

**South Dakota State University**  
**Open PRAIRIE: Open Public Research Access Institutional**  
**Repository and Information Exchange**

---

Civil and Environmental Engineering Reports and  
Manuals

Department of Civil and Environmental  
Engineering

---

8-2018

# Respirometry Test Manual: WEERC Laboratory

Sepideh Sadeghi

*South Dakota State University*, [sepideh.sadeghi@jacks.sdstate.edu](mailto:sepideh.sadeghi@jacks.sdstate.edu)

Suzette Burckhard

*South Dakota State University*, [Suzette.Burckhard@sdstate.edu](mailto:Suzette.Burckhard@sdstate.edu)

Christopher Schmit

*South Dakota State University*, [Christopher.Schmit@sdstate.edu](mailto:Christopher.Schmit@sdstate.edu)

Follow this and additional works at: [https://openprairie.sdstate.edu/cvlee\\_manual](https://openprairie.sdstate.edu/cvlee_manual)

 Part of the [Civil and Environmental Engineering Commons](#)

---

## Recommended Citation

Sadeghi, Sepideh; Burckhard, Suzette; and Schmit, Christopher, "Respirometry Test Manual: WEERC Laboratory" (2018). *Civil and Environmental Engineering Reports and Manuals*. 1.  
[https://openprairie.sdstate.edu/cvlee\\_manual/1](https://openprairie.sdstate.edu/cvlee_manual/1)

This Other is brought to you for free and open access by the Department of Civil and Environmental Engineering at Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. It has been accepted for inclusion in Civil and Environmental Engineering Reports and Manuals by an authorized administrator of Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. For more information, please contact [michael.biondo@sdstate.edu](mailto:michael.biondo@sdstate.edu).

# Respirometry Test Manual

## WEERC Laboratory

**Sepideh Sadeghi**

*South Dakota State University, Sepideh.sadeghi@jacks.sdstate.edu*

**Suzette Burckhard**

*South Dakota State University, Suzette.Burckhard@sdstate.edu*

**Christopher Schmit**

*South Dakota State University, Christopher.schmit@sdstate.edu*

South Dakota State University  
Jerome J. Lohr College of Engineering  
Department of Civil and Environmental Engineering and  
Water and Environmental Engineering Research Center  
August 2018

## Table of Contents

Introduction .....	1
Equipment .....	1
Nutrient Preparation .....	2
Substrate Preparation .....	2
Aerobic Test Nutrient Application .....	4
Test Procedure .....	5
References .....	13

## **Introduction**

Biological treatment methods are the basis for waste water treatment worldwide. Among biological treatment systems, activated sludge processes are most widely used to remove the oxygen demand and nutrients such as nitrogen (N) and phosphorous (P) from wastewater. The activated sludge process uses naturally occurring bacteria at high concentrations in tanks. The bacteria in the activated sludge process remove small molecules of organic carbon, N, and P as a consequence of bacterial growth and the waste water is purified. The effluent can then be safely released to the environment.

Respirometry techniques are an effective tool to measure the respiration of living organisms (Young and Cowan, 2004). These techniques are associated with readily biodegradable organic carbon (Vanrolleghem et al., 1994). Modern respirometry has the ability to automate data collection. Therefore, it is considered a simple and effective tool to measure the oxygen uptake or the rate of substrate (organic carbon) depletion. The oxygen consumption is directly associated with substrate removal and biomass growth in respirometry (Grady Jr et al., 1989). This makes it a useful technique for monitoring, modeling and controlling the activated sludge process. The respirometric experiments described in this manual are conducted in a batch reactor which is shown in Figure 1. The chemicals needed for the test include a test substrate, nutrient solution, microbial seed and a CO<sub>2</sub> absorbent (Naziruddin et al., 1995).

## **Equipment**

Figure 1 shows all the respirometry equipment used for the respirometry test. A PF-8000 pulse flow respirometer is designed specifically to measure oxygen uptake for aerobic respirometry, and gas production for anaerobic, and anoxic biological reactions. The respirometer was associated along with a laptop computer which is operational with the respirometry software, pressure regulator module (PR-5010 model), respirometer control module, water bath cooler, stirring base, incubator, vessels, syringe, and oxygen tank. The entire system also had a battery backup surge protector. This ensures every test is undisrupted from power surges or sudden power failure.

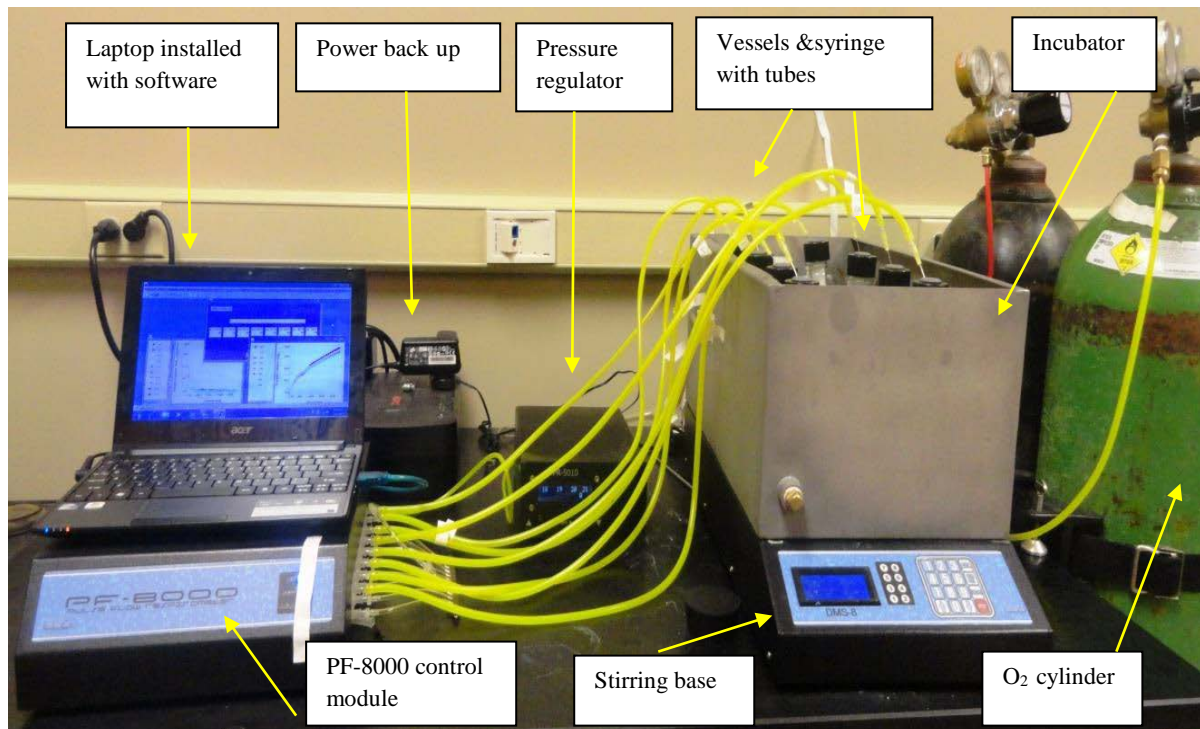


Figure 1. Respirometry equipment test set up

### Nutrient Preparation

A nutrient solution, two mineral base solutions and a buffer solution were added to each vessel to support the biomass growth just before the test. This ensures that the biomass is able to fully consume the substrate without lacking minerals. Table 1 shows the composition of all four solutions, and the amounts required are given subsequently.

### Substrate Preparation

After the first endogenous decay respiration occurred, the substrate was added to each bottles to support the biomass growth. A calculation example for evaluating the required amount of initial substrate (here as Acetate) is shown in the following:

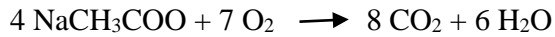
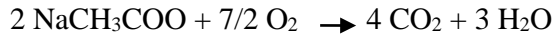
Mixed liquor COD = 2000 mg/L and MLVSS = 1400 mg/L were first measured from the activated sludge sample.

Initial substrate COD (mg)/Biomass (mg) ratio is used as  $S_0/X_0 = 0.2$  in the following calculation for high rate extant tests (Young and Cowan, 2004).

Remember that  $VSS \cdot 1.42 = COD$  and  $S_0 = 0.2 X_0$ , So:

$S_0$  (mg COD) =  $0.5^{\#}$  (MLVSS mg/L)(1.42 mg COD/mg VSS)(0.2 mg sub COD/mg biomass COD)  $\rightarrow S_0$  - 200 mg COD required for test

***Calculation of theoretical COD for Acetate***



***Calculation of mass acetate required to achieve desired  $S_0/X_0$  ratio (0.2)***

$$7(\text{O}_2 \text{ MW})/4(\text{CH}_3\text{COO} \text{ MW}) = 7(32)/4(59) = 0.95 \text{ mg COD/mg acetate}$$

$S_0$  (mg COD)/0.95 (mg COD/mg acetate) =  $200/0.95 \sim \underline{210}$  mg acetate in 500 ml bottle,  
or 420 mg acetate/L.

***Calculation of acetate volume required to achieve desired  $S_0/X_0$  ratio (0.2)***

Sodium acetate salt ( $\text{NaCH}_3\text{COO}$ ) MW = 82.03 mg, and acetate ( $\text{CH}_3\text{COO}^-$ ) MW = 59 mg

(420 mg acetate/L) (82.03 mg  $\text{NaCH}_3\text{COO}$ ) / (59 mg  $\text{CH}_3\text{COO}^-$ )  $\sim 584$  mg/L (0.584 g/L)  
 $\text{NaCH}_3\text{COO}$  salt required.

A bulk solution of 50 g/L  $\text{NaCH}_3\text{COO}$  was already made in the lab,

Remember that  $C_1V_1 = C_2V_2 \rightarrow 0.584 \text{ g/L} * 0.5 \text{ L} = 50 \text{ g/L} * \text{required volume}$   
 $\rightarrow \text{required V} = 5.84 \times 10^{-3} \text{ L}$  or 5.84 mL

---

<sup>#</sup> 500 mL (1/2 L) Bottle used in the test (assuming the whole bottle is MLVSS)

**Table 1. Nutrient/Mineral/Buffer formulations for aerobic respirometric tests (Young and Cowan, 2004)**

<b>Mineral Base I:</b> Added to 800 mL of reagent quality water and diluted to 1L. (Should be agitated vigorously before transferring to avoid the formation of light precipitate)	
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.25 g
FeCl <sub>3</sub> . 6H <sub>2</sub> O	5.0 g
MnCl <sub>2</sub> . 4H <sub>2</sub> O	0.05 g
NaMoO <sub>4</sub> . 2H <sub>2</sub> O	0.005 g
NiCl <sub>2</sub> . 6H <sub>2</sub> O	0.025 g
CuCl <sub>2</sub> . 2H <sub>2</sub> O	0.007 g
ZnCl <sub>2</sub>	0.025 g
H <sub>3</sub> BO <sub>3</sub>	0.025 g
Na <sub>2</sub> SeO <sub>4</sub>	0.025 g
<b>Mineral Base II:</b> Dissolved to 800 mL distilled water and diluted to 1L.	
CaCl <sub>2</sub>	27.7 g
MgCl <sub>2</sub> . 7H <sub>2</sub> O	101 g
<b>Nutrient Base:</b> Dissolved to 800 mL distilled water, neutralize to pH 7 using 50% NaOH and diluted to 1L.	
NH <sub>4</sub> Cl	38.2 g
Na <sub>2</sub> SO <sub>4</sub>	30 g
<b>Buffer Base:</b> Dissolved to 800 mL distilled water, neutralize to pH 7.0-7.2 using NaOH and diluted to 1L.	
KH <sub>2</sub> PO <sub>4</sub>	204 g
KOH (30%)	30 g in 100 mL

**Aerobic test nutrient Application (Young and Cowan, 2004)**

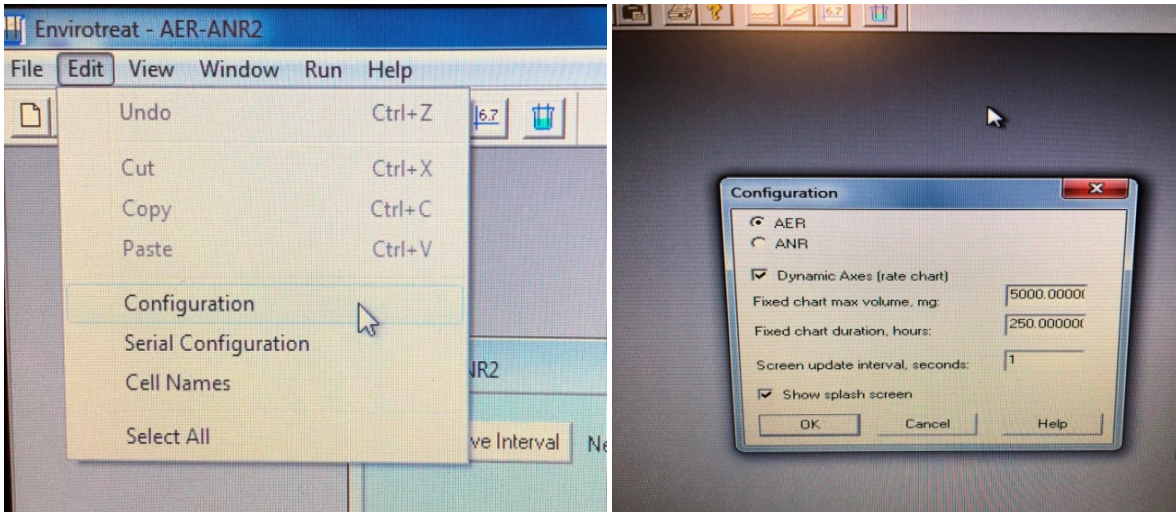
The following list is the amount of nutrients and minerals that are added for each test vessel.

1. Add 5 ml each of Mineral Base I and Mineral Base II per 1000 mg of COD of substrate.
2. Add sufficient Nutrient Base to give a COD:N ratio of 60:1. (Remember to include existing N in the waste)
3. Add 1 ml Buffer Base per 50 mg of COD of substrate.

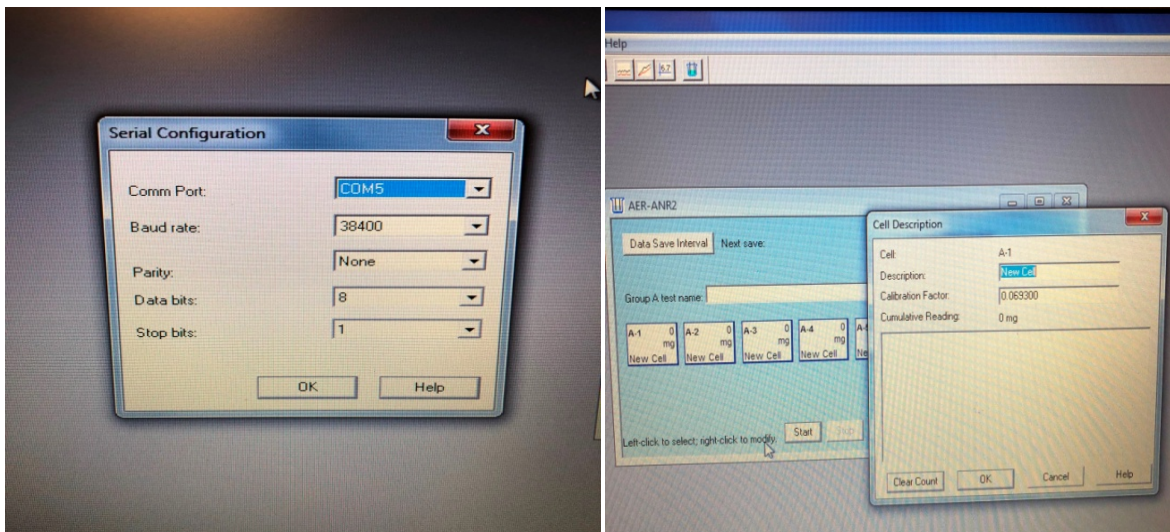
## Test Procedure

1. Bubble air in the activated sludge sample for 30 minutes to remove any remaining soluble substrate (Note: after bubbling and removing the desired amount of sample, the rest may be kept in the cooler for no more than 2 days. For any remaining tests over this time, no air bubbling is required), and in the mean time turn the water bath cooler for the respirometer on and adjust to the desired temperature.
2. Start the PF-8000 data acquisition software on the data acquisition computer. Select **Configuration** under the **Edit** on the menu bar. Select the **AFR** item for aerobic tests and leave other numbers as they are (Figure 2a). Select **Serial Configuration** under **Edit** on the menu bar. Check the **Device Manager** on the **Control Panel** of the computer; **Control Panel/Hardware/Device Manager**; open the ports (COM and LPT) option to see which virtual port has been assigned. With some computers the COM 3 is the highest setting available. In this case set **Comm Port** as **Com 5**, and **Baud rate** as 38,400 and leave the rest of the numbers as they are (Figure 2b). Click the left mouse button on the position 1 box to highlight that box. Click the right mouse button with the cursor on the green box to get a position menu. Select the **Cell history** item. Enter calibration factors for each cell as 0.0693, 0.0667, 0.0704, 0.0681, 0.0698, 0.0685, 0.0705, 0.0674 for Cell 1, 2, 3, 4, 5, 6, 7, 8, respectively (Figure 2c). These values are specific to this respirometer and are given by the manufacture. It will vary for other respirometers. You also have to make sure to use the calibration values for the right pressure (20 psi). Under **Edit** on the menu bar, check **Select All**. Click the right mouse button with the cursor on any green box to get a menu that allows you to add all boxes to a **rate and volume charts** (Figure 2d). Select **Cell Names** under Edit on the menu bar and name the cells as desired based on sample identification (Figure 2e). Set the **Data Save Interval** at 1 second (Figure 2f). Leave the software in the stop mode at this time.
3. After finishing bubbling the activated sludge, pour 500 ml of sludge into 500 ml bottles.
4. Insert a magnetic stirring bar in each bottle (use 5 cm magnets in 500 ml bottle to facilitate good mixing)
5. Add the calculated amount of nutrient, buffer and minerals described previously.
6. Add 5 ml of KOH (not diluted) to each carbon dioxide adsorption tube (Figure 3). This will adsorb CO<sub>2</sub> from the headspace to make sure that 100% oxygen uptake is being measured.





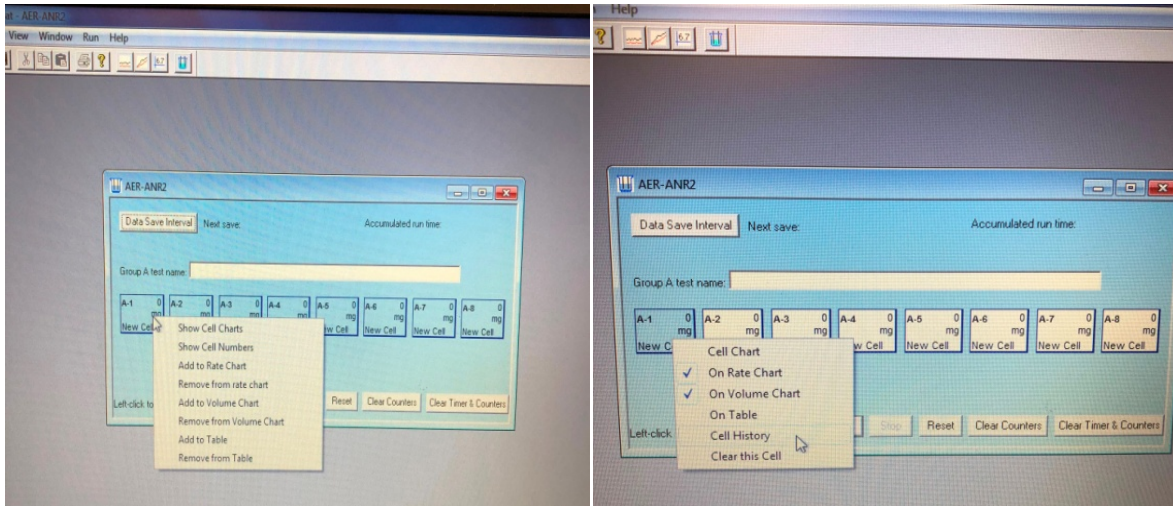
a) Configuration set up



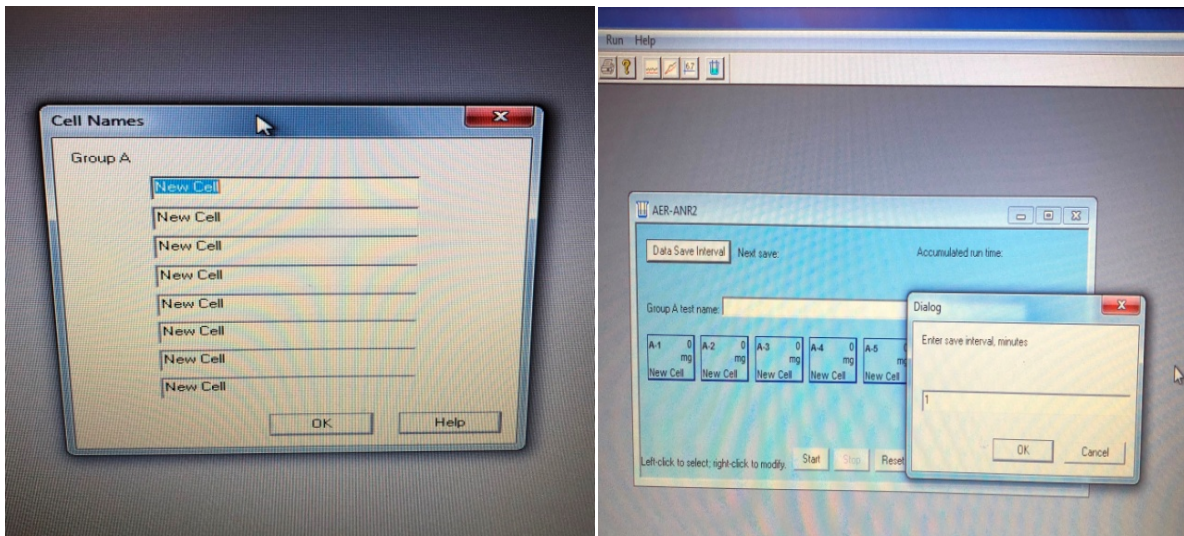
b) Serial configuration set up

c) Calibration factors set up

Figure 2: Software set up



d) Adding rate and volume charts



e) Adding cell names

f) Data save interval

Figure 2: Software set up (cont.)



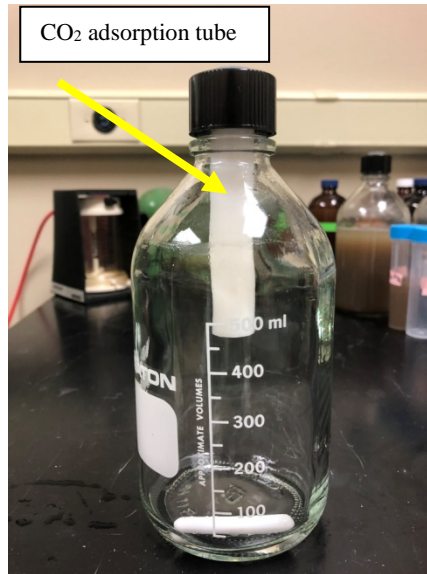


Figure 3. CO<sub>2</sub> adsorption tube

7. Add screw cap with inserted rubber septum to each test bottle. Tighten snugly to seal.
8. Place test bottles in the incubator.

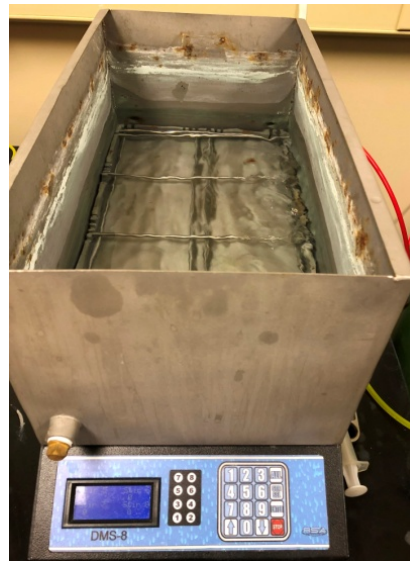


Figure 4. Incubator

9. Turn on the O<sub>2</sub> cylinder and set the pressure regulator to 20 psi which is matched with the calibration values.
10. Turn on the PF-8000 Control Module with all tubing open to the atmosphere.
11. Insert all the needles in the edge of the septum at about 60° angle from the horizontal surface as shown in Figure 5. (Note: 1.5 inch, 20-gage needles are recommended)



Figure 5. Needle angle

12. Press the **Start** button on the control screen and name the file.
13. Turn on the stirring base and adjust the stirring rate to produce a vortex that touches the mixing magnet (700-750 rpm) as shown in the Figure 6. (Note: Some of the mixing magnets are affected by the other bottle's magnetic field and lose their smooth spinning when other magnets come on. To overcome this; those affected bottles should be moved in different directions to find the position that ensures smooth spinning of the mixing magnets. Another way to achieve smooth spinning is to decrease the speed of the bottles adjacent to the bottle that is having problems. Adjust the stirring base every time before testing. Close monitoring is required.



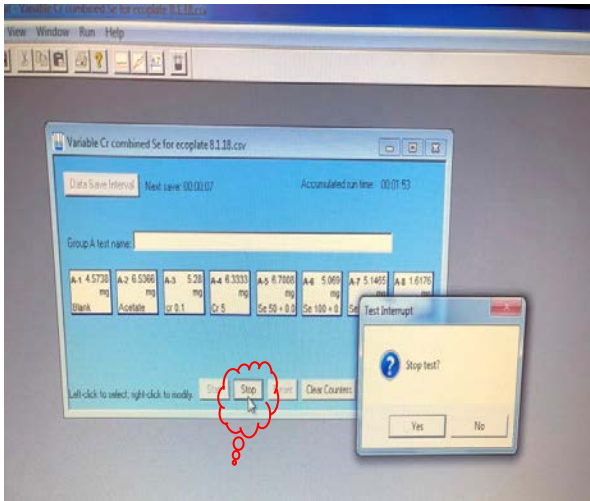
Figure 6. Vortex in 750 rpm

- Using a 20-ml syringe and 20-gage needle, withdraw headspace gas from each test bottle (as shown in Figure 7) until one or two counts appear on the software. After all bottles have been purged, then **Stop** the data acquisition software and press **Reset** on the control screen, and initiate data acquisition by clicking on the **Start** button as shown in Figure 8.

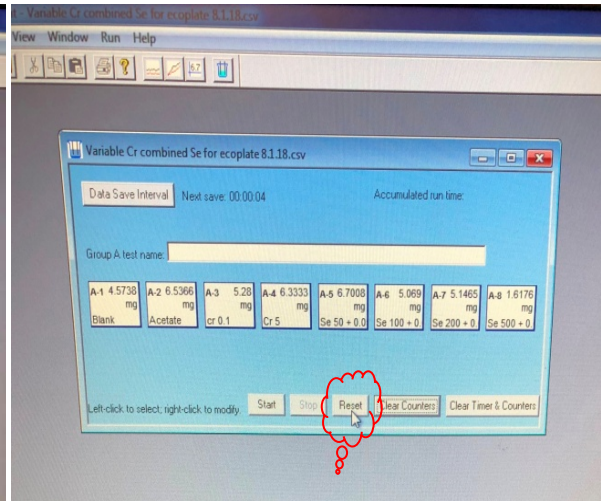


Figure 7. Withdrawing the headspace gas from the test bottle

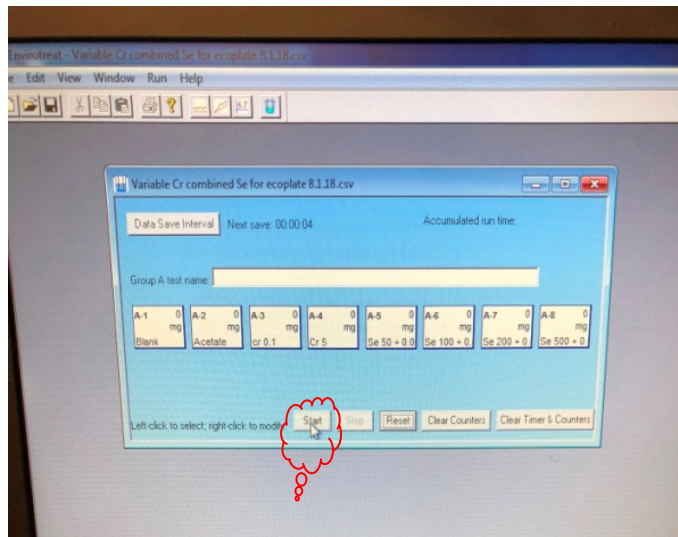
- After a few hours (3-4 hours) while the oxygen uptake rate became constant, the first endogenous decay respiration occurred, in this time **Stop** the data acquisition software and **Stop** the stirrer base. Open all the caps up and inject desired amount of the substrate and tighten the caps and start the data acquisition software and stirrer base (No purging is required at this time for two reasons; 1. the substrate amount is high enough for the system to continue the test by itself, and 2. the previous data may be lost by resetting the data following the purging).
- Each test will take 24 hours or less and will end when the bottle goes back into endogenous phase.



a) Stopping the test



b) Resetting the data



c) Starting the test

Figure 8. Data acquisition procedure

Figure 9 is an example of oxygen consumption measurement data for sample receiving two different types of heavy metals in aerobic respirometer.

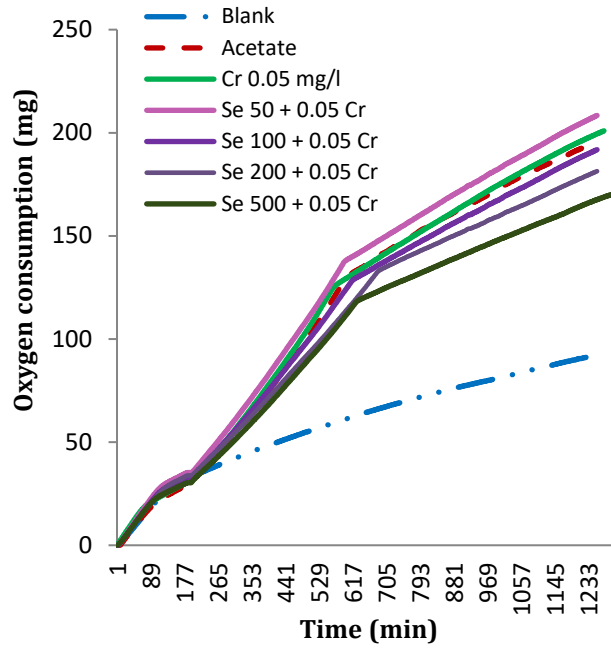


Figure 9. Activated sludge fed with the combination of Cr and Se

## References

Young, J. C., & Cowan, R. M. (2004). *Respirometry for environmental science and engineering*: SJ Enterprises Springdale, AR.

Vanrolleghem, P., & Van Daele, M. (1994). Optimal experimental design for structure characterization of biodegradation models: on-line implementation in a respirographic biosensor. *Water Science & Technology*, 30(4), 243-253.

Grady Jr, Leslie, C. P., Dang, J., Harvey, D., Jobbagy, A., & Wang, X.-L. (1989). Determination of biodegradation kinetics through use of electrolytic respirometry. *Water Science & Technology*, 21(8-9), 957-968.

Naziruddin, M., Grady Jr, C. L., & Tabak, H. H. (1995). Determination of biodegradation kinetics of volatile organic compounds through the use of respirometry. *Water environment research*, 151-158.