Michaelis-Menten

The objective of this case study is to investigate how enzyme velocity changes as substrate concentration increases and to quantify that relationship using nonlinear curve fitting in Isalos Analytics Platform. In this case study, the **Michaelis-Menten** model is used to estimate the maximum enzyme velocity and the Michaelis constant using a standard substrate-velocity enzyme kinetics framework.

The dataset used in this case study is an enzyme kinetics dataset describing the hydrolysis of sucrose by the enzyme **invertase,** isolated from dry yeast, reported by Al-Odat in *Journal of Microbiology & Biology Education* (2024). In this model, the independent variable (X) is the **substrate concentration** $[Sucrose]$ in **μmol/mL,** and the dependent variable (Y) is the **average initial velocity** V_0 in **μmol/min/mL.**

The **Michaelis-Menten** model describes a saturable hyperbolic relationship in which enzyme velocity increases with substrate concentration and approaches a limiting maximum at higher substrate levels. The equation used in this analysis is:

$$\boldsymbol{Y = Vmax \times \frac{X}{Km + X}}$$

In this model, **Vmax** represents the maximum enzyme velocity in the same units as **Y**, and **Km** represents the substrate concentration required to achieve half-maximal velocity in the same units as **X**.

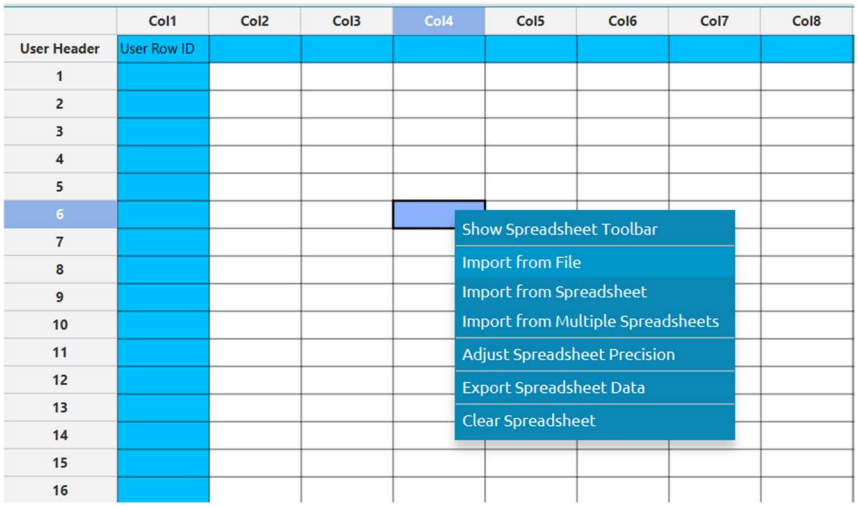
The purpose of this analysis is to determine whether the observed data are consistent with a Michaelis-Menten enzyme kinetics mechanism and to convert the measured velocity values into biologically meaningful quantitative outputs. The main results obtained from the fit are **Vmax** and **Km**, where **Vmax** reflects the maximal catalytic capacity of invertase under the assay conditions and **Km** reflects the apparent substrate concentration required for half-maximal catalytic activity. These results are useful because they allow direct comparison between enzyme preparations, assay conditions or substrates supporting interpretation of catalytic efficiency and substrate dependence.

Isalos version used: 2.0.2

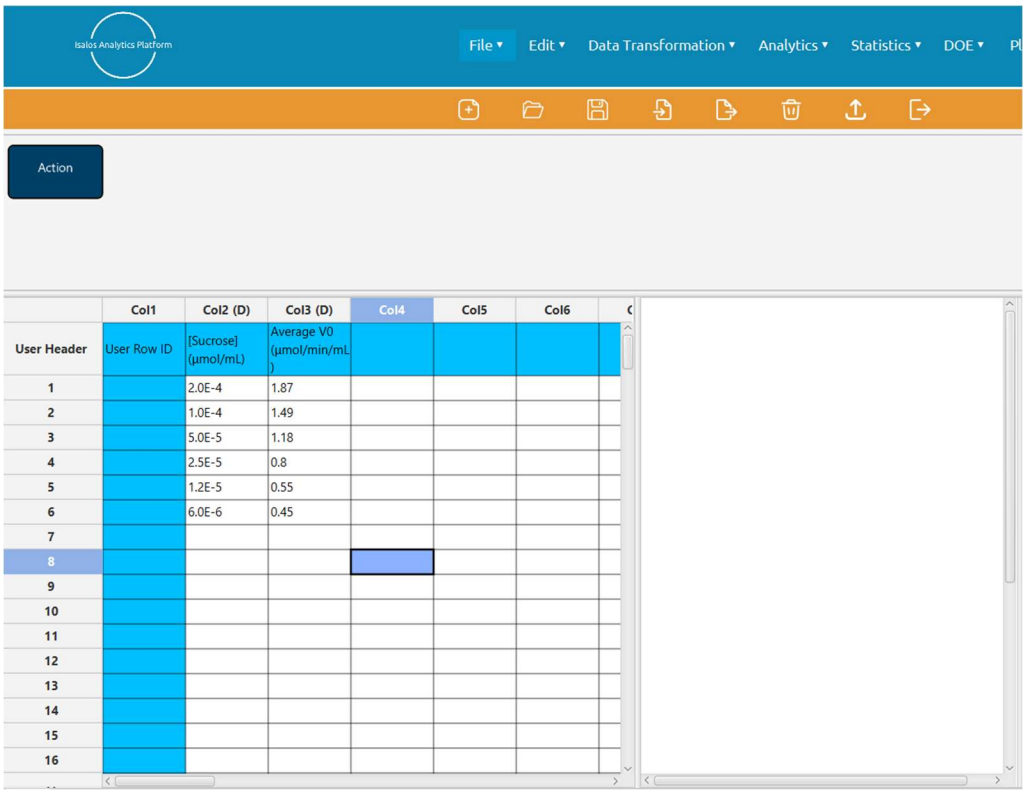
Scientific Article: https://journals.asm.org/doi/10.1128/jmbe.00050-24

Step 1: Import data from file

Right click on the input spreadsheet (left) and choose the option "**Import from File**". Then navigate through your files to load the one with a XY dataset in which each row corresponds to one radiation dose value and one measured surviving fraction value.

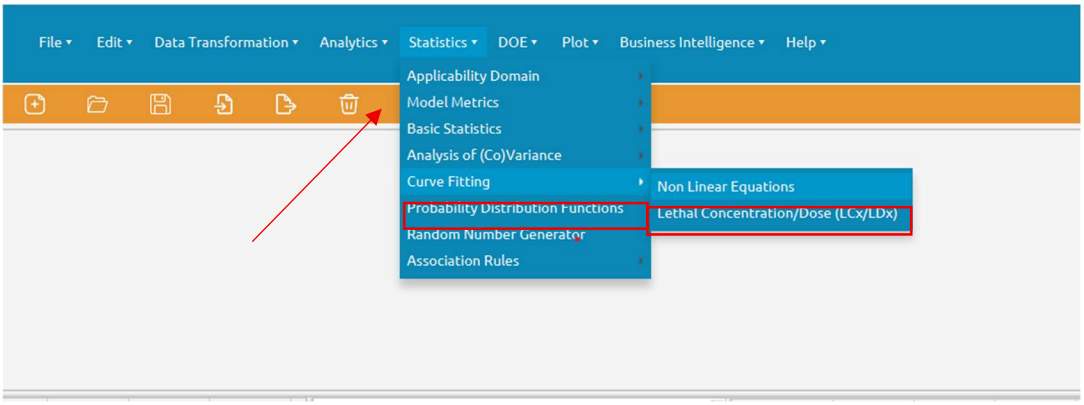


The data will appear on the left spreadsheet

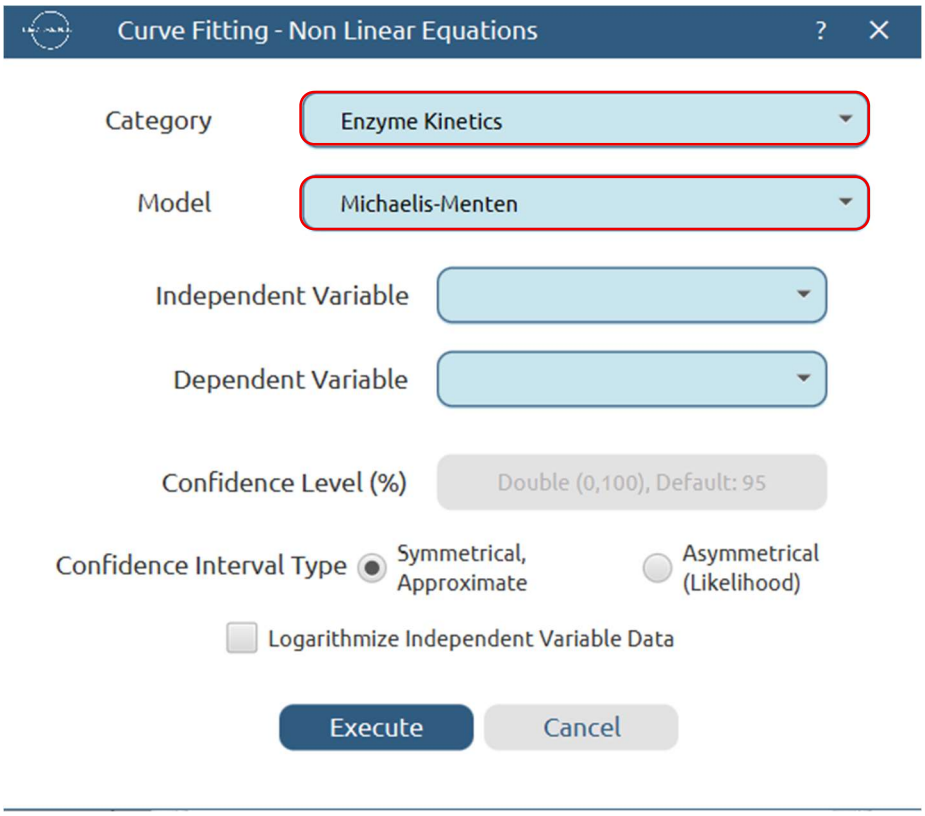


Step 2: Select the desired statistical analysis model

From the toolbar, open the **Statistics** drop-down list and navigate through *Statistics > Curve Fitting > Non Linear Equations*.

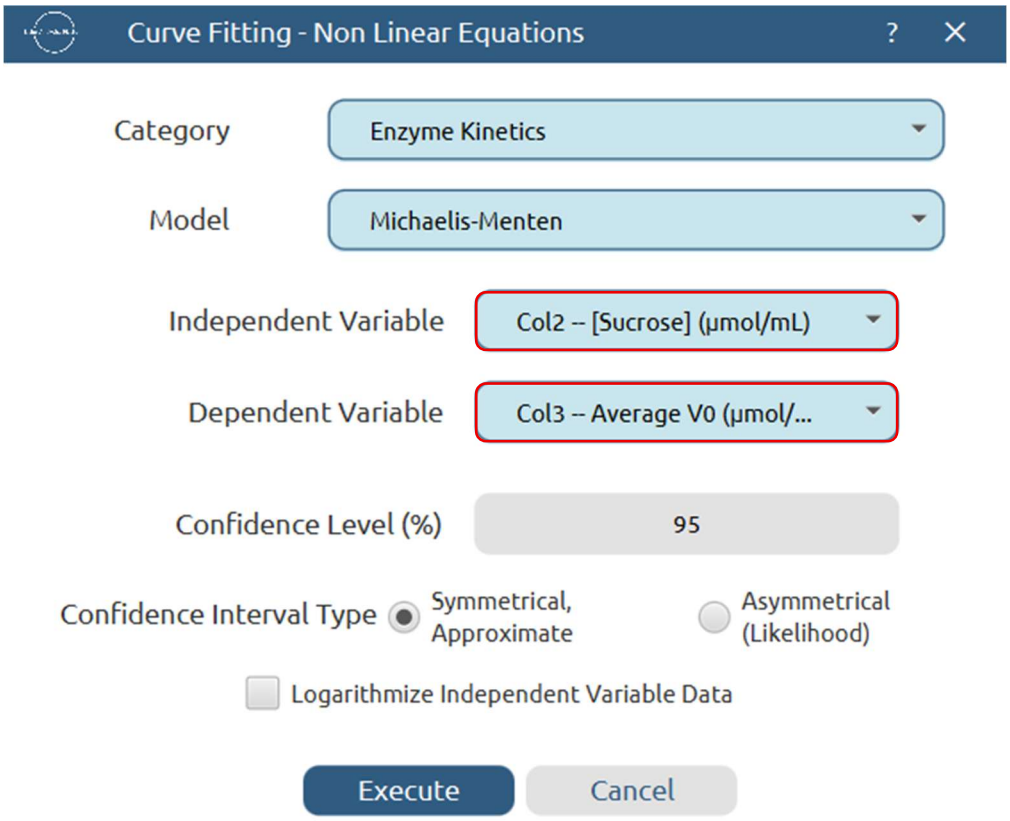


In the category list, select **Enzyme Kinetics** and then choose the **Michaelis Menten** model on the model menu.



Step 3: Configure variables and confidence intervals

Set the column containing the **"[Sucrose](µmol/mL)"**as the independent variable (X), and set the column containing the **"Average V0 (µmol/mL/min)"** values as the dependent variable (Y). Set the confidence level to **95%** and choose **Symmetrical Approximate** as the confidence interval type.

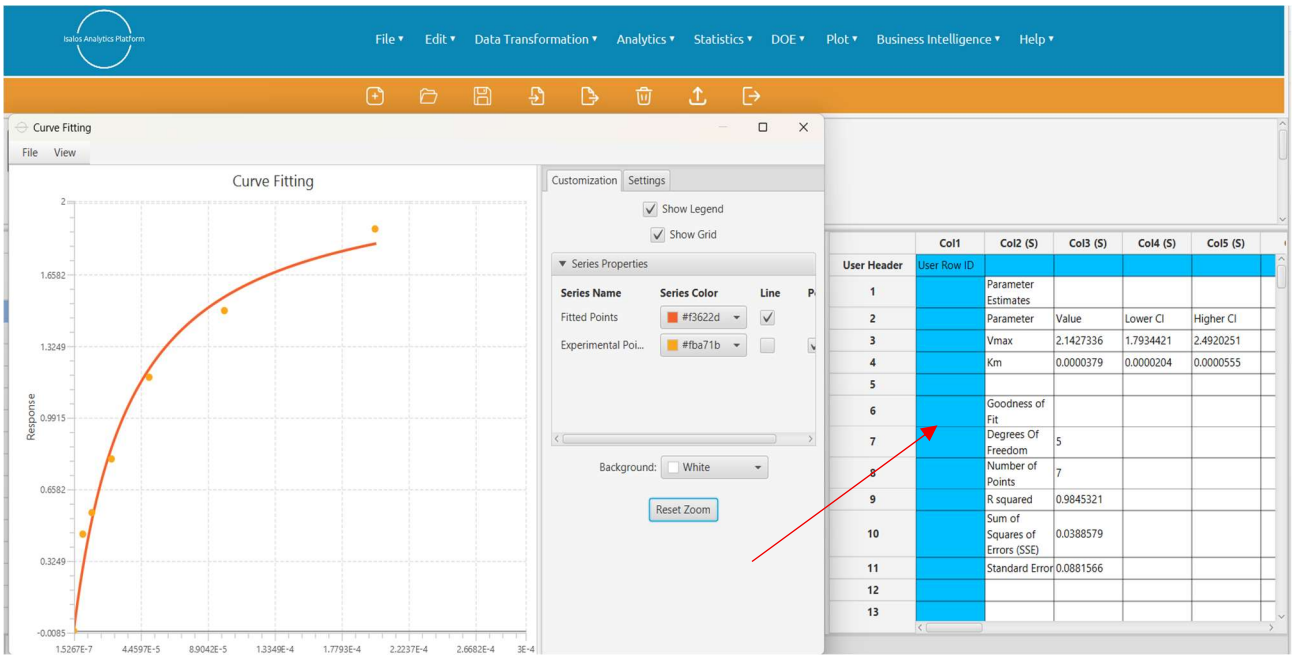


Step 4: Analyze the output and fitted curve

Once the analysis is completed, Isalos presents the fitted Michaelis-Menten curve together with the experimental data points, allowing direct visual assessment of how well the model captures the observed enzyme kinetics pattern. The results page presents the estimated model parameters with their confidence limits, together with goodness-of-fit statistics and the corresponding fitted plot. In the **Michaelis-Menten** model, the main fitted parameters are **Vmax** and **Km**, and the graphical output should display an increasing hyperbolic curve in which enzyme velocity rises with substrate concentration and gradually approaches a plateau at higher substrate concentrations.

The **Goodness of Fit** section of the table summarizes key fitting statistics such as the **number of data points used**, **degrees of freedom**, **residual sum of squares**, and the **standard error** of

the regression. These outputs should be interpreted together with the fitted plot in order to evaluate how well the model describes the data and how reliable the estimated parameters are.



The fitted results indicate that the invertase dataset is broadly consistent with a Michaelis-Menten enzyme kinetics model, in which reaction velocity increases with sucrose concentration and approaches a limiting maximum at higher substrate levels.

The estimated **Vmax** of **2.143 µmol/min/mL** suggests the maximal catalytic rate achievable under the assay conditions, while the estimated **Km** of $\mathbf{3.79 \times 10^{-5}}$ **µmol/mL** indicates the substrate concentration associated with half-maximal enzyme velocity. The relatively high $\boldsymbol{R^2}$ value of **0.985** and the low standard error suggest that the model provides a reasonable description of the observed data.

Referenes:

(1) Al-Odat, I., 2024. Educational activity of enzyme kinetics in an undergraduate biochemistry course: invertase enzyme as a model. *Journal of Microbiology and Biology Education*, *25*(2), pp.e00050-24.