

Title: Bioconversion of D-glucose into D-glucosone by immobilized glucose 2-oxidase from *Coriolus versicolor* at moderate pressures

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Abstract: The immobilized glucose 2-oxidase (pyranose oxidase, pyranose:oxygen-2-oxidoreductase, EC 1.1.3.10) from *Coriolus versicolor* was used to convert D-glucose into D-glucosone at moderate pressures, up to 150 bar, with compressed air in a modified commercial batch reactor. Several parameters affecting biocatalysis at moderate pressures were investigated as follows: pressure, different forms of immobilized biocatalysts, glucose concentration, pH, temperature and the presence of catalase. Glucose 2-oxidase (GOX2) was purified by immobilized metal affinity chromatography on epoxy-activated Sepharose 6B-IDA-Cu(II) column at pH 6.0. Purified enzyme and catalase were immobilized into a polyethersulfone (PES) membrane in the presence of glutaraldehyde and gelatin. Enhancement of the bioconversion of D-glucose was done by the pressure since an increase in the pressure with compressed air increases the conversion rates. The optimum temperature and pH for bioconversion of D-glucose were found to be 62 degrees C and pH 6.0, respectively and the activation energy (E_a) was 28.01 kJ mol⁻¹. The apparent kinetic constants (V_{max}), K_m , K_{cat} and K_{cat}/K_m for this bioconversion were 2.27 U mg⁻¹ protein, 11.15 mM, 8.33 s⁻¹ and 747.38 s⁻¹ M⁻¹, respectively. The immobilized biomass of *C. versicolor* as well as crude extract containing GOX2 activity were also useful for bioconversion of D-glucose at 65 bar with a yield of 69.9 +/- 3.8% and 91.3 +/- 1.2%, respectively. The immobilized enzyme was apparently stable for several months without any significant loss of enzyme activity. On the other hand, this immobilized enzyme was also stable at moderate pressures, since such pressures did not affect significantly the enzyme activity. (C) 2010 Elsevier Ltd. All rights reserved.

Author Keywords: Biocatalysis at Moderate Pressures; Immobilized Glucose 2-Oxidase from *Coriolus Versicolor*; Immobilization in Polyethersulfone Membranes; Catalase; Bioconversion of D-glucose into D-Glucosone; Apparent Kinetic Constants

KeyWords Plus: Pyranose 2-Oxidase; Chromatographic Behavior; Enzymatic Oxidation; Fungal Strains; Oxidase; Purification; Products; Enzymes; Design

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