Process considerations for use of galactose oxidase as an industrial biocatalyst

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Publication date:
2015

Document Version
Peer reviewed version

Process considerations for use of Galactose Oxidase as an industrial biocatalyst

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Introduction

In nature galactose oxidase (GOase, EC.1.1.3.9) catalyses the oxidation of the C6 hydroxyl group of D-galactose to the corresponding aldehyde, while reducing molecular oxygen to hydrogen peroxide. In recent years a great effort has been made to broaden the substrate scope, enabling GOase to oxidize C6-OH of glucose and fructose, as well as secondary alcohols to ketones. The widened substrate scope of GOase opens up many important industrial applications, such as synthesis of industrially relevant compounds containing aldehydes and ketones (e.g. the oxidation of 5-hydroxymethylfurfural to diformylfuran), deracemization of secondary alcohols, and modification of a wide range of naturally occurring polysaccharides [1,2]. Despite these promising characteristics of GOase, application at industrial scale has not been achieved so far. This can in part be ascribed to the process challenges experienced when performing oxidative biocatalysis at a large scale.

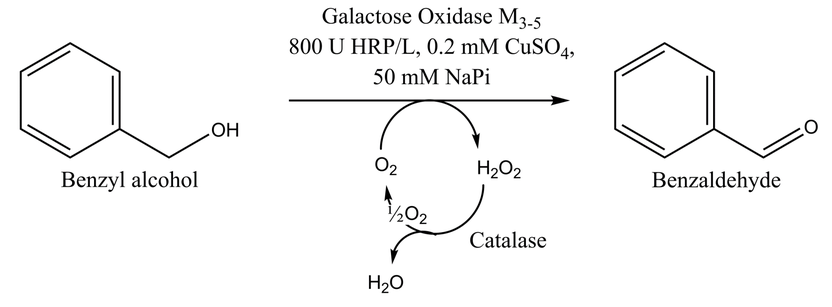
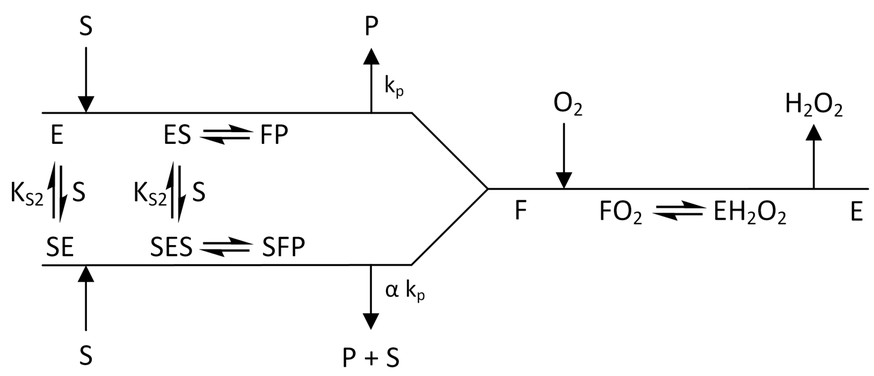


Figure 1. Model reaction used to study the potential of galactose oxidase.

Kinetic modelling



$$\frac{\mathrm{r}}{V_{\max}} = \frac{S\,O\left(1+\alpha\frac{S}{K_{S2}}\right)}{(S\,O + \mathrm{K_{mO}}\,S\, + \mathrm{K_{mS}}\,O)\left(1+\frac{S}{K_{S2}}\right)}$$

Table 1. Kinetic parameters obtained by non-linear regression of the rate expression to initial rate data.

Parameters	Estimated value
k_{cat}	15 µmol/min/mg CFE
K_{mS}	36.8 mM
K_{mO}	3.0 mM
K_{S2}	196.5 mM
α	3.06 mM

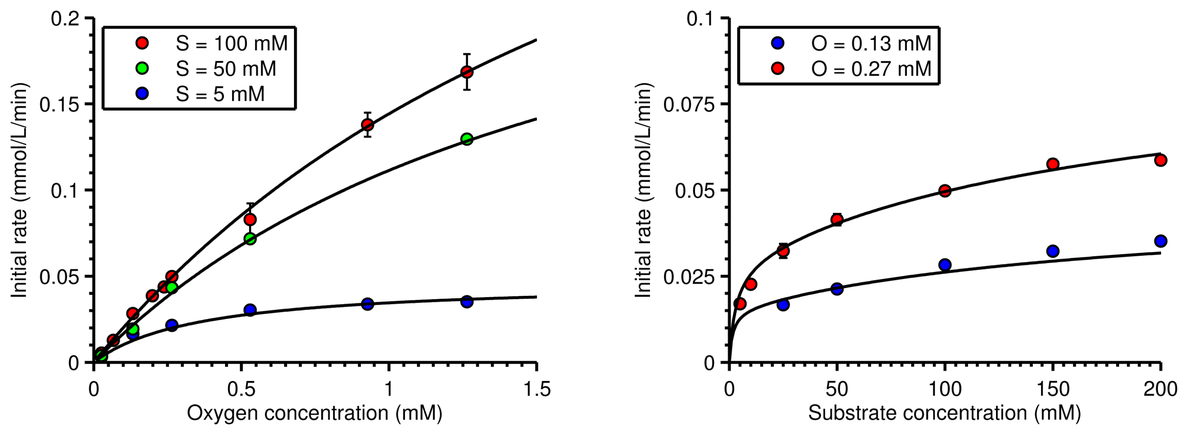


Figure 2. Fit of ping-pong bi bi mechanism with substrate activation to initial rate data.

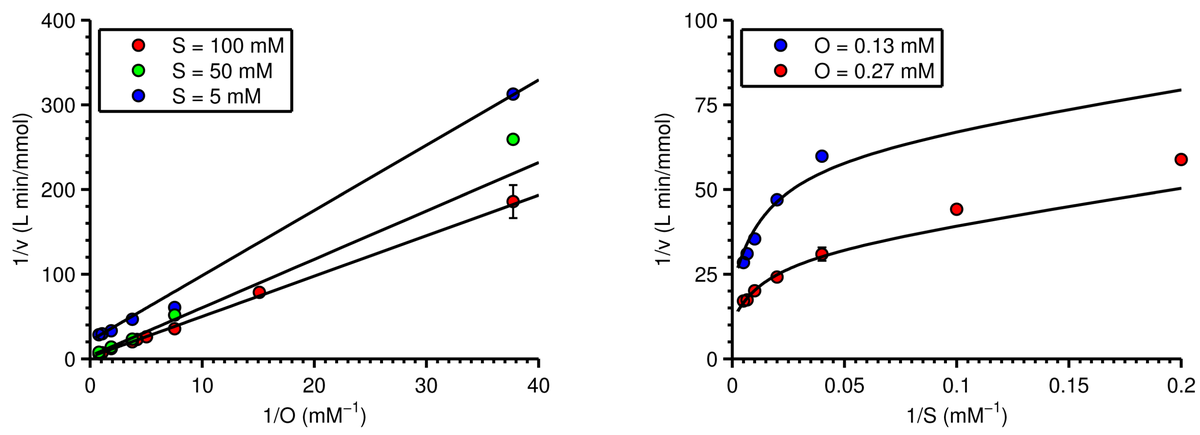


Figure 3. Lineweaver-Burk plot of initial rate data. Clearly, the data does not produce parallel straight lines as dictated by the standard ping pong bi bi mechanism.

Process considerations

Oxygen supply

The high K_m for oxygen relative to the solubility of oxygen reveals a trade-off between supplying oxygen sufficiently fast and utilizing the enzyme most efficiently:

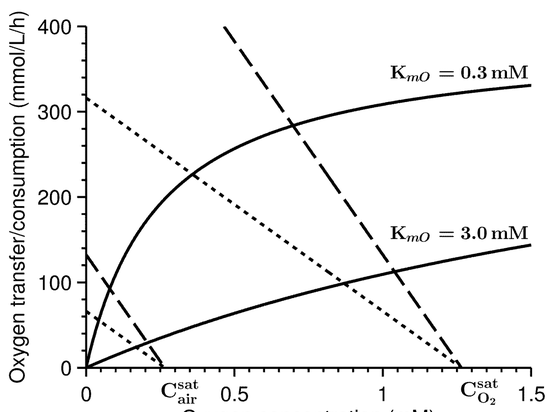


Figure 4. Rate of oxygen transfer to the reactor using air ($C^{sat}_{air} = 0.265$ mM) or pure oxygen ($C^{sat}_{air} = 1.27$ mM) at two different volumetric mass transfer coefficients (--) k_La = 250 h^{-1} (– –) k_La = 500 h^{-1}, and the enzymatic rate of oxygen consumption at two different $K_{m,O}$ as a function of oxygen concentration.

Enzyme stability

The stability of enzymes are known to be affected by process related parameters such as the gas-liquid interface created upon aerating with air. This is not the case for GOase.

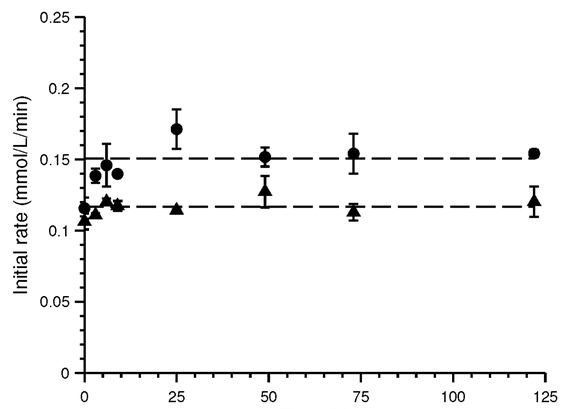


Figure 5. Stability of GOase in the absence of substrate or product exposed to stirring at 250 rpm with aeration (air; 0.5 vvm) at 25 °C (●) and stirring without aeration at 25 °C (▲). (--)The average value in the interval 3-122 h.

Substrate and product volatility

Oxygen supply by bubbling with air might cause volatile compounds in the reaction mixture to be stripped out of solution.

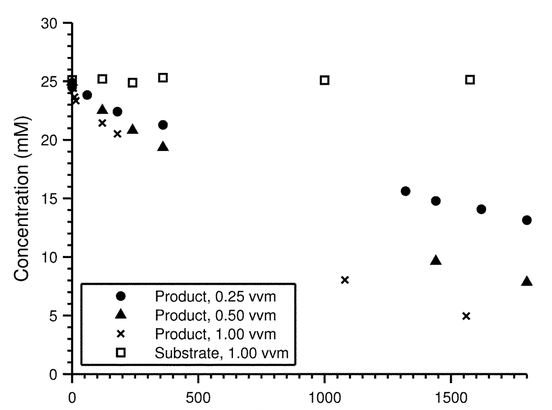


Figure 6. Evaporation of benzyl alcohol (substrate) and benzaldehyde (product) at 25 °C with different aeration rates and 300 rpm stirring.

Conclusions and further challenges

- The high K_m for oxygen relative to the solubility of oxygen results in poor utilization of the enzyme at standard operating conditions. Therefore, the benefits of using enriched air or increased reactor pressure are large.
- The apparent stability of GOase towards bubbling makes the choice of aeration method less critical. However, the operating stability has to be investigated, since this might be significantly different from the stability of non-catalytically active enzyme.
- Volatility of the product is a limiting factor. This may be avoided by using alternative aeration methods, such as dead-end membrane aeration. On the other hand the volatility could be utilized to selectively remove and concentration the product from the reaction mixture.

References:

[1] Escalettes F, Turner NJ, **2008,** Directed evolution of galactose oxidase: generation of enantioselective secondary alcohol oxidases, ChemBioChem 9:857-860

[2] McKenna S, Leimkühler S, Herter S, Turner NJ, Carnell A, **2015**, Enzyme cascade reaction: Synthesis of furandicarboxylic acid (FDCA) and carboxylic acids using oxidases in tandem, Green Chem 44:1-5

BIOOX

"Developing a validated technology platform for the application of oxygen dependent enzymes in synthesis and transformation of alcohols"

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 613849.