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A METHODOLOGICAL COMPARISON OF LINEAR AND NON-LINEAR REGRESSION FOR KINETIC ANALYSIS OF HYDROGEN PEROXIDE DECOMPOSITION BY CATALASE

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Abstract: *This study presents a comprehensive kinetic analysis of hydrogen peroxide decomposition by bovine liver catalase, with particular emphasis on methodological evaluation of parameter estimation techniques. Kinetic analysis revealed that under the experimental conditions employed, the decomposition exhibited apparent second-order behavior (R^2>0.993), with rate constants of 0.13 $L{\cdot}mmol^{-1}{\cdot}min^{-1}$ at 24°C and 0.064 $L{\cdot}mmol^{-1}{\cdot}min^{-1}$ at 6°C. The apparent activation energy was estimated as 28.5 kJ/mol from Arrhenius behavior. A critical comparison of regression methods revealed striking discrepancies: linear transformation approaches (Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf methods) produced statistically problematic results, including physiologically implausible negative values for Michaelis-Menten kinetic parameters, V_m and K_m. In contrast, non-linear regression, which minimizes the residual sum of squares (RSS), yielded robust, meaningful parameters (V_m = 659 and 25.6 mmol/L.min; K_m = 962 and 69.9 mmol/L at 24°C and 6°C, respectively) and a significantly better fit to the experimental data. However, the analysis also highlights the necessity for cautious interpretation of regression-derived parameters due to inherent correlation between V_m and K_m, experimental design limitations, and model simplification constraints. This work demonstrates that while non-linear regression is indispensable for accurate kinetic analysis, its outputs must be interpreted within appropriate statistical and experimental contexts to ensure biologically relevant conclusions.*

Key words: Enzyme Kinetics; Hydrogen Peroxide; Catalase; Non-Linear Regression; Michaelis-Menten Parameters, Methodological Comparison

1 Introduction

The quantitative determination of enzyme kinetic parameters including reaction order, rate constants, and activation energy represents a fundamental aspect of enzymology, providing critical insights into catalytic mechanisms, enzyme efficiency, and functional responses to environmental variables (Cornish-Bowden, 2013). For both basic research and applied biotechnology, from drug discovery to industrial biocatalysis, reliable kinetic data serves as the foundation for predictive modeling and process optimization (Lehninger et al., 2005). The catalase-catalyzed decomposition of hydrogen peroxide (H_2O_2) presents an ideal model system for examining the crucial relationship between experimental methodology and parameter estimation accuracy. As a well-characterized, biologically essential reaction central to cellular antioxidant defense systems (Chelikani et al., 2004), its kinetics have been extensively investigated in both research and educational contexts. The reaction's reproducibility and relatively simple experimental setup make it particularly suitable for evaluating how different analytical approaches influence derived kinetic parameters (Berg et al., 2023).

Historically, the field of enzyme kinetics has been dominated by linear transformations of the Michaelis-Menten equation, notably the Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf plots (Lineweaver & Burk, 1934; DeLisa et al., 2017). These methods

gained widespread adoption primarily due to their straightforward graphical interpretation, making them accessible teaching tools (Goličnik, 2012). However, this apparent simplicity conceals significant statistical limitations. Extensive analysis over decades has demonstrated that these linearization techniques introduce substantial systematic errors by disproportionately weighting data points and amplifying experimental noise, particularly at low substrate concentrations where measurement uncertainty is typically greatest (Dowd & Riggs, 1965; Motulsky & Ransnas, 1987). The consequence is frequently biased, inaccurate estimates of the kinetic parameters that can yield physiologically meaningless results, including negative values for these fundamental constants (Ritchie & Prvan, 1996). In stark methodological contrast, non-linear regression techniques fit the original Michaelis-Menten equation directly to untransformed experimental data, thereby preserving the intrinsic error structure and providing statistically superior parameter estimates (Johnson & Goody, 2011). This approach minimizes the residual sum of squares between observed and predicted reaction rates without introducing the mathematical distortions inherent in linear transformations (Johnson, 1992). Despite these demonstrated advantages and the widespread availability of computational tools, many contemporary studies persist in using linear methods, perpetuating a legacy of potential inaccuracy in kinetic characterization (Transtrum et al., 2015).

While the theoretical limitations of linear transformations are well-documented, very few studies, if any, have provided direct experimental comparison using a single, well-characterized enzyme system under controlled conditions to quantitatively demonstrate the magnitude of resulting errors. Furthermore, the catalase-H_2O_2 system, despite its widespread use in research and teaching, has not been systematically examined to illustrate how temperature-induced kinetic variations interact with methodological choices to affect parameter estimates. To address these gaps, this study was designed with three specific objectives: first, to determine the apparent kinetic order of catalase-mediated H_2O_2 decomposition under the defined experimental conditions; second, to quantify the influence of temperature variation on the kinetic rate constants and derived activation energy; and third, to conduct a rigorous comparative analysis evaluating the extent to which traditional linear transformation methods, specifically Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf plots, produce divergent and potentially invalid estimates of the Michaelis-Menten parameters (K_m and V_m) when contrasted with estimates obtained through non-linear regression of identical experimental data.

Through this approach, the present study provides empirical evidence quantifying the degree to which linear methods can generate statistically invalid parameters, including physiologically meaningless negative values for fundamental constants, while simultaneously demonstrating how non-linear regression preserves biologically interpretable parameter estimates across varying experimental conditions.

2 Materials and Method

2.1 Materials and Analytical Equipment

All chemicals utilized in this study were of analytical grade. Fresh bovine liver was procured from a local abattoir in Kano, Nigeria, and transported to the laboratory on ice within 30 minutes. Hydrogen peroxide (H_2O_2, 70% w/v) used as the substrate was obtained from Sigma-Aldrich (USA). Sodium phosphate monobasic (NaH_2PO_4, ≥99.0%) and sodium phosphate dibasic (Na_2HPO_4, ≥99.0%) for the preparation of phosphate buffer (0.1 M, pH 7.0) were also sourced from from Sigma-Aldrich (USA). All solutions were prepared using distilled water produced by a Mon Scientific SZ-96 water distiller (Mon Scientific Ltd., Lagos, Nigeria). General laboratory apparatus included a sports digital stopwatch (PULIVIA, USA) and Class A glassware. The enzymatic decomposition of hydrogen peroxide was monitored using a Jenway 6314 UV/Visible Scanning Spectrophotometer (Jenway 6314, UK). The pH was measured and adjusted using an Ohaus ST2100-B benchtop pH meter (Ohaus ST2100-B, USA). Blending was carried out using a mechanical blender (TT-I777, China). Mass measurements were performed on a weighing balance (Ohaus SP202, Scout Pro, USA).

2.2 Extraction of Catalase

Fresh bovine liver tissue was immediately processed upon arrival at the laboratory. The tissue was dissected into small pieces and homogenized using a mechanical blender with 0.1 M phosphate buffer (pH 6.9) in a 1:3 tissue-to-buffer ratio (w/v) to preserve

enzyme activity(Bergmeyer, 2012). The resulting homogenate was sequentially filtered through double-layered muslin cloth to remove coarse tissue debris (Bisswanger, 2017). The clear, reddish-brown supernatant was carefully decanted and served as the crude catalase extract for all subsequent kinetic assays.

2.3 Preparation of Calibration Curve

A calibration curve was established to correlate hydrogen peroxide concentration with spectrophotometric absorbance. A stock solution of 10 mM H_2O_2 was prepared in 0.1 M phosphate buffer. This stock was subsequently diluted to create a series of standard solutions with concentrations ranging from 0.5 mM to 10.0 mM. The absorbance of each standard solution was measured at 240 nm against a phosphate buffer blank using spectrophotometer. All absorbance measurements were performed using standard 1 cm path length quartz cuvettes. Absorbance values for the calibration standards ranged from 0.001 to 1.999, remaining within the linear range of the spectrophotometer. Resulting data were subjected to linear regression analysis, yielding a calibration curve with the equation Absorbance = 0.1714 × [H_2O_2] (R^2 = 0.9986), which was subsequently used to calculate H_2O_2 concentrations from absorbance readings during kinetic experiments.

2.4 Kinetic Experiment

The enzymatic decomposition of hydrogen peroxide was monitored using a standardized assay protocol. For each experimental run, a 20 mL reaction mixture containing 10 mM H_2O_2 in 0.1 M phosphate buffer (pH 7.0) was prepared in a 50 mL glass beaker. The reaction was initiated by the rapid addition of 1 mL of crude catalase extract, followed by immediate and vigorous manual shaking to ensure homogeneous mixing. Immediately, 3 mL of the reaction mixture was quickly transferred to a glass cuvette, which was immediately placed in the spectrophotometer. The decrease in absorbance at 240 nm was recorded at 10-second intervals until a stable absorbance reading was recorded where subsequent measurements showed no much changes in the values. This procedure was performed under two distinct temperature conditions 24.0°C and 6.0°C. The initial substrate concentration ($[S]_0$) was measured prior to adding of the enzyme extract.

2.5 Mathematical Framework

2.5.1 Determination of Reaction Order

The reaction order with respect to hydrogen peroxide concentration was established using the integrated rate law method (Connors, 1990; Nyachwaya & Wood, 2014). The concentration-time data were fitted to zero-order, first-order, and second-order kinetic models to identify the most appropriate reaction mechanism.

For zero-order kinetics, the concentration-time relationship is described by:

$$[S] = [S]_o - k_o t \quad (1)$$

where $[S]$ is the concentration at time t, $[S]_0$ is the initial concentration, and k_0 is the zero-order rate constant (Marangoni, 2003; Atkins, 2006). A linear plot of $[S]$ versus t with a negative slope confirms zero-order kinetics.

For first-order kinetics, the integrated rate law is expressed as:

$$ln[S] = ln[S]_o - k_1 t \quad (2)$$

Where k_1 is the first-order rate constant (Justi & Gilbert, 1999; Marangoni, 2003). A linear plot of $\ln[S]$ versus t with a negative slope indicates first-order behavior.

For second-order kinetics, the relationship follows:

$$\frac{1}{[S]} = \frac{1}{[S]_o} + k_2 t \quad (3)$$

where k_2 is the second-order rate constant (Espenson, 1995; Marangoni, 2003). A linear plot of $1/[S]$ versus t with a positive slope confirms second-order kinetics.

The optimal reaction order was determined by comparing the coefficient of determination (R^2) values obtained from linear regression analysis of each transformed dataset (Motulsky & Ransnas, 1987). The model yielding the highest R^2 value, accompanied by a random residual distribution, was selected as the best representation of the reaction kinetics at each experimental temperature.

2.5.2 Activation Energy Determination

The activation energy was calculated using the two-point form of the Arrhenius equation (Horie, 2013):

$$E_a = \frac{T_1 T_2 R \ln \frac{k_2}{k_1}}{T_2 - T_1} \quad (4)$$

where k_1 and k_2 are the rate constants at temperatures T_1 and T_2 (in Kelvin), respectively, and R is the universal gas constant (8.314 J/mol·K).

2.6 Kinetic Parameter Estimation Technique

2.6.1 Linear Regression

Lineweaver-Burk (double-reciprocal), Eadie-Hofstee, and Hanes-Woolf plots were constructed to linearize the Michaelis-Menten equation and estimate specific kinetic parameters V_m (maximum reaction rate) and K_m (Michaelis Menten's constant) as described by (Marangoni, 2003; DeLisa et al., 2017).

The Michaelis-Menten equation is given by:

$$V = \frac{V_m [S]}{K_m + [S]} \quad (5)$$

i. Lineweaver-Burk (Double-Reciprocal) Plot: The Lineweaver-Burk method involves taking the reciprocal of both sides of the Michaelis-Menten equation, resulting in a linear form.

$$\frac{1}{V} = \frac{K_m}{V_m} \frac{1}{[S]} + \frac{1}{V_m} \quad (6)$$

A plot of $1/V$ vs $1/[S]$ yields a straight line with positive slope as K_m/V_m and y-intercept as $1/V_m$.

2.5.1.1 ii. Eadie-Hofstee Plot:

$$V = V_m - K_m \frac{V}{[S]} \quad (7)$$

A plot of V vs $V/[S]$ yields a straight line with negative slope as K_m and positive y intercept as V_m.

2.5.1.2 iii. Hanes-Woolf Plot:

$$\frac{[S]}{V} = \frac{K_m}{V_m} + \frac{1}{V_m}[S] \quad (8)$$

A plot of $[S]/V$ vs $[S]$ yields a straight line with positive slope as $1/V_m$ and positive y intercept K_m/V_m.

2.6.2 Non-Linear Regression

Non-linear regression analysis was performed to directly fit the experimental data to the Michaelis-Menten equation without data transformation. The parameter estimation was conducted by minimizing the residual sum of squares (RSS) between the experimentally observed reaction rates and the model-predicted values using the Levenberg-Marquardt optimization algorithm (Pum, 2020; Tellinghuisen, 2008). The objective function was defined as:

$$RSS = \sum_{i=1}^{n} (V_{i,obs} - V_{i,pred})^2$$

where $V_{i,obs}$ represents the experimentally determined reaction rate, $V_{i,pred}$ denotes the predicted rate calculated from the Michaelis-Menten (equation 5), and n is the number of experimental observations. The computational fitting was implemented using the Solver add-in in Microsoft Excel, which iteratively adjusted the parameters V_m and K_m to minimize the RSS value to a minimum.

3 Results and Discussion

3.1 Reaction Order and Temperature Dependence

The concentration-time profiles obtained at the two experimental temperatures (Figure 1) demonstrate a pronounced temperature dependence of the reaction rate, with hydrogen peroxide consumption occurring approximately twice as rapidly at 24°C compared to 6°C. This observed acceleration of substrate decomposition with increasing temperature follows fundamental principles of chemical kinetics as described by the Arrhenius equation, which governs the temperature dependence of reaction rates in both

chemical and biological systems (Pum, 2020; Kohout, 2021).

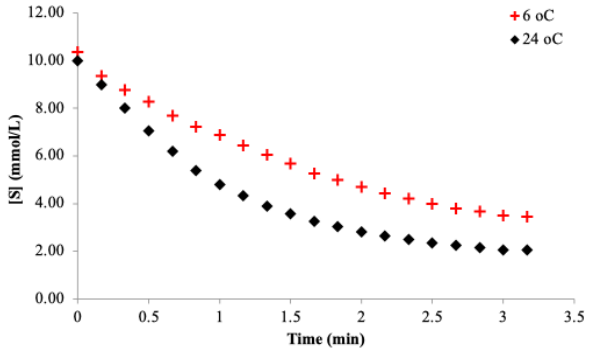

Figure 1. Concentration-time profiles for H_2O_2 decomposition at 6°C and 24°C

The optimal reaction order was determined by comparing the coefficient of determination (R^2) values obtained from linear regression analysis of each transformed dataset, supplemented by visual inspection of residual plots to assess random versus patterned error distribution (Motulsky & Ransnas, 1987). While both zero-order (R^2 = 0.861–0.953) and first-order (R^2 = 0.965–0.993) models provided good correlations, the second-order model not only provided the highest R^2 values (>0.993) but also exhibited the most random residual distribution, whereas zero-order and first-order models showed clear systematic deviations in residual plots, as demonstrated in Figure 2. The significantly higher correlation for the second-order model indicates that the reaction rate is most accurately described as proportional to the product of the enzyme and substrate concentrations. The second-order rate constants, determined from the linear regression of 1/[S] versus time plots, were 0.13 $L \cdot mmol^{-1} \cdot min^{-1}$ at 24°C and 0.064 $L \cdot mmol^{-1} \cdot min^{-1}$ at 6°C. This two-fold reduction in the rate constant with an 18°C temperature decrease reflects the significant thermal sensitivity of the catalytic process, consistent with established principles of enzyme temperature dependence (Daniel et al., 1996; Piskulich et al., 2019).

The apparent second-order kinetics observed in this system can be attributed to several factors. First, the substrate concentrations examined (0–10 mM) may lie within a transitional region where neither the first-order nor zero-order approximation adequately describes the behavior. Second, the use of crude enzyme extract introduces multiple proteins and cellular components that could influence the reaction progress. Third, the integrated rate law method applied to progress curves differs from initial velocity measurements typically used for Michaelis–Menten analysis; the former reflects the entire reaction time course, including periods when both enzyme and substrate concentrations are changing. This distinction is important: while initial velocity studies of catalase typically show Michaelis–Menten saturation behavior, the integrated analysis of complete progress curves under these specific conditions yields apparent second-order characteristics. The observed temperature dependence of the second-order rate constants, with a two-fold reduction upon 18°C cooling, is consistent with the expected thermal sensitivity of enzymatic reactions.

This kinetic pattern aligns with the well-established catalytic mechanism of catalase, which involves the formation of a distinct enzyme-substrate complex during the catalytic cycle (Ogura, 1955; Chelikani et al., 2004). The bimolecular nature of this initial step, where one molecule of H_2O_2 interacts with the heme group of the enzyme, provides the theoretical foundation for the observed second-order kinetics (Aebi, 1974). The temperature dependence of the second-order rate constants conformed to classical Arrhenius behavior, with the rate constants' sensitivity to temperature providing further evidence of the reaction's elementary bimolecular character.

BJET, 2026, Vol. 201(1), 123-133

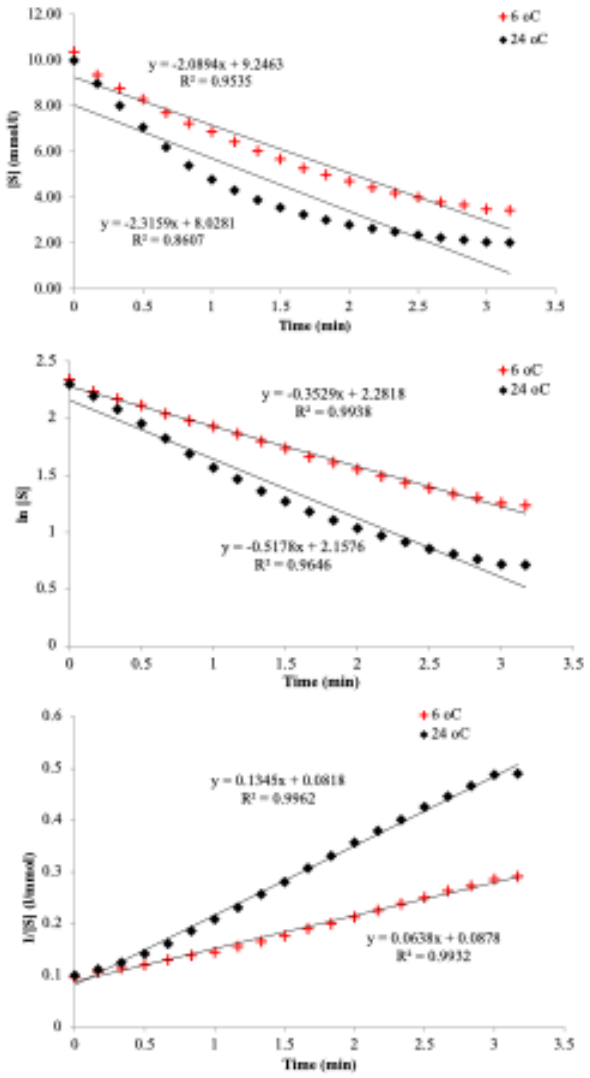

Figure 2. Zero-order (top), First-order (middle) and second-order (bottom) kinetic plots for reactions at 24°C and 6°C

Application of the two-point Arrhenius equation yielded an activation energy (E_a) of 28.5 kJ·mol⁻¹. This apparent value falls within the characteristic range reported for enzyme-catalyzed reactions (20 – 80 kJ·mol⁻¹) and is consistent with previously reported activation energies for catalase-mediated reactions, which typically range from 20-50 kJ·mol⁻¹ depending on the enzyme source and experimental conditions (Aebi, 1974; Cornish-Bowden, 2013). The magnitude of this activation energy represents the energy barrier that must be overcome for the reaction to proceed and provides a quantitative explanation for the observed temperature sensitivity, where lower temperatures result in fewer enzyme-substrate complexes possessing sufficient energy to achieve the transition state (Segel, 1975).

3.2 Critical Comparison of Regression Methods

The performance of linear transformation methods and non-linear regression was rigorously evaluated by comparing the estimated kinetic parameters (V_m and K_m) and their respective goodness-of-fit measures. The comparative analysis revealed substantial methodological discrepancies, with linear transformation techniques producing statistically problematic results across both experimental temperatures.

3.2.1 Linear Regression

As illustrated in Figure 3, all linear transformation methods yielded physiologically implausible kinetic parameters, including negative values for both K_m and V_m, accompanied by poor correlation coefficients (R^2 values as low as 0.416). These atypical results are consistent with theoretical predictions regarding error amplification inherent in reciprocal data transformations (Segel, 1975; Motulsky & Ransnas, 1987; Cornish-Bowden, 2013). A notable observation was the superior fitting performance at 24°C compared to 6°C across all linear methods, as evidenced by higher R^2 values, suggesting that temperature-induced variations in reaction rates further exacerbate the limitations of linear transformations.

BJET, 2026, Vol. 201(1), 123-133

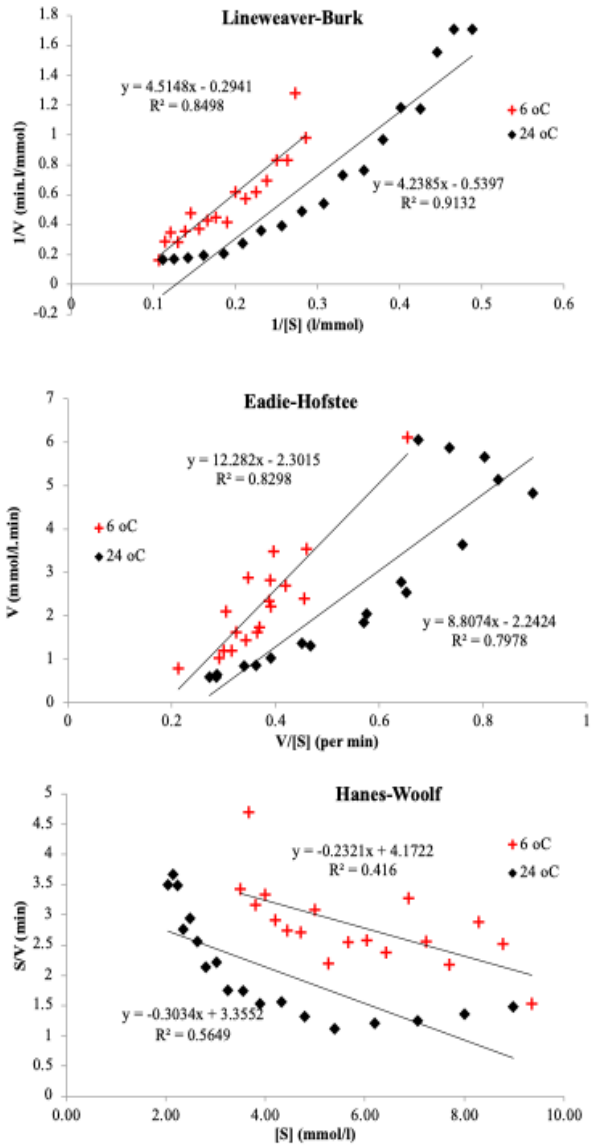

Figure 3. Linearized Plots (Lineweaver-Burk, Eadie-Hofstee, Hanes-Woolf) at 6°C and 24°C.

The comprehensive results summarized in Table 1 demonstrate the systematic failure of linear transformation methods. The graphical representations further highlight the methodological deficiencies. The Lineweaver-Burk plots exhibited negative intercepts, contradicting the theoretical requirement of positive slope and intercept (DeLisa et al., 2017). Similarly, Eadie-Hofstee plots demonstrated positive slopes and intercepts, directly opposing the expected negative slope and positive y-intercept configuration. The Hanes-Woolf method produced negative slopes with positive intercepts and exhibited particularly poor correlation (R^2 = 0.41 – 0.56). The consistent emergence of negative values for fundamental kinetic parameters underscores the inherent limitations of linear transformation approaches for enzymatic kinetic analysis (Maini, 2003; Marasović et al., 2017).

Table 1. Comparison of kinetic parameters obtained by linear transformation methods

Method	Temperature °C	K_m (mM)	V_m (mM/min)	R^2
Lineweaver-Burk	6	15.38	-3.41	0.85
	24	7.82	-1.85	0.91
Eadie-Hofstee	6	-12.28	-2.30	0.83
	24	-8.80	-2.24	0.80
Hanes-Woolf	6	-17.93	-4.30	0.41
	24	-112	-33.30	0.56

The emergence of negative kinetic parameters from linear transformation methods represents a systematic failure of these approaches rather than a consequence of random experimental error. While data scatter and the specific substrate concentration range employed certainly contributed to the magnitude of the bias, the consistent production of physiologically impossible negative values across all three linear methods, despite high-quality raw data that yielded meaningful parameters via non-linear regression, demonstrates that the problem is methodological rather than data-quality related. The transformations inherently distort the error structure: at low substrate concentrations, small absolute errors in 1/*S* become greatly magnified in *1/V* space, and these high-leverage points disproportionately influence the regression line. Additionally, if the substrate range does not adequately bracket the true K_m, extrapolation to the axes can produce intercepts with incorrect signs. The fact that the same dataset, when appropriately analyzed by non-linear regression, yielded positive, physiologically meaningful parameters confirms that the negative values are artifacts of the transformation process itself.

3.2.2 Non-Linear Regression

The robustness of the non-linear regression fitting is visually demonstrated in Figure 4, which presents a scatter plot comparing observed versus predicted reaction rates. The tight clustering of data points along the line of identity. The physiologically meaningful parameters obtained through non-linear regression, which logically reflect expected temperature effects, contrast sharply with the nonsensical negative values often produced by linear transformation methods. This

methodological advantage proves crucial for detecting biologically significant changes in kinetic parameters and enables accurate modeling of enzyme behavior across environmental conditions (Transtrum et al., 2015; Marasović et al., 2017).

The residual plots indicated a proper fit with values reported in Table 2 as V_m and K_m at 24°C as 659 mmol·L^{-1}·min^{-1} and 962 mmol·L^{-1} respectively. However, the values obtained at 6°C were 25.6 mmol·L^{-1}·min^{-1} and 69.9 mmol·L^{-1}respectively. The 26-fold reduction in V_m with decreasing temperature follows Arrhenius behavior and reflects the temperature dependence of the catalytic rate constant (Johnson, 1992; Piskulich et al., 2019) . Concurrently, the 14-fold decrease in K_m suggests enhanced enzyme-substrate affinity at lower temperatures, potentially resulting from improved stability of the enzyme-substrate complex under reduced thermal energy conditions (Daniel et al., 1996). This phenomenon aligns with thermodynamic principles governing enzyme-substrate interactions, where lower temperatures can shift equilibrium toward complex formation (Segel, 1975).

Table 2. Michaelis-Menten Parameters Estimated by Non-linear Regression of Progress Curve Data

Temperature °C	K_m (mM)	V_m (mM/min)	RSS
6	69.9	25.6	2.844
24	962	659	8.579

The K_m values obtained from non-linear regression (962 mM at 24°C and 69.9 mM at 6°C) are substantially higher than typically reported for purified catalase preparations, which generally range from 20–110 mM depending on source and assay conditions (Chelikani et al., 2004). Several factors may explain this discrepancy. First, the use of crude extract rather than purified enzyme introduces competing substrates, alternative hydrogen peroxide-consuming reactions (e.g., peroxidase activity of hemoglobin), and potential enzyme inhibitors that can increase apparent K_m (Heckmann & Paradisi, 2025). Second, the progress curve method employed differs from initial velocity measurements; integrated analysis over the entire reaction course can yield different parameter estimates compared to initial rate approaches, particularly when enzyme stability or product inhibition become factors (Fernley, 1974; Murugan, 2025). Recent theoretical work confirms that progress curve methods require careful consideration of reaction timescales to obtain reliable estimates, and parameter accuracy depends strongly on whether data collection captures regimes with maximum curvature in the kinetic trajectory (Murugan, 2025). Third, the substrate concentration range examined may not have adequately captured the approach to saturation, making precise K_m estimation difficult, a common limitation when K_m exceeds the experimentally accessible concentration range. Fourth, the pronounced temperature sensitivity of K_m (14-fold decrease from 24°C to 6°C) suggests that enzyme-substrate affinity is highly temperature-dependent (Siddiqui et al., 2005), and literature values obtained at different temperatures may not be directly comparable. Recent studies emphasize that temperature can alter K_M by several orders of magnitude (Erkanli et al., 2025), underscoring the importance of considering thermal effects when comparing kinetic parameters across studies. These considerations reinforce the importance of interpreting kinetic parameters as condition-dependent estimates rather than absolute constants.

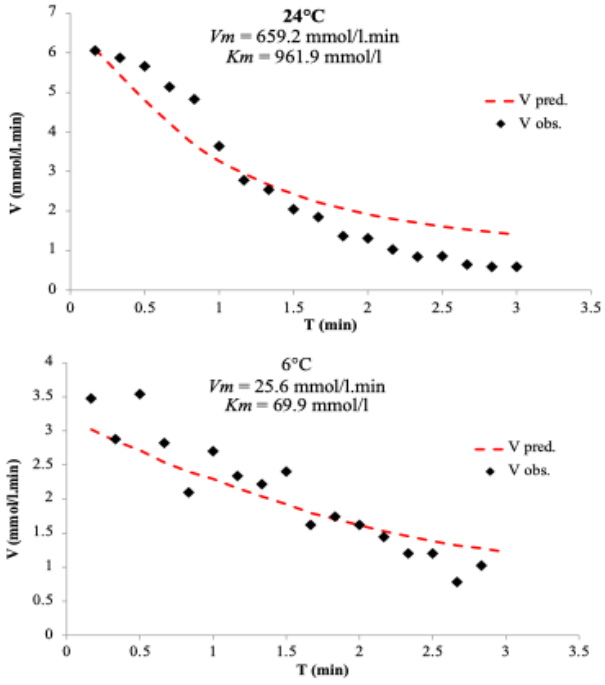

Fig. 4. Observed and predicted values of V from non-linear regression at 24°C and 6°C.

3.3 The Importance of Cautious Interpretation

While non-linear regression provides statistically superior estimates of V_m and K_m compared to

linearization methods, it is crucial to exercise caution in their interpretation. Regression analysis, by its nature, is an optimization algorithm that finds the parameter values that minimize the error (e.g., the sum of squared residuals) between the model (the Michaelis-Menten equation) and the experimental data. As such, the resulting parameters are the statistically most likely values given the model and the specific dataset, but they are not necessarily the absolute, physiologically "true" values (Motulsky & Ransnas, 1987).

The potential for exaggerated or skewed parameter estimates arises from several factors:

i. Inherent Parameter Correlation: In the Michaelis-Menten equation, V_m and K_m are highly correlated. The algorithm can often achieve a similarly good fit by increasing both V_m and K_m or decreasing them together (Atkins, 2006). This means there can be a "ridge" of nearly equivalent solutions in the parameter space, and the final values can be sensitive to small variations or noise in the data (Johnson, 1992). The dramatic 14-fold change in K_m observed between 24°C and 6°C, while plausible, must be viewed in this context.

ii. Limitations of the Experimental Design: The accuracy of the estimated parameters is heavily dependent on the range and distribution of the substrate concentrations used. If the data does not adequately bracket the true K_m value, for instance, if most data points are collected at saturating conditions ([S]>>K_m), the estimate for K_m can be highly uncertain and potentially exaggerated, as the curve-fitting process will extrapolate to find the half-saturation point with limited constraint (Cornish-Bowden, 2013).

iii. Model Assumptions and Oversimplification: The Michaelis-Menten model itself is a simplification of enzyme kinetics. It assumes a single catalytic pathway and does not account for more complex phenomena like substrate inhibition, enzyme cooperativity, or the presence of isoenzymes with different kinetic properties. If such complexities are present but unaccounted for, the regression will still force a simple hyperbolic fit, potentially resulting in skewed V_m and K_m values that represent a "compromise" rather than a true mechanistic constant (Goličnik, 2012).

In a nutshell, while non-linear regression is the gold standard for parameter estimation, its outputs are best estimates, not infallible truths. The values of V_m and K_m should be interpreted as a coupled pair that defines the curve's shape, with a clear understanding that their individual magnitudes can be influenced by experimental design and data variance. The dramatic temperature effect observed is compelling, but its quantitative magnitude should be considered within these statistical and methodological constraints.

4 Conclusions

This investigation has unequivocally established that, under the specific experimental conditions employed, the catalase-mediated decomposition of hydrogen peroxide exhibits apparent second-order kinetics, with rate constants of 0.13 $L \cdot mmol^{-1} \cdot min^{-1}$ at 24°C and 0.064 $L \cdot mmol^{-1} \cdot min^{-1}$ at 6°C. The calculated activation energy of 28.5 $kJ \cdot mol^{-1}$ falls within the characteristic range for enzyme-catalyzed reactions and provides a quantitative explanation for the observed temperature sensitivity, wherein an 18°C temperature reduction resulted in a two-fold decrease in the reaction rate. Methodologically, this study demonstrates the fundamental superiority of non-linear regression over linear transformation techniques for estimating enzyme kinetic parameters. The traditional linear methods, Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf plots, consistently produced statistically invalid results, including physiologically meaningless negative values for both V_m and K_m across all transformations. In stark contrast, non-linear regression of the identical dataset yielded robust, physiologically interpretable parameter estimates (V_m = 659 and 25.6 $mmol \cdot L^{-1} \cdot min^{-1}$; K_m = 962 and 69.9 $mmol \cdot L^{-1}$ at 24°C and 6°C, respectively). This study further demonstrates that while non-linear regression is indispensable for accurate kinetic analysis, its outputs must be interpreted within appropriate statistical and experimental contexts to ensure biologically relevant conclusions

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