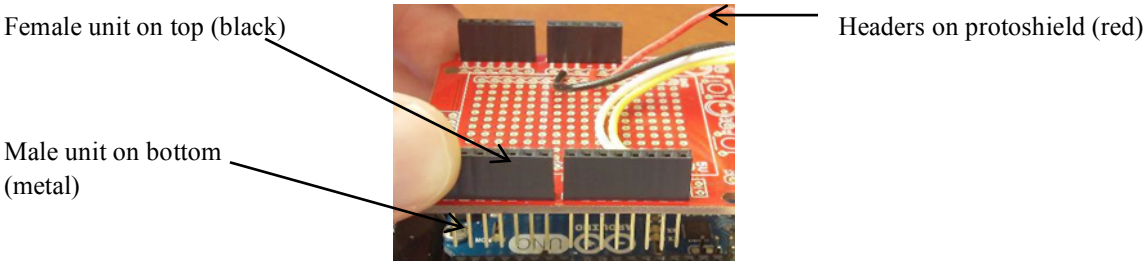

Figure 4. Stackable headers (1 unit : female on top –black and male on bottom - metal) need to be soldered to the protoshield (red).

- RJ45 Breakout Boards and Protoshield
  - Set up a map of connections for the SparkFun RJ45 Breakout Boards to consistently allocate a position for each of these connections on the RJ45 (**must be the same every time**):
    - 5 volt (red)
    - Ground (GND=black)
    - Transmit (TX=(yellow)
    - Receive (RX = white or orange)

Figure 5. Assembling the breakout boards (red board).

- Solder wires to **two** SparkFun RJ45 Breakout boards to the designated positions and colors:
  - 5 volt (red)
  - Ground (GND=black)
  - Transmit (TX=(yellow)
  - Receive (RX = white or orange)

- Connect a RJ45 Pin connector to the RJ45 Breakout boards by pressing the connector into the board.

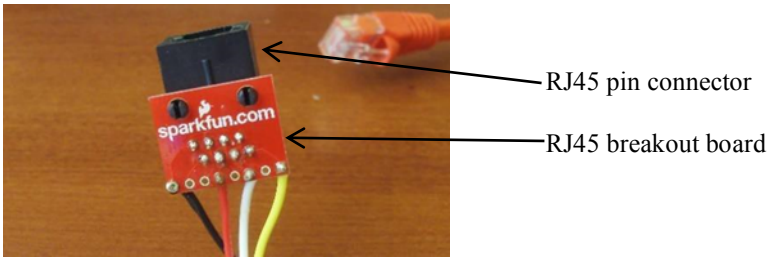

Figure 6. RJ56 Pin connector (black) and RJ45 Breakout Board (red).

- Repeat with the other RJ45 connector and board.
- Use one of the RJ45 breakout boards and solder the 4 wires to their respective holes on the Protoshield (5v, GND, TX, RX)

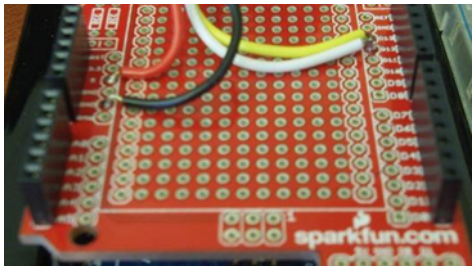

Figure 7. Soldering wire connections to the protoshield (red).

- The other RJ45 board will be soldered to the K30 Sensor. See the K30 data sheet for the placement of the wires on the K30 sensor (CO2sensor.com – K30 datasheet). Solder the sensor last after you have had a chance to practice soldering. Leaving the soldering iron next to or on the K30's circuit board can damage it. Use caution here.

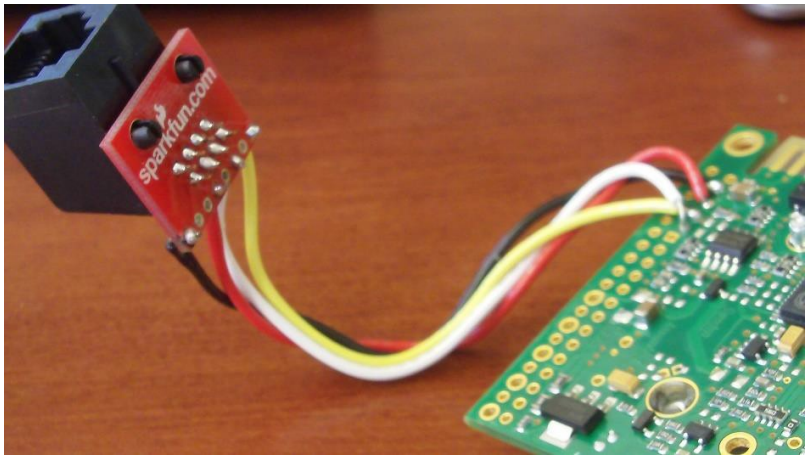

Figure 8. Attaching the assembled RJ45 board to the K30 sensor.

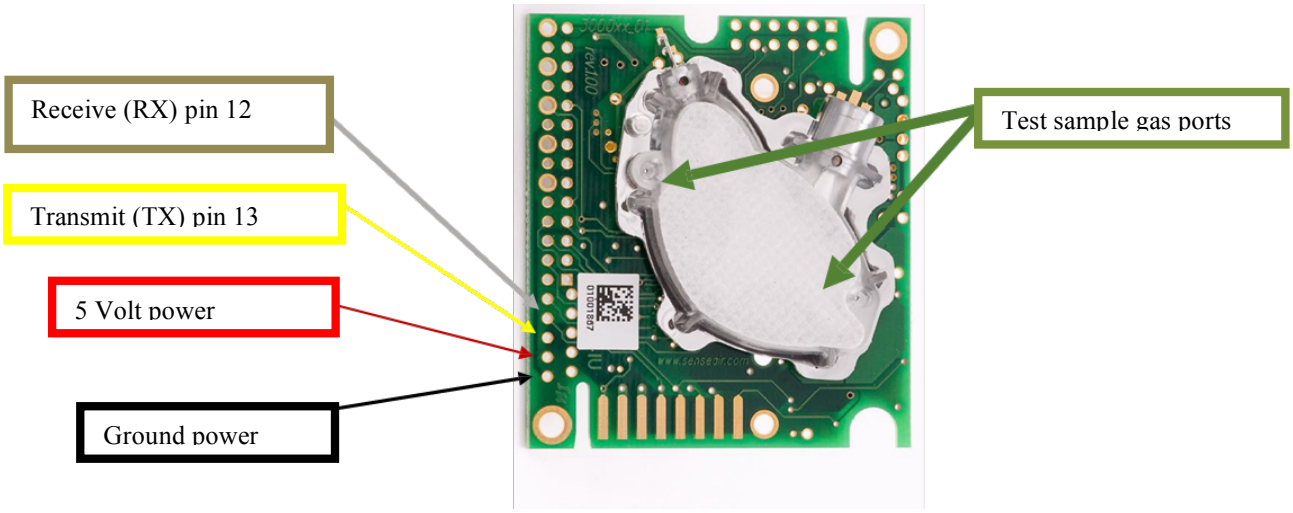

Figure 9. K30 CO2 Sensor (Basic model) and the location of the wire connections.

- Repeat this process and create another set of connector-protoshield, and connector-K30 sensor components. Add the K30 Sensor Tube Cap Adapter to a K30 Sensor. Fit a 3-4 inch length of auto vacuum hose onto the nipple of the adaptor, and fit the other end over the glass elbow tube. (BE CAREFUL!!! Do this in a manner as to not break the glass or puncture your finger while pushing the tubing over the glass rod).

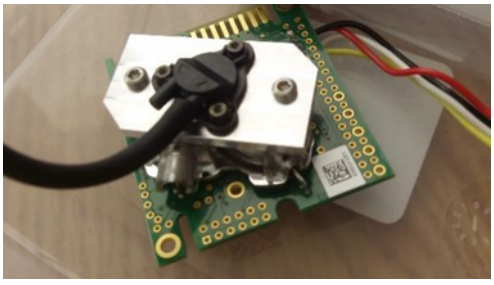

Figure 10. The K30 Tube Cap Adaptor (metal plate with blackturret/nozzle).

Figure 11. Setup in pencil box for direct measurement with the K30 Tube Cap Adaptor.

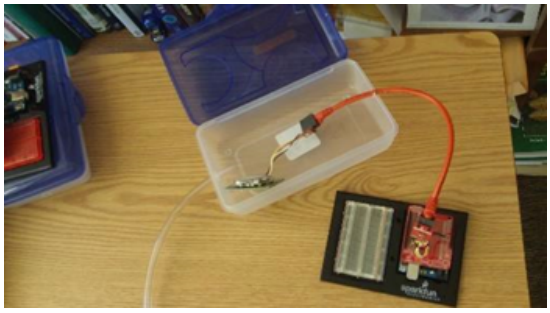

Figure 12. Setup to measure yeast. The CO_2 will enter and fill the pencil box and be sensed by the K30 Sensor. The Arduino connected to the K30 Sensor relays the reading to the laptop.

- To build the CO2 Chambers (yeast chamber and pea chamber), use an old soldering iron to melt and manipulate the plastic under a fume hood.

Yeast Chamber

The yeast chamber is a pencil box with a large hole that will accommodate a PVC tube with a very tight fit. Melt this large hole with a soldering iron under a fume hood. Start small and test the size of the hole and the fit of the PVC tube.

Figure 13. Hole created in yeast chamber for PVC hole.

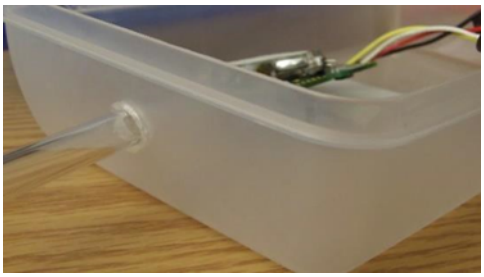

Figure 14. PVC tube fitting.

- A second hole needs to be made to accommodate the Ethernet cable leaving the chamber. This Ethernet cable connects the K30 sensor in the chamber to the Arduino on the outside of the chamber. Again, melt a divot on the bottom part at the edge where the top and bottom portion of the pencil box come together. This is best accomplished by laying the soldering iron on the edge where the top and bottom come together. Again, only melt the smallest amount to accommodate the Ethernet cable and allow closing the box so that the fit is still tight.

Pea Chamber

The pea chamber needs only one hole to accommodate the Ethernet cable leaving the chamber. This Ethernet cable connects the K30 sensor in the chamber to the Arduino on the outside of the chamber. Again, melt a divot on the bottom part at the edge where the top and bottom portion of the pencil box come together. This is best accomplished by laying the soldering iron on the edge where the top and bottom come together. Again, only melt the smallest amount to accommodate the Ethernet cable and allow closing the box so that the fit is still tight.

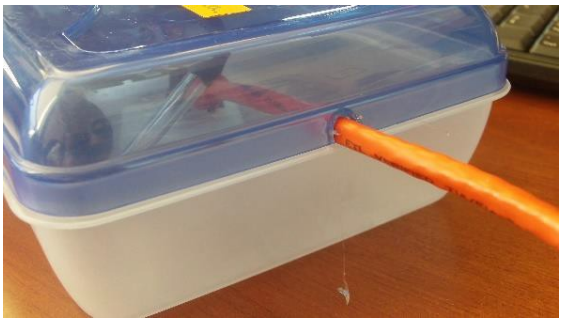

Figure 15. Hole created in pea chamber for cable.

Figure 16. The fit of the cable for the pea sensor.

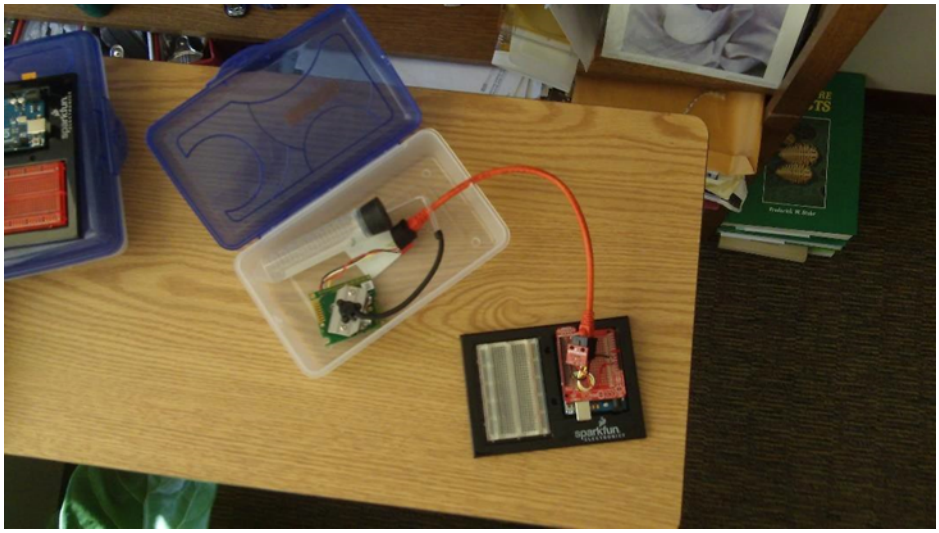

Figure 17. Complete setup for the direct measurement (peas) **.** The germinating peas are loaded into the centrifuge tube, the stopper is replaced, and the box is closed. The CO_2 diffuses from the centrifuge tube into the K30 sensor.

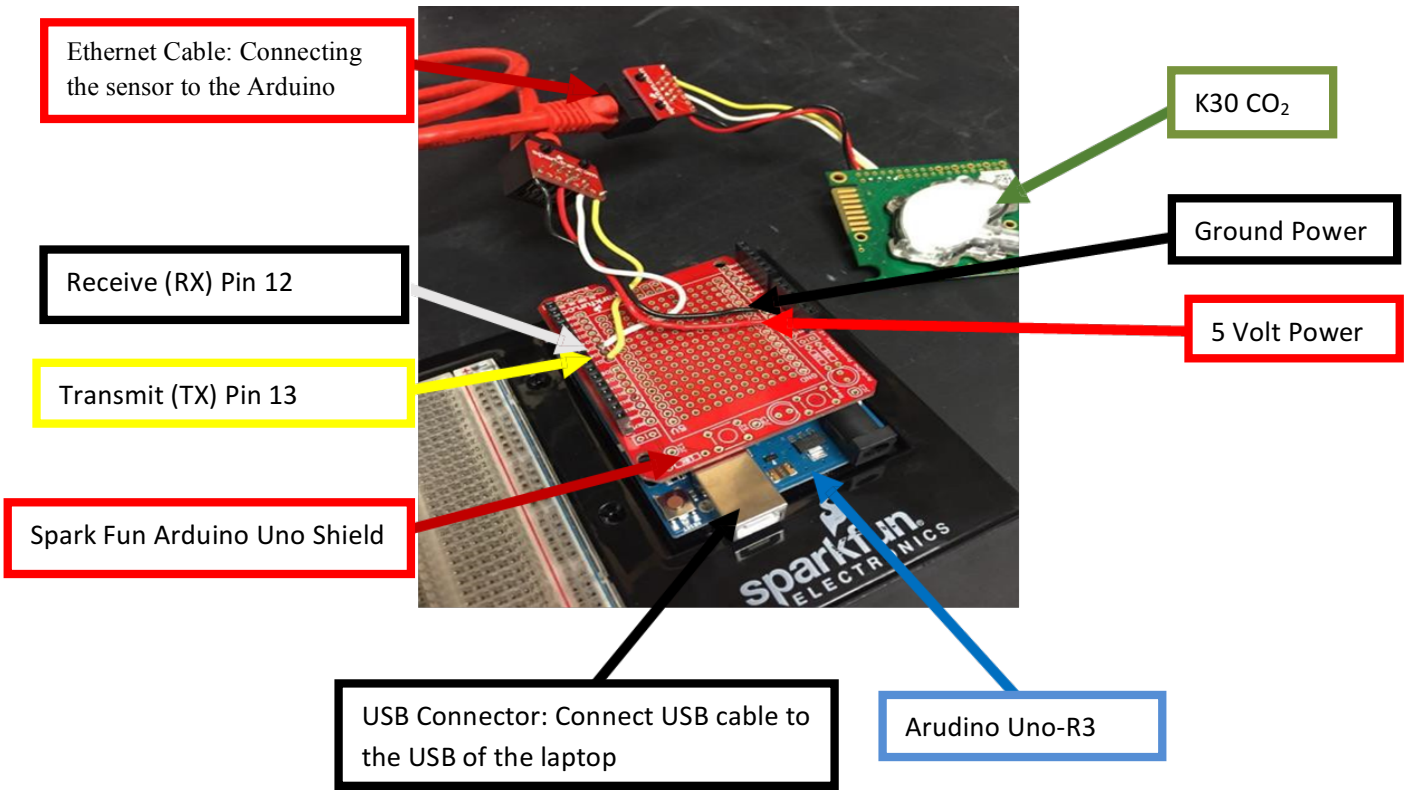

Figure 18. Fully assembled K30-Arduino sensing system. Here the K30 sensor could detect the CO2 ppm in the room.

Downloading the k30 code from here:
http://www.co2meters.com/Documentation/AppNotes/AN126-K3x-sensor-arduino-uart.pdf

- Copy the code from the "/*" to the last "}" . Just after the last "}" the document reads "**END CODE EXAMPLE #1**"
- Paste the code into the Arduino IDE and save it to a new folder named "K30 Sensor" (you will be prompted to do this. Save to your Desktop).
- Open the Serial Port Monitor for Data collection of CO2 in PPM

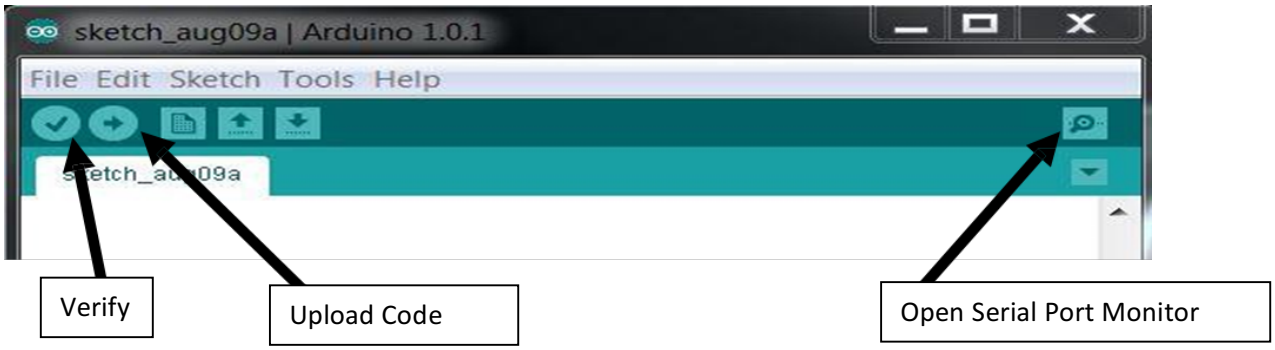

Figure 19. Arduino IDE and the indication of where to open a serial window, which is a window that shows the data output in ppm. Once you verify the code, and upload it to the Arduino (The K30 sensor must be attached to the Arduino).

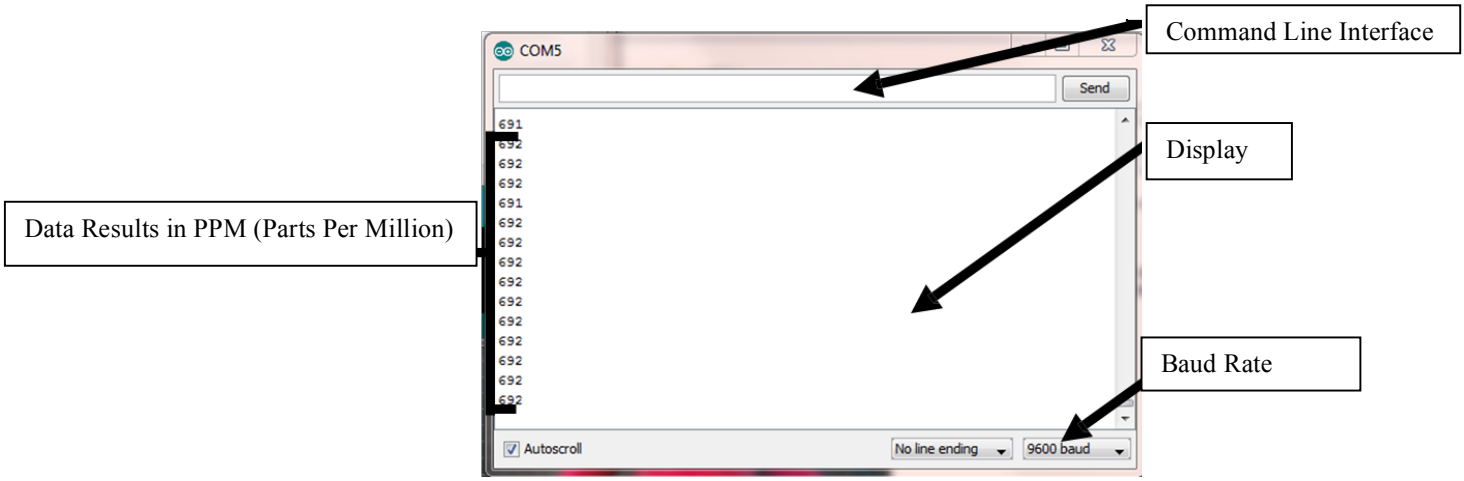

Figure 20. Here is the open Serial Port Monitor to view the data collection.

Appendix C
Code to Upload to Arduino for Use with K30 Sensor

Copy and paste this code into the Arduino IDE from /* to }. The Arduino IDE can be obtained from Arduino.cc.

```
/*
Basic Arduino example for K-Series sensor
Created by Jason Berger
Co2meter.com
*/
#include "SoftwareSerial.h"
SoftwareSerial K_30_Serial(12,13); //Sets up a virtual serial port
                    //Using pin 12 for Rx and pin 13 for Tx
byte readCO2[] = {0xFE, 0X44, 0X00, 0X08, 0X02, 0X9F, 0X25}; //Command packet to read Co2 (see app note)
byte response[] = {0,0,0,0,0,0,0}; //create an array to store the response
//multiplier for value. default is 1. set to 3 for K-30 3% and 10 for K-33 ICB
int valMultiplier = 1;
void setup()
{
// put your setup code here, to run once:
Serial.begin(9600);          //Opens the main serial port to communicate with the computer
K_30_Serial.begin(9600);    //Opens the virtual serial port with a baud of 9600
}
void loop()
{
sendRequest(readCO2);
unsigned long valCO2 = getValue(response);
Serial.print("Co2 ppm = ");
Serial.println(valCO2);
delay(2000);
```

```
}
void sendRequest(byte packet[])
{
while(!K_30_Serial.available()) //keep sending request until we start to get a response
{
K_30_Serial.write(readCO2,7);
delay(50);
}
int timeout=0; //set a timeoute counter
while(K_30_Serial.available() < 7 ) //Wait to get a 7 byte response
{
timeout++;
if(timeout > 10)      //if it takes to long there was probably an error
{
    while(K_30_Serial.available()) //flush whatever we have
    K_30_Serial.read();

    break;                        //exit and try again
}
delay(50);
{
for (int i=0; i < 7; i++)
{
response[i] = K_30_Serial.read();

  }
}
unsigned long getValue(byte packet[])
{
   int high = packet[3];                  //high byte for value is 4th byte in packet in the packet
```

```
    int low = packet[4];                    //low byte for value is 5th byte in the packet
    unsigned long val = high*256 + low;             //Combine high byte and low byte with this formula to get value
    return val* valMultiplier;
}
```