Key References:

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Oxygen Supply to Biooxidation Reactions:
Quantification of Glucose Oxidase Reaction Kinetics

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1. Motivation & Objective

In order to study the oxygen supply for a reaction catalysed by an oxidase, the kinetic parameters for the enzyme such as k_{cat}, $K_{m\ gluc}$ and $K_{m\ O}$ have to be quantified in order to precisely assess the oxygen requirements towards realising a greater efficiency of the enzyme.

This contribution aimed to **determine the kinetic parameters** (k_{cat}, $K_{m\ gluc}$ and $K_{m\ O}$) **for two glucose oxidases (GOXs) form two different organisms, GOX-I and GOX-II.** GOX catalyses the oxidation of glucose to glucono-lactone with the production of hydrogen peroxide (H_2O_2), using molecular oxygen.

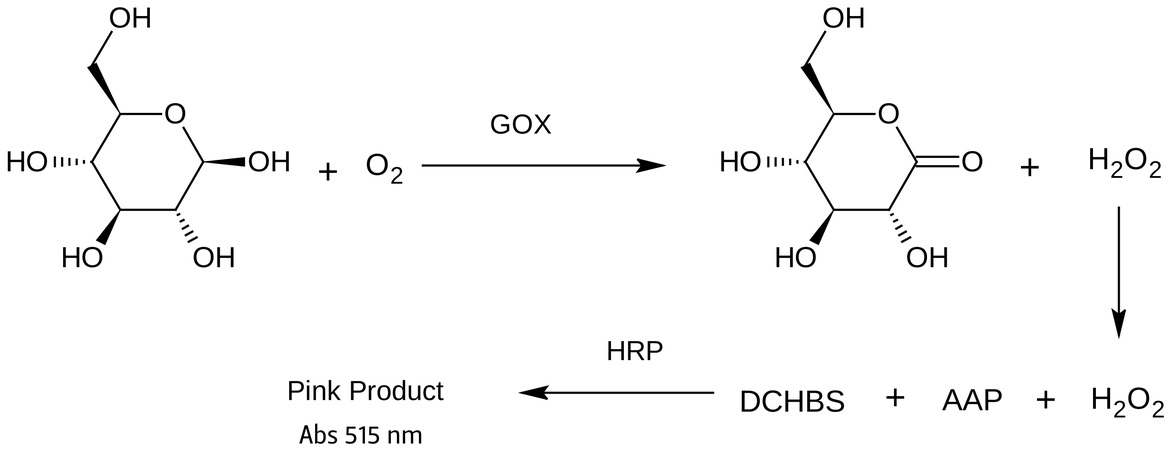
2. Methods

With regards to characterising the GOXs, the enzyme kinetics as function of substrate concentration were studied.

The kinetic parameters were found by two different methods:

- Spectrophotometric Activity Assay (under atmospheric conditions)

The activity of GOX was measured using an assay that describes the quantification of glucose oxidase activity by a secondary reaction with the hydrogen peroxide. Horseradish peroxidase (HRP) catalyses the reaction between 4-aminoantipyrine (AAP), sodium 3,5-dichloro-2-hydroxybenzenesulfonate (DCHBS) and hydrogen peroxide that forms a pink product. The formation of product can be measured by an increase in absorbance at 515 nm in a spectrophotometer.



- Tube-in-Tube reactor (TiTr)

The experiments were performed in a tubular flow reactor where the initial rates were found for different glucose concentrations and different oxygen concentrations. One of the particular features of this apparatus is the possibility to operate under pressure, which allows an increase and control of the oxygen concentration in the reaction. Gluconic acid was measured by UV-Vis detection, as carried out in HPLC.

3. Results and Discussion

The kinetic constants for the two GOXs, k_{cat} $K_{m\ S}$ and $K_{m\ O}$, were determined. The $K_{m\ S}$ and k_{cat} were found by the analytical assay as well as in the Tube-in-Tube reactor. $K_{m\ O}$ was found in the Tube-in-Tube reactor.

Table 1 – Kinetic parameters estimated for the two GOXs by two different methods.

	k_{cat} (µmol/min/$mg_{protein}$)		$K_{m\ S\ apparent}$ (mM)		$K_{m\ S}$ (mM)	$K_{m\ O}$ (mM)
	Assay	TiTr	Assay	TiTr	TiTr	TiTr
GOX-I	35,0*1	97,56	7,21	5,99*2	29,59	1,073
GOX-II	65,3*1	120,9	31,4	20,8*2	73,59	0,6932

*1 These are apparent k_{cat} values estimated based on lower concentration of oxygen

*2 These values were estimated based on the $K_{m\ S}$ obtained in the TiTr by the following equation: $K_{m\ S\ apparent} = K_{m\ S} / (1 + (K_{m\ O}/p_{O2}))$

The parameters estimated in the TiTr were fitted following the equation: $v = (k_{cat} C_{O2} C_S) / (C_{O2} C_S + K_{m\ S} C_{O2} + K_{m\ O} C_S)$

The Tube-in-Tube reactor was pressurized to 6 bar in order to achieve oxygen concentrations above that ofatmospheric pressure. With this system it was possible to quantify the different kinetic constants without the enzyme being under oxygen limiting conditions (oxygen solubility in water **0.268 mM**, air at 1 atm, 25 °C). The apparent $K_{m\ S}$ was estimated for the Tube-in-Tube reactor allowing a comparison with the results obtained from the spectrophotometric activity assay. The results from the two different methods are consistent.

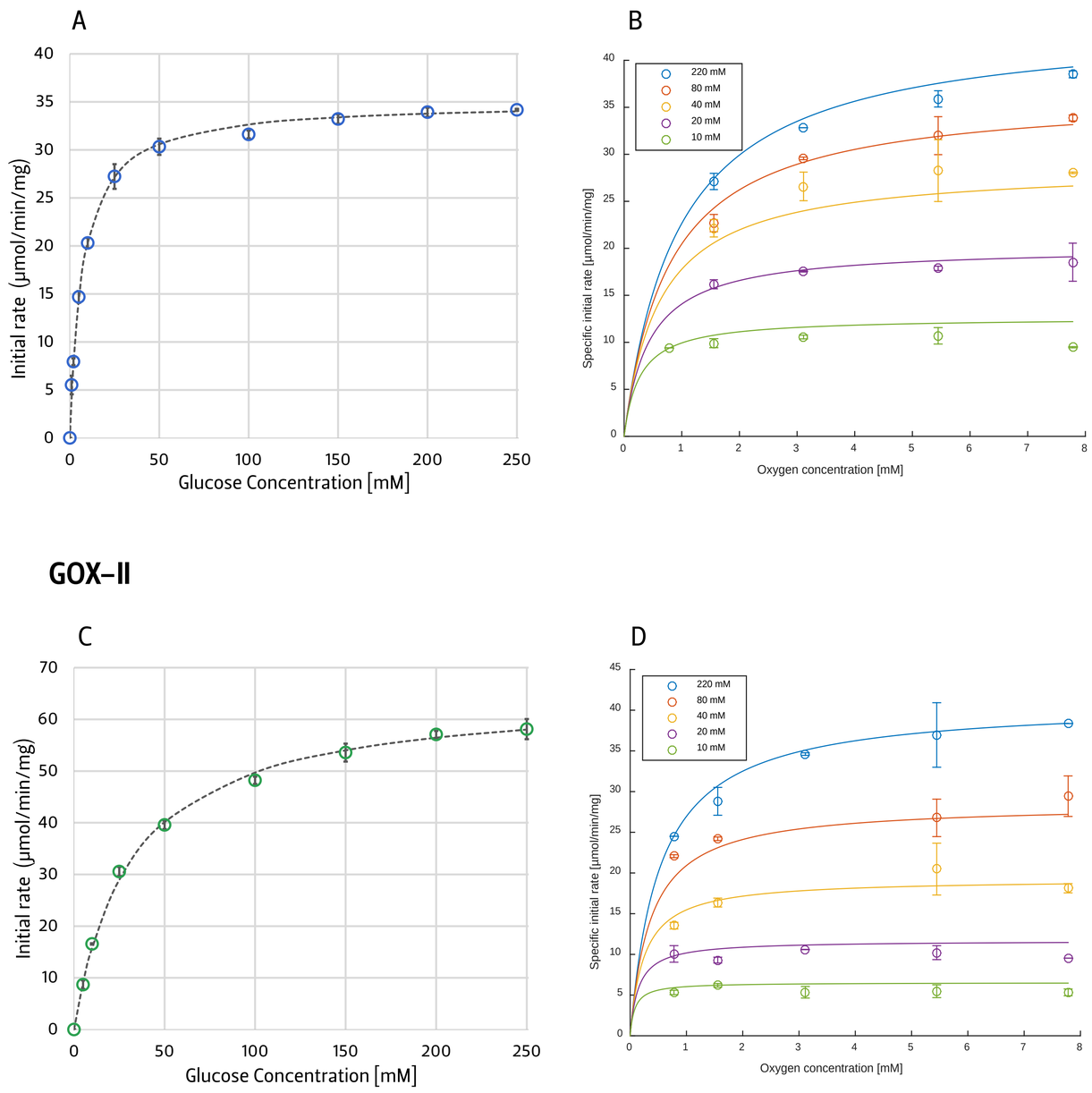


Figure 1 – Enzyme activity as a function of glucose concentration for GOX-I (A) and GOX-II (C), obtained by the spectrophotometric assay. The assay was performed at room temperature and under atmospheric pressure in 1 mL cuvettes with phosphate buffer at pH 7.5. The reaction for each substrate concentration was performed in triplicate; error bars reflect standard deviation. The Michaelis-Menten kinetic estimation is represented by the dotted line (A and C).
Enzyme activity as a function of oxygen concentration for GOX-I (B) and GOX-II (D), obtained in the Tube-in-Tube reactor. The experiments were carried at 25 °C and up to 6 bar pressure.

6. Conclusions

- **The Tube-in-Tube reactor is an elegant and convenient apparatus to quickly determine enzyme kinetics under varying oxygen conditions**
- **For both enzymes the $K_{m\ O}$ is higher than the solubility of oxygen in water under atmospheric pressure. Under this condition, the enzyme kinetics is oxygen limited. Therefore, the $K_{m\ O}$ can be an interesting target for protein engineering research**
- **To work at a higher efficiency of these enzymes, reactions must be carried at concentrations above the $K_{m\ O}$ and $K_{m\ S}$, and alternative methods to bubbling air to a reactor for oxygen supply should be considered**

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