Date: Tuesday, September 10, 2019

Topic(s): Reaction kinetics, oxygen uptake rates by bacteria

Instructor: D. Sills

Report: Memo lab report due, Tuesday, September 17, 2019

Preparation: 1. Read lab handout
2. Calculate mass of sodium acetate to prepare solutions (see Prelab, question 1, page 3).
Mass of sodium acetate in 100 mL = _____ g

Instructional Objectives of Lab: After this lab, students should be able to:

1. Measure the dissolved oxygen (DO) concentration in environmental samples.
2. Measure concentrations of reactants over time.
3. Determine the reaction rate order for a set of environmental data.
4. Calculate kinetic rate coefficients for an environmental reaction.
5. Present the data in a clear, well formatted graph.

Introduction and Background

Oxygen is a reactant in many environmental systems, because it is required for aerobic respiration by mammals, fish, and even bacteria. Oxygen dissolved in water, also called dissolved oxygen or DO, is essential for water quality and ecosystem health in waterways. In many cases dissolved oxygen can be depleted in lakes, rivers and estuaries when too much organic matter is discharged into the waterway. This occurs because bacteria in the water consume that organic matter as food or substrate, and as they consume food, they consume oxygen according to the following reaction:

$$\text{Substrate} + O_2 + \text{Microorganisms} \rightarrow CO_2 + H_2O + \text{More Microorganisms} \qquad \text{Eq. 1}$$

The rate at which organisms take up oxygen can be an indicator of the rate of substrate consumption. If too much substrate is discharged to a waterway, bacteria can deplete the oxygen at rates that are typically faster than the oxygen can be replenished from the atmosphere. This can result in very low DO concentrations that kill fish and other aquatic organisms that need the DO to breathe. For example, warm water fish need at least 5 mg/L of DO or more to be happy, and some cold water species prefer concentrations greater than 7-8 mg/L, although fish can survive for short periods at lower concentrations. Some interesting information and pictures can be found on Wikipedia (http://en.wikipedia.org/wiki/Fish_kill), and you should read the first three paragraphs to provide additional background for this topic.

Because DO in waterways is a very important water quality parameter, the discharge of organic matter to rivers, lakes, and oceans is tightly regulated. To determine the impact of organic matter on water quality, engineers and scientists typically perform experiments to determine the oxygen uptake rates (OURs) of different chemicals. This is basically a test in which the chemicals are fed to bacteria and oxygen concentrations are measured over time. Based on the data, the reaction rate order and the reaction rate coefficients can be determined. This data can then be used to model the degradation of the chemical as well as DO concentrations in a system such as a river or an engineered system. Later the class material on reactor design will rely on these kinetic parameters.

For this lab, a fresh sample of aerobic (oxygen consuming) bacteria from a local wastewater treatment facility will be fed a readily biodegradable substrate, acetate (vinegar). As these microorganisms biodegrade this substrate, they will consume oxygen as represented by the reaction in Equation 1. The goal of the experiment is to determine the rates of the reaction and the kinetic parameters, n and k, such that this information can be used in a model to determine how long it will take for acetate to be consumed by the bacteria.

Experimental Method

1. Turn on and calibrate the DO meter.
2. Prepare 100 mL of a 80 mM sodium acetate (CH_3COONa), using the dry chemical, deionized water, and the 100 mL volumetric flask provided. (amount of sodium acetate was calculated as part of the pre-lab)
3. Measure ~230 mL of activated sludge (bacteria from the sewage treatment plant) using a graduated cylinder.
4. Pour the activated sludge into the plastic bottle provided.
5. Pour the entire 100 mL flask of substrate into the plastic bottle with the activated sludge. Cap the bottle and shake vigorously for ~15 seconds.
6. Pour the contents of the plastic bottle into a glass BOD bottle provided. The contents may overflow slightly; this is ok. Allow any air bubbles to rise to the top.
7. Insert the calibrated DO electrode and turn on the mixer. Immediately start a stopwatch and begin to record oxygen concentrations at regular intervals (15-30 seconds if decreasing rapidly, 1-2 minute intervals if decreasing more slowly).
8. Record DO concentrations (in the Google Data Sheet for Lab 3) until the DO concentration has dropped to 0.05 mg/L.
9. Remove the DO electrode from the glass BOD bottle and pour the contents back into the plastic bottle.

When finished:

a. Empty the contents of the BOD bottle into the sink.
b. Rinse your glass BOD bottle, plastic bottle, 100 ml volumetric flask, and 250 mL graduated cylinder first with tap water, then with deionized water from the wash bottle. Store inverted on the lab bench to dry.
c. Rinse the DO electrode with deionized water and store in the BOD bottle containing ~half tap water. Turn off the DO meter.

For your analysis of the data,

1. Using the data sets on the Google Sheet that each team has posted, choose two data sets that appear similar for the analysis below.

2. Create a plot of the DO concentration versus time (symbols only, no lines) for the tests as multiple series on the same graph. Be sure to label your axes, including units and use symbol types to

distinguish the first test from the second test, etc. I've posted the conventions of good graphs on the course website (under Lab 3 on the course landing page). You may remember this from ENGR 101.

3. Determine the reaction order (zero or first), (follow instructions in lab) and visually inspecting the fits.

4. For the reaction order you determined in part 3, determine the reaction rate coefficient, including units (follow instructions in lab).

5. Using the reaction order and coefficient, plot the equation through the data points (for two trials) to see how well it fits the data, and show both data sets on the same plot with the best fit line through each data set. Kaliedegraph does this automatically.

6. Create a table to summarize your data, including the two values of kinetic coefficients, the average and the standard deviation and the coefficient of variation.

7. You will turn in a hard copy of your memo at the beginning of lab next week.

This is an individual assignment, due one week from the lab period in which we perform the experiment. Follow the guidelines discussed in lab for producing quality graphs.

Prelab:

1. Calculate the mass of sodium acetate (CH_3COONa) needed to prepare 100 mL of a 23.4 mM solution of sodium acetate. Hint mM stands for millimoles per liter, and the molecular weight of sodium acetate is 82 g/mole.
2. Be prepared to describe today's lab procedure to the class. I will call on one group of students.
