Process requirements of galactose oxidase catalyzed oxidation of alcohols - DTU Orbit (08/11/2017)

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Biocatalytic oxidation reactions have the potential to substitute many chemically catalyzed oxidations in the pharmaceutical and fine chemical industry due to their superior regio- and stereoselectivity and low environmental impact. Galactose oxidase (GOase) has been shown to be a promising biocatalyst for the oxidation of primary and secondary alcohols to their corresponding aldehydes and ketones, respectively. However, GOase requires a number of additives to sustain its catalytic function, such as the enzyme catalase for degradation of the byproduct hydrogen peroxide as well as single-electron oxidants to reactivate the enzyme upon loss of the amino acid radical in its active site. In this work, the addition of catalase, single-electron oxidants, and copper ions was investigated systematically in order to find the minimum concentrations required to obtain a fully active GOase. Furthermore, it was found that the concentration and type of buffer is essential for the activity of GOase, which was significantly more active in sodium phosphate buffer than in other buffers investigated. Enzyme stability and oxygen requirements are of crucial importance for the implementation of oxidase based processes. GOase was shown to be completely stable for 120 h in buffer with stirring at 25 °C, and the activity even increased 30% if the enzyme solution was also aerated in a similar experiment. The high K_m for oxygen of GOase (>5 mM) relative to the solubility of oxygen in water reveals a trade-off between supplying oxygen at a sufficiently high rate and ensuring a high degree of enzyme utilization (i.e., ensuring the highest possible specific rate of reaction). Nevertheless, the good stability and high activity of GOase bode well for its future application as an industrial biocatalyst.

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