



Quantification of Chloride Inhibition of Oxygen Reduction in Bilirubin Oxidases using Protein Film Electrochemistry

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Background

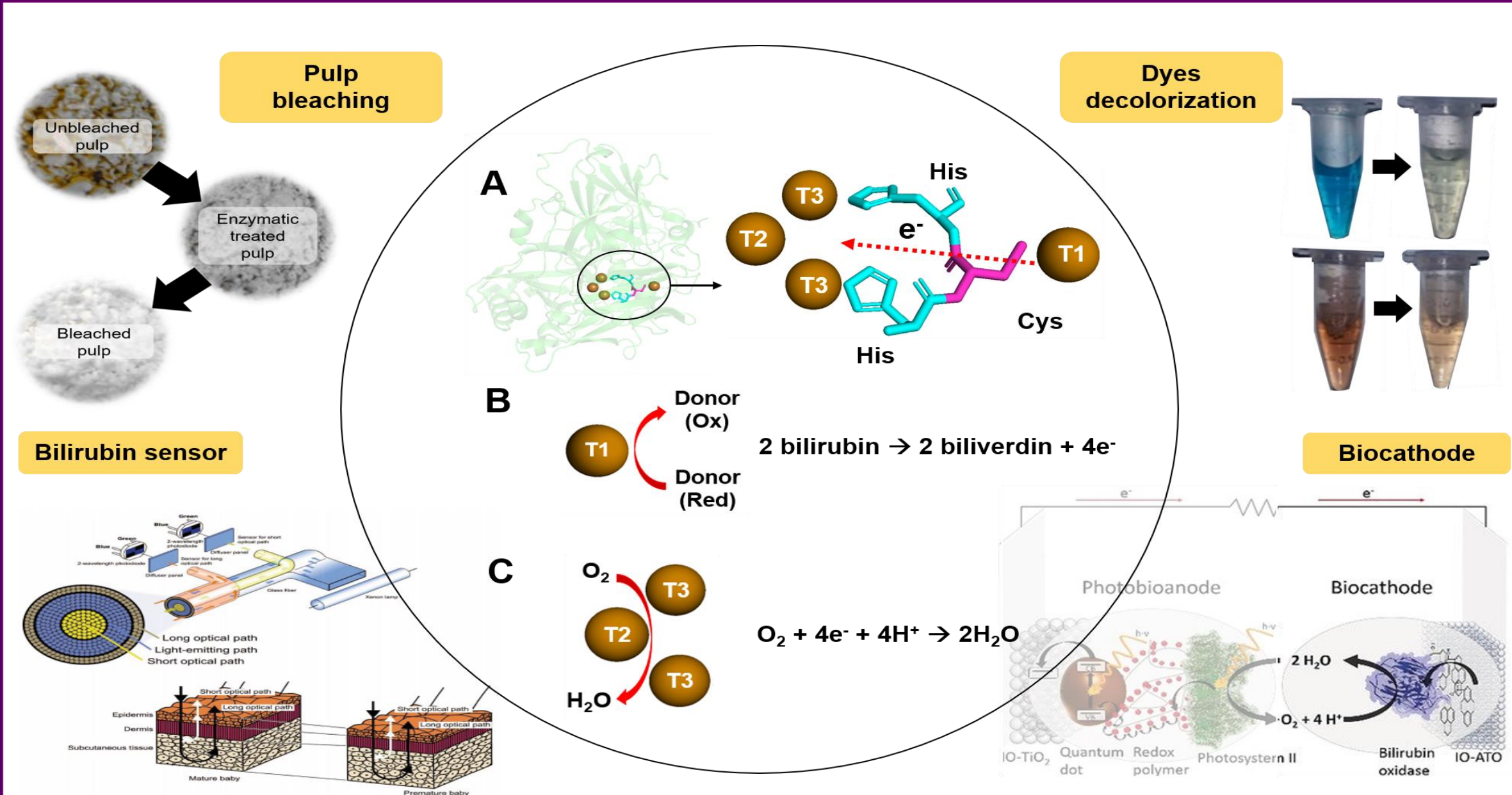


Fig. 1 (A) Structure of bilirubin oxidase from *Magnaporthe oryzae* (PDB: 2L9Y) and of the active sites, (B) Oxidation of bilirubin at T1Cu site and (C) Reduction of oxygen at TNC, surrounded by its industrial applications.¹⁻³

Objectives

1. To investigate the effect of temperature on BODs-catalysed ORR measurements.
2. To quantify chloride inhibition in MoBOD and BpBOD using PFE technique.

Techniques – Protein Film Electrochemistry and Model Discrimination

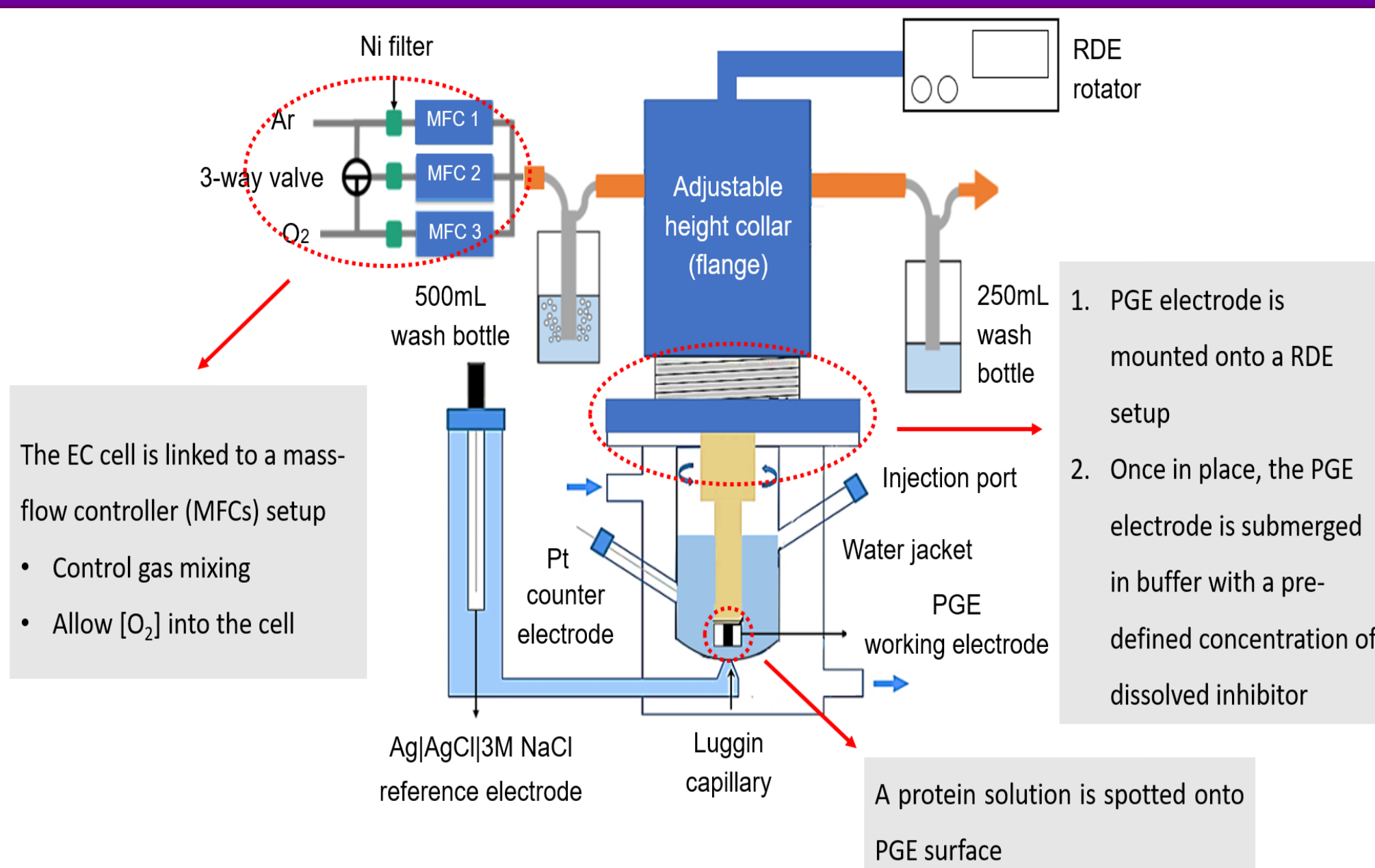


Fig. 2 Schematic of electrochemical cell used for bioelectrocatalytic assays. Orange arrows represent the gas flow path. The blue arrows show the path for the temperature-controlled water flowing around the analysis compartment.

Model	Reaction Scheme	Eadie-Hofstee Plot
Competitive (CI)	$E + S \xrightleftharpoons{K_S} ES \xrightarrow{k_{cat}} E + P$ $K_i + I \rightleftharpoons EI$	
Uncompetitive (UCI)	$E + S \xrightleftharpoons{K_S} ES \xrightarrow{k_{cat}} E + P$ $-1 \rightleftharpoons EI \xrightleftharpoons{\alpha K_i} EIS$	
Mixed (MI)	$E + S \xrightleftharpoons{K_S} ES \xrightarrow{k_{cat}} E + P$ $K_i + I \rightleftharpoons EI \xrightleftharpoons{\alpha K_i} EIS$	
Hyperbolic (HI)	$E + S \xrightleftharpoons{K_S} ES \xrightarrow{k_{cat}} E + P$ $EI \xrightleftharpoons{\alpha K_S} EIS \xrightarrow{\beta k_{cat}} EI + P$	

Data Analysis

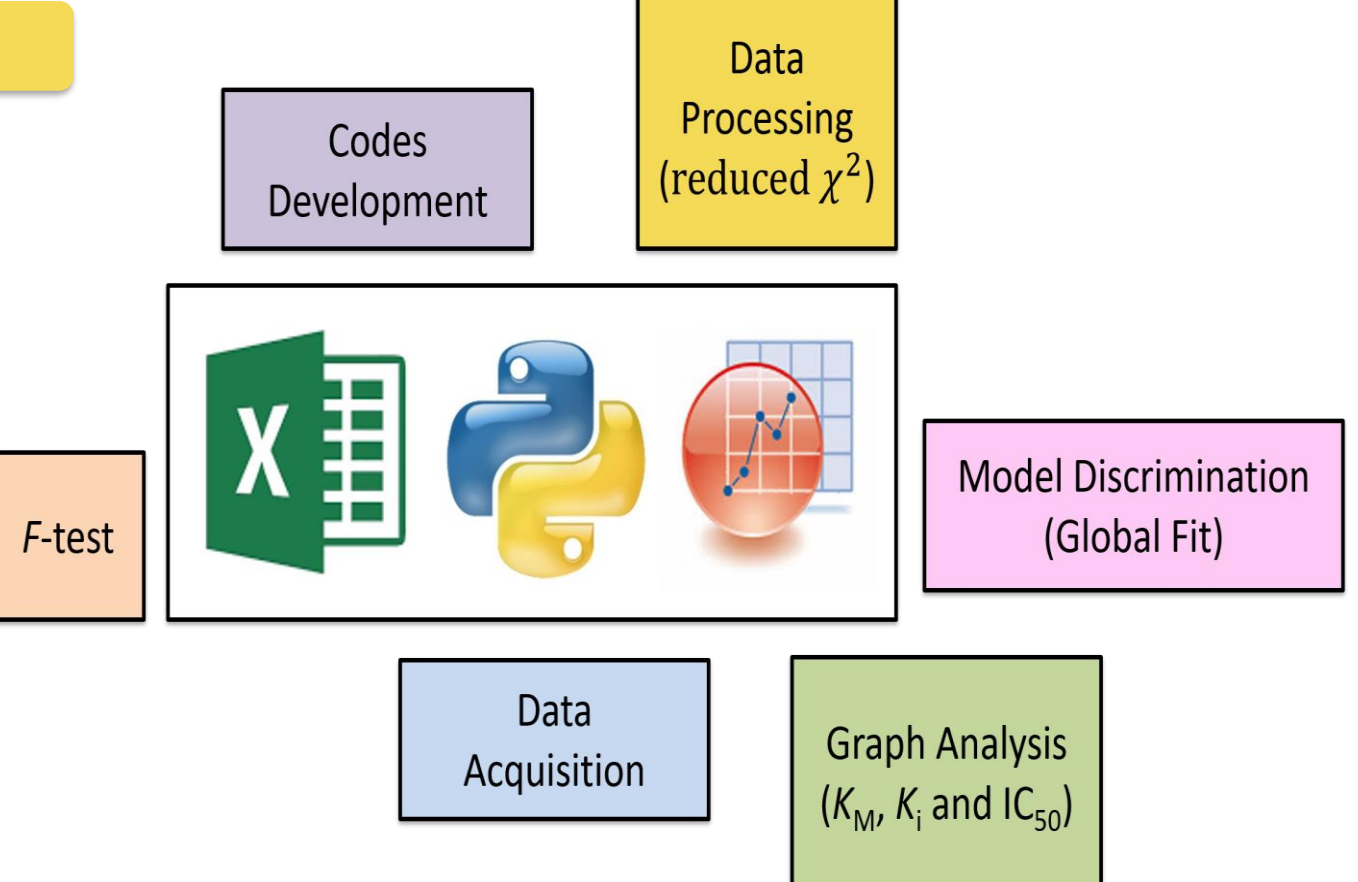


Fig. 3 Effects of EH plot on relative reaction rates. Black: no inhibitor, dark green: $[X^-] = 0.5$ mM, green: $[X^-] = 2.5$ mM, pink: $[X^-] = 10$ mM). Lines in MI indicate the value of α when $\alpha \neq 0$ (solid: $\alpha > 1$, dash: $\alpha = 1$, dot: $\alpha < 1$). Lines in HI represent α and β values when $\alpha > 1$ and $\beta \neq 0$ (solid: $\beta = 10^1$, dash: $\beta = 10^{0.4}$, dot: $\beta = 10^{-1}$)

Results – Data Acquisition and Model Discrimination

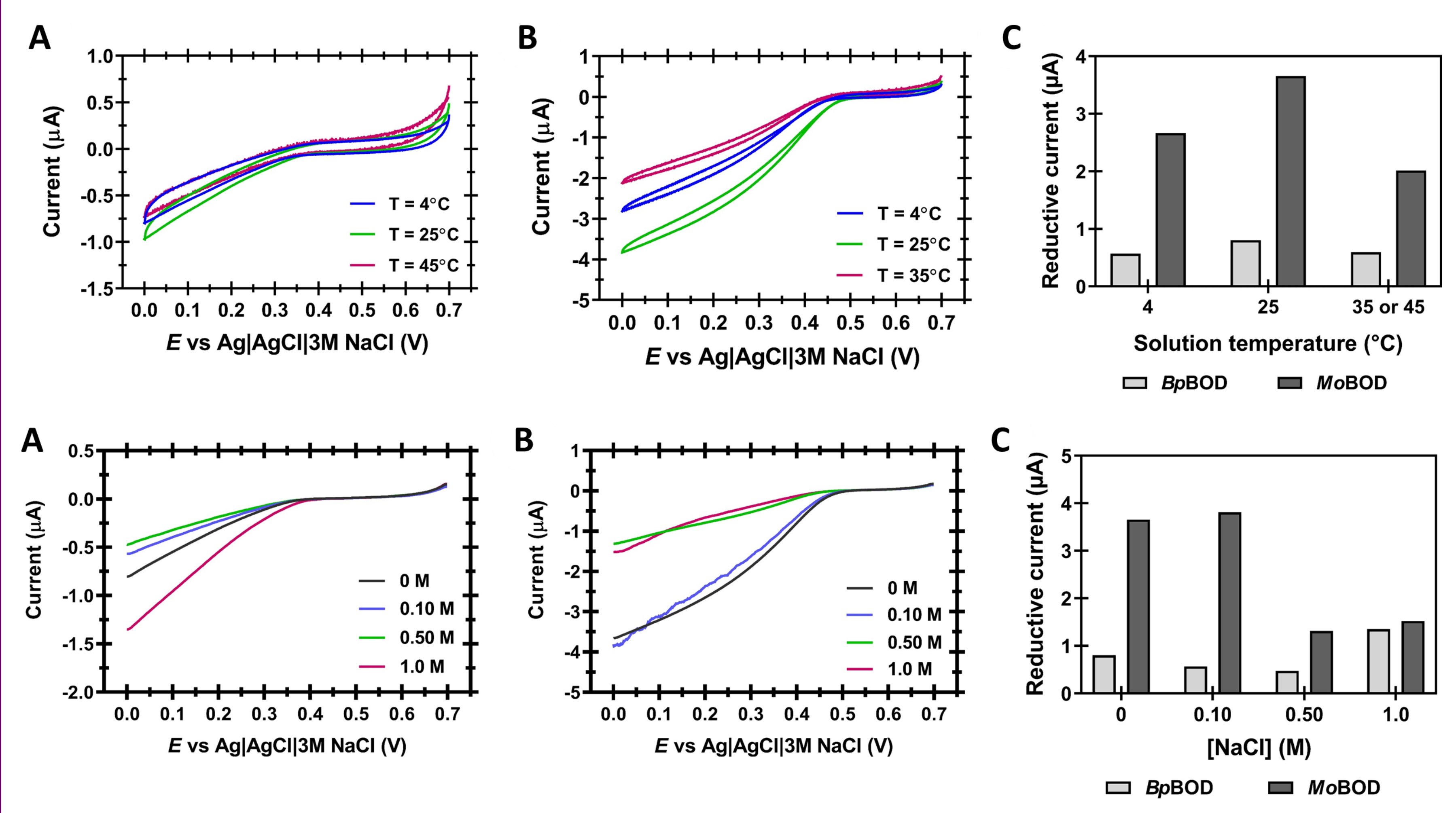


Fig. 4 Cyclic voltammograms of PGE electrode modified with (A) *BpBOD*, and (B) *MoBOD* under 100% O_2 at various temperatures. (C) Dependence of the catalytic currents on the Cl^- concentrations for both BODs recorded at 0 V vs ref based on grouped temperatures. Conditions: A = 0.02 cm^2 , 200 mM phosphate-100 mM citrate buffer, pH 7.0, RDE speed = 1630 rpm, scan rate = 10 $mV s^{-1}$, total gas flow rate = 1920 sccm.

Fig. 5 Cyclic voltammograms of PGE electrode modified with (A) *BpBOD*, and (B) *MoBOD* under 100% O_2 at 0 M Cl^- (black), 0.10 M Cl^- (blue), 0.50 M Cl^- (green) and 1.0 M Cl^- (red). (C) Dependence of the catalytic currents on the Cl^- concentrations for both BODs recorded at 0 V vs ref based on grouped Cl^- concentrations. Conditions: A = 0.02 cm^2 , 200 mM phosphate-100 mM citrate buffer, pH 7.0, T = 25°C, RDE speed = 1630 rpm, scan rate = 10 $mV s^{-1}$, total gas flow rate = 1920 sccm.

Model Discrimination

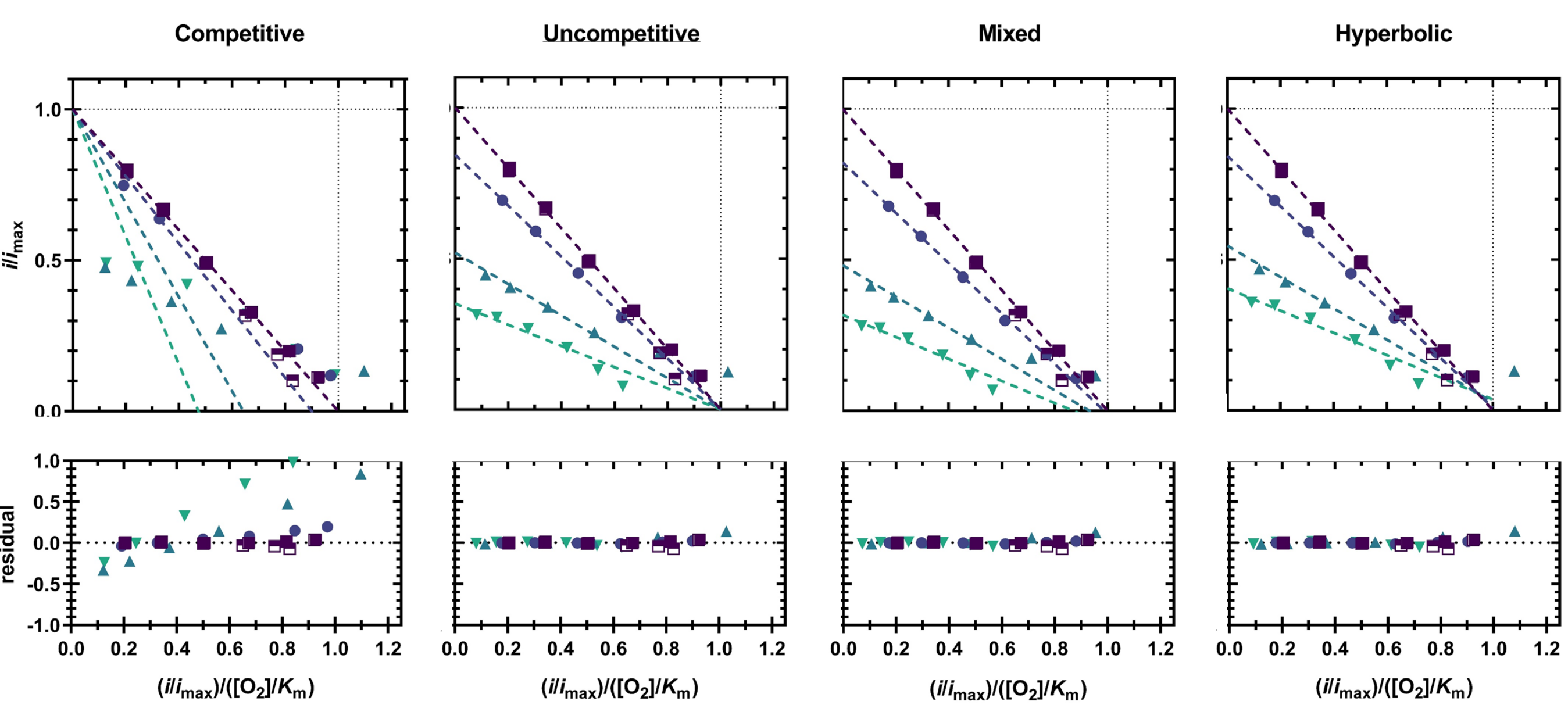


Fig. 6 Graphical representation of how the four inhibition models represented by Eadie-Hofstee plot (solid lines) fit the normalised currents (scatter) collected for *MoBOD*-catalysed O_2 reduction inhibited by NaCl (above) and residual of the goodness of fit plots (bottom). No Cl^- (■), 0.10 M Cl^- (●), 0.50 M Cl^- (▲), and 1.0 M Cl^- (▼). Conditions: $E = 0.10$ V vs ref, pH = 7, T = 25°C, RDE speed = 3560 rpm, 200 mM phosphate-100 mM citrate buffer pH 7.0, scan rate = 10 $mV s^{-1}$, total gas flow rate = 1920 sccm.

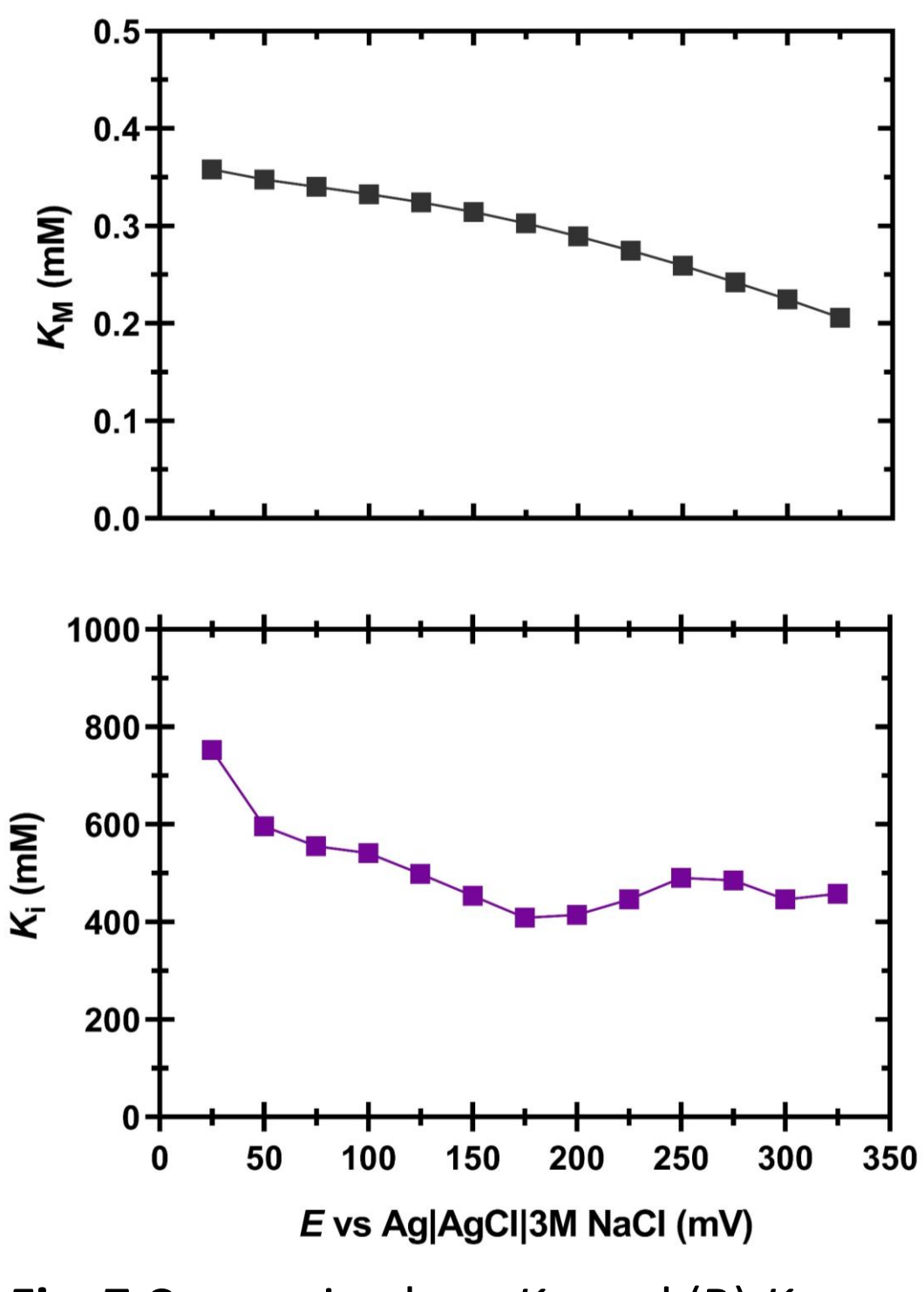


Fig. 7 Comparing how K_M and (B) K_i vary with increasing potential for Cl^- inhibition of *MoBOD* at pH 7.0 and T = 25°C. The data shown in both panels represent the favoured uncompetitive inhibition model.

References

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References

