REB, The Course
Class 25 Learning Activity Solution

The enzymatic dehydration of substrate S to product P, reaction (1), was studied in a 50 ml BSTR. Three experiments were performed. The temperature was the same in all three experiments, but the initial concentration of substrate S differed. At the start of each experiment product P was not present in the reactor. The reaction takes place in aqueous solution, so the concentration of water may be assumed to be constant. The concentration of product P was measured at 10 min intervals during 2 h of reaction in each of the experiments. Use the resulting data to assess the accuracy of the Michaelis-Menten rate expression, equation (2), for this reaction.

$$S \rightarrow P + H_2O \tag{1}$$

$$r = \frac{V_{max} C_S}{K_m + C_S} \tag{2}$$

The experimental data are provided in the file, activity_25_data.csv. The first few data points from that file are shown in Table 1.

Table 1. First 6 of the 72 Isothermal BSTR Data Points.

$C_{S,0}$ (mmol L^{-1})	t_{meas} (min)	$C_{P,meas}$ (mmol L^{-1})
15.0	5.0	0.64
15.0	10.0	1.15
15.0	15.0	1.57
15.0	20.0	2.38
15.0	25.0	2.87
15.0	30.0	2.78

Assignment Summary

The information provided in the assignment narrative is summarized below. The listed deliverables include the estimated parameters and their confidence intervals, the coefficient of determination, and parity and residuals plots. These were not specifically requested in the assignment narrative, but they are needed for assessing the accuracy of the proposed rate expression.

Reaction

$$S \rightarrow P + H_2O \tag{1}$$

Rate Expression

$$r = \frac{V_{max} C_S}{K_m + C_S} \tag{2}$$

Reactor: Isothermal BSTR

Given Constants: V = 50 ml, $\nu_{sampling}$ = 10 min, and $t_{f,expt}$ = 2 h.

Given Adjusted Experimental Inputs: $\underline{C}_{S,0}$ and $\underline{t}_{meas}$

Given Experimental Responses: $\underline{C}_{P,meas}$

Parameters to Estimate: V_{max} and K_m

Deliverables: Parameter and 95% confidence interval. estimates, coefficient of determination, parity and residuals plots, and an assessment of the accuracy of the proposed model.

BSTR Model Function

The purpose of the BSTR model function is to solve the design equations for the BSTR that was used in the experiments. This is done numerically by calling an IVODE solver. The additional uncomputable unknowns listed below must be passed to the BSTR model function as arguments. If necessary, it will then make them available to the BSTR derivatives function as as global variables. The BSTR derivatives function described here also must be written.

<u>BSTR Model Equations</u>:

$$\frac{dn_S}{dt} = -rV \tag{3}$$

$$\frac{dn_P}{dt} = rV \tag{4}$$

<u>BSTR Model Variables</u>: $\underline{t}$, $\underline{n}_S$, and $\underline{n}_P$

<u>Additional Computable Unknowns</u>: r, C_S

$$C_S = \frac{n_S}{V} \tag{5}$$

<u>Additional Uncomputable Unknowns</u>: $C_{S,0}$, t_{meas}, V_{max} and K_m

<u>IVODE Solver Inputs</u>:

1. Initial values of the reactor model variables

$$t_0 = 0 \tag{6}$$

$$n_{S,0} = C_{S,0}V \tag{7}$$

$$n_{P,0} = 0 \tag{8}$$

2. Stopping criterion

$$t_f = t_{meas} \tag{9}$$

3. Derivatives function that
    a. receives the BSTR model variables at the start of an integration step
    b. has access to V_{max} and K_m
    c. calculates the additional unknowns using equations (2) and (5)
    d. evaluates and returns the BSTR derivatives using equations (3) and (4)

Deliverables Function

The first purpose of the deliverables function is to use the BSTR model function above to estimate the V_{max} and K_m, their 95% confidence intervals, the coefficient of determination, and the model-predicted responses for the experiments. This is done numerically by calling a fitting function, and that will require writing the predicted responses function described below. To improve the likelihood of numerical convergence, the base-10 logarithms of the parameters are estimated instead of the parameters them selves. The second purpose of the deliverables function is to calculate the experiment residuals and generate a parity plot and residuals plots.

<u>Deliverables Function</u>

1. Define guesses for [parameters]

$$\beta_{V_{max},guess} = 0.0 \tag{10}$$

$$\beta_{K_m,guess} = 0.0 \tag{11}$$

2. Call a numerical fitting function.
    a. Arguments
        i. The experimental data: $\underline{C}_{S,0}$, $\underline{t}_{meas}$, and $\underline{C}_{P,meas}$
        ii. The Initial guesses for the parameters, equations (10) and (11)
        iii. A predicted responses function as described below
    b. Returned values
        i. estimates and confidence intervals for V_{max} and K_m
        ii. the coefficient of determination

iii. the predicted responses: $\underline{C}_{P,pred}$

3. Calculate V_{max} and K_m and their confidence intervals

$$V_{max} = 10^{\beta_{V_{max}}} \tag{12}$$

$$K_m = 10^{\beta_{K_m}} \tag{13}$$

$$V_{max,CI} = 10^{\beta_{V_{max,CI}}} \tag{14}$$

$$K_{m,CI} = 10^{\beta_{K_{m,CI}}} \tag{15}$$

4. Calculate the experiment residuals

$$\underline{\epsilon}_{expt} = \underline{C}_{P,meas} - \underline{C}_{P,pred} \tag{16}$$

5. Generate
   a. a parity plot showing $\underline{C}_{P,pred}$ vs. $\underline{C}_{P,meas}$
   b. residuals plots showing $\underline{\epsilon}_{expt}$ vs. $\underline{C}_{S,0}$ and $\underline{t}_{meas}$

Predicted Responses Function

1. receives the adjusted experimental inputs and guesses for the parameters
2. calculates V_{max} and K_m using equations (12) and (13)
3. loops through all of the experiments
   a. calls the BSTR model function to get the model variables
   b. calculates the model-predicted response

$$C_{P,pred} = \frac{\underline{n}_P\Big|_{t=t_{meas}}}{V} \tag{17}$$

4. returns the predicted responses for all of the experiments

Implementation of the Calculations

The Python script, activity_25.py, that was written to perform the calculations accompanies this solution. It is structured as follows.

1. Import necessary libraries.
2. Make the given constants, adjusted experimental inputs, and measured responses available wherever they are needed.
3. Declare global variables for quantities that cannot be passed to the derivatives function as arguments.
4. Define the BSTR model, BSTR derivatives, predicted responses, and deliverables functions as described above.
5. Call the deliverables function.

Results and Discussion

The parameter estimation results are presented in Table 2, the parity plot in Figure 2, and the residuals plots in Figure 3. The fitted model is quite accurate. Looking at the parity plot, the deviations of the data from the parity line are very small, and in the residuals plots, the deviations are random with no apparent trends. The difference between the upper and lower limits of the 95% confidence intervals are small relative to the estimated values of V_{max} and K_m, and the coefficient of determination is nearly equal to 1.0.

Table 2. Michaelis-Menten Parameter Estimates.

Parameter	Value	95% CI
V_{max} (mmol L^{-1} min^{-1})	0.12	[0.115, 0.124]
K_m (mmol L^{-1})	2.52	[2.17, 2.93]
R^2	0.994	

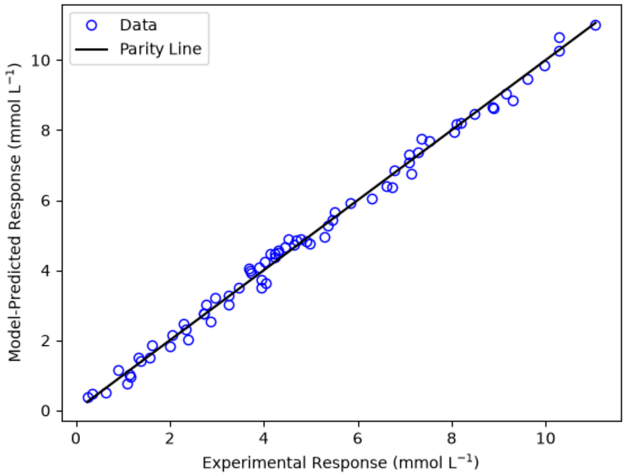

Figure 1. Parity plot.

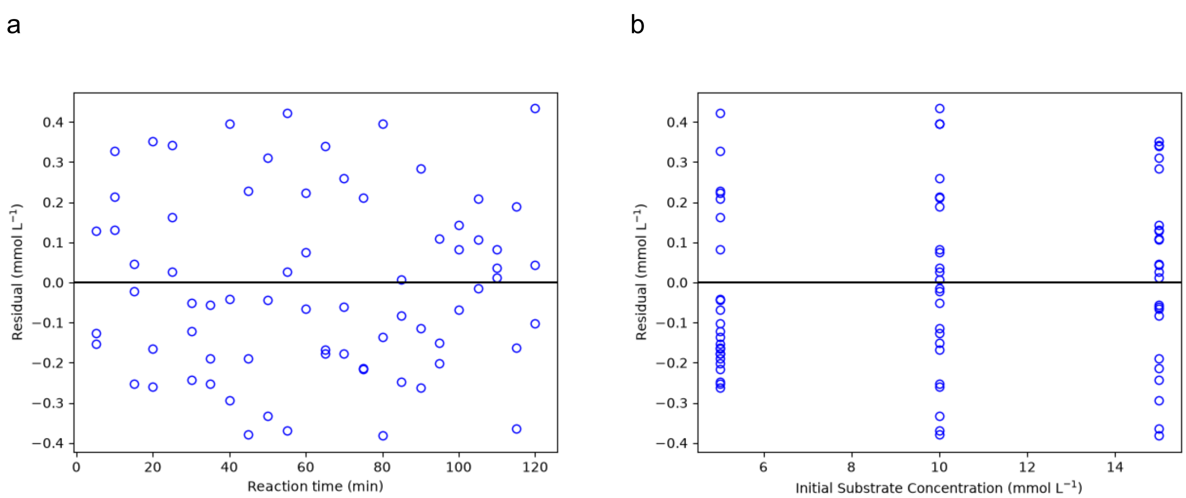

Figure 2. Residuals plots for a) t_{meas} and b) $C_{S,0}$

Accuracy Assessment

When the values of V_{max} and K_m shown in Table 2 are used in the proposed rate expression, equation (2), it is acceptably accurate.