CE421/521 Environmental Biotechnology Laboratory
Demonstration of Method to Determine Extant Kinetics Using Respirometry

In this class period we will be demonstrating a new procedure for determining the biodegradation kinetics of a particular synthetic organic compound (SOC), ethylene glycol. Ethylene glycol and other SOCs are used in many industrial processes and are therefore a potential concern for wastewater treatment plants that receive an industrial component. In order to predict the effluent concentration of a particular SOC, we need to know its biodegradation kinetics. We can do this several ways, but one of the quickest and most reproducible methods is to use respirometry. If we measure the kinetics in a respirometric test with a high biomass concentration and a low substrate concentration, we call the kinetic parameters extant, having the meaning "currently existing" (see Ellis *et al*., 1996). An outline of the experimental procedure and data analysis is provided below.

As a group assignment (in groups of 3 or 4), analyze the data set from the demonstration using the following outline. Turn in a brief description of the procedure, a plot of the normalized data and model fit, an estimate of the kinetic parameters, an estimate of the effluent concentration of ethylene glycol from a facility operating at a 10 day SRT (use the following equation and assume that the decay coefficient is 0.007 h^{-1}), and a brief conclusion.

$$S = \frac{K_S(\frac{1}{\theta_C} + k_d)}{\hat{\mu} - (\frac{1}{\theta_C} + k_d)}$$

1. Obtain biomass from a continuous activated sludge treatment plant, place in respirometer vessel, and aerate until a stable background oxygen uptake rate has been achieved.
2. Inject a known concentration of the test compound into the vessel and observe the respirometric response.
3. Once the respiration (metabolism) of the test compound is complete, average the resulting raw data to a time interval that will result in approximately 150 to 300 data points.
4. Import the raw data into a spreadsheet program of your choice (this usually involves importing the ASCII text file and parsing the data into separate columns).
5. Once you have the raw data in a spreadsheet format, you will need to manipulate the data in order to achieve a normalized curve (with the background oxygen uptake rate subtracted out). To do this you will need to calculate a slope on the portion of the response either before the test compound was injected or after the compound was completely respired (your choice). Most spreadsheet programs have a linear regression function to accomplish this. Use the data in Column A for the independent variable (X-series) and Column B for the dependent variable. Have the output of the regression written so that you can refer to the value of the slope in a formula as follows:

Spreadsheet Columns

A	B	C	D
time data	DO response of Channel 1 See panel A in Figure on back.	DO response of Channel 2 Not used in demonstration.	Normalized response for Channel 1, write in the following equation: +B1-A1*slope where slope is the cell used by l.r. (e.g. I10). See panel B in Figure on back of sheet for an example of a normalized response (and model fit).

6. Once you have a curve with good initial and ending plateaus, you are ready to copy those values into the curve fitting template (0MONOD.WK1). Use the Edit Value command (or equivalent)

to copy the values (not the formulas) into the template in the column indicated "DO" (J32).

7. Go to the end of the data series that you just copied in. If it is longer than the formulas in the columns A through I and K, then copy the formulas in A through I and K down to the end of the data series.
8. Adjust the range for the final DO (DOf is in cell M33) and SSE (SSE is in cell J28) values to accommodate the entire data series (if the data series goes to row 200, adjust these cells to row 200). The final DO range should include 10 to 20 rows at the end of the response.
9. Insert the values for the concentration of the test compound, So, in cell J13, biomass concentration, X, in cell J14, and time interval, del T, in cell J15. At this point you may want to plot the data along with the predicted data (pDO in column I) versus time. From this plot you need to adjust the initial values of the time of response, TR, in cell J24, and mu max (J27) and Ks (J26) to get the predicted response or model line to match up as close as possible with the actual DO values.
10. Once you have obtained a reasonable fit by eye, you will need to minimize the SSE value. In Excel you can do this using the Solver function. Go to the Tools/Solver function, set the target cell to the SSE cell (J28) and have the mu max and Ks cells that it changes subject to the constraints that they are both positive values. Set the solver function to minimum. Click on OK to let the solver function do its thing. The solver routine will minimize the SSE function by adjusting the mu max and Ks values. If you are using Lotus or Qpro, you can do a grid search to find the mu max and Ks values that minimize SSE (see me if you want to know how to do this in Lotus or Qpro).

Data From Demonstration:
Test Compound: _____
Injection Concentration (So): _____mg/L as COD
Biomass Concentration (X): _____mg/L as COD