Kinetic Measurements of Oxidase Enzymes

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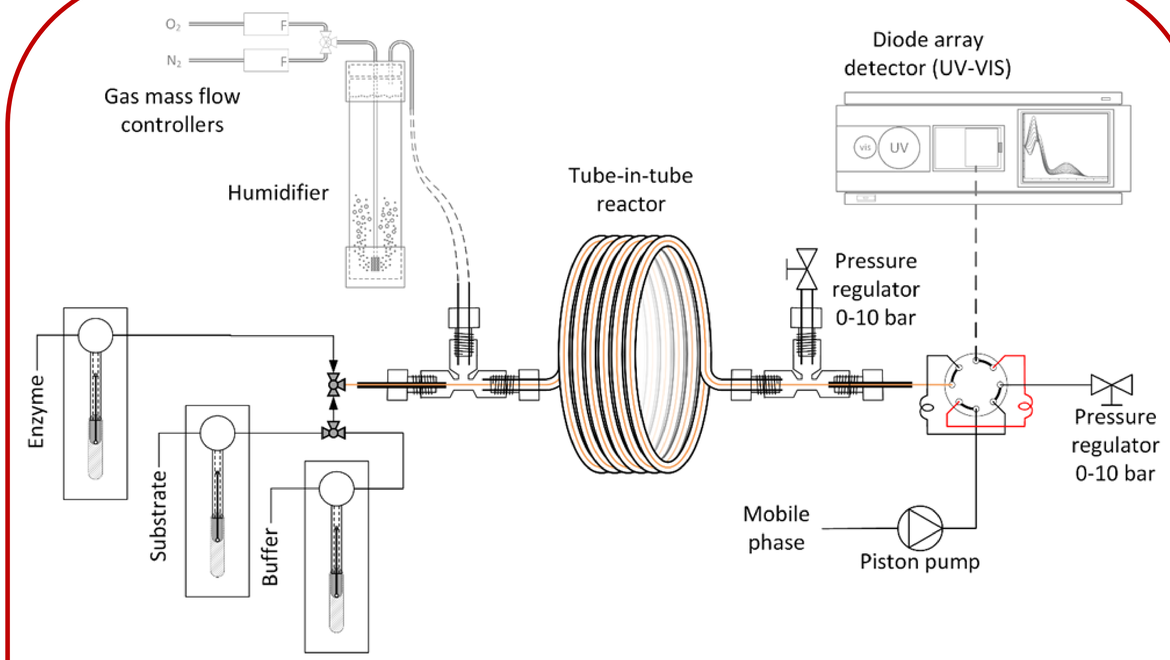
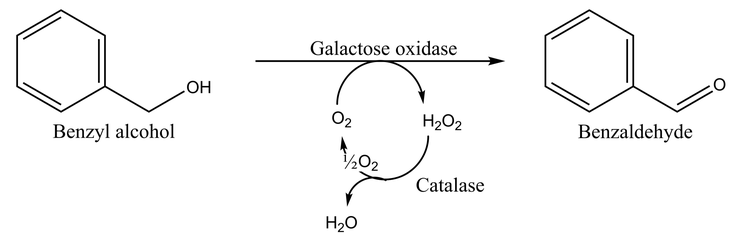
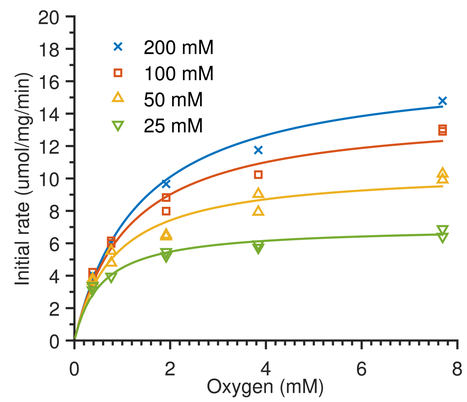
Oxidases follow the well-established ping-pong bi-bi mechanism made up of two half-reactions. Accordingly, this forms the basis for the rate law, which is dependent upon both the concentration of the primary substrate, as well as the concentration of oxygen. Since the solubility of oxygen in aqueous medium is rather low (265 µM at 25 °C in equilibrium with air at 1 atm), it follows that the supply rate of oxygen will be also be limited. However of even greater importance for effective use of the enzyme is that concentrations of both substrates should be above their respective Michaelis constants for the majority of the reaction time. Interestingly, the BRENDA database indicates that the Michaelis constant for oxygen (K_{MO}) of many oxidases is relatively high (in comparison to its solubility). Consequently, many oxidases cannot reach their maximum rate of reaction and determining the K_{MO} for a given enzyme reveals this. We have developed a novel automated instrument, based on tube-in-tube flow technology to achieve rapid and accurate estimates of the constants.

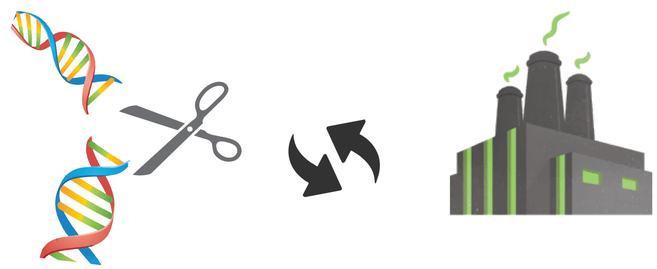


- Precise control of oxygen concentration from 0 to 12.8 mM (10 bar O_2)
- Completely autonomous operation
- Full enzyme characterization in 12 h (40 initial rates)



- Measured K_{MO} values sufficiently high to challenge the effective use of the enzyme
- Provides guidance to operate at high oxygen partial pressure and/or to lower K_{MO} by protein engineering





- Building a database of K_{MO} values
- Interest in studing other cases, including different biocatalyst formats
- Interest in studying cases in different media

Ringborg, R.H. and Woodley, J.M. (2016). Reaction Chem Engng. **1**, 10-22

Ringborg, R.H., Toftgaard Pedersen, A. and Woodley, J.M. (2017). *ChemCatChem,* **9**, 3285-3288

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The ROBOX project has received funding from the European Union (EU) project ROBOX (grant agreement n° 635734) under EU's Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1. Any statements herein reflects only the author's views and that the European Union is not liable for any use that may be made of the information contained therein.