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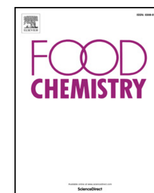
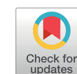


Co-localization of glucose oxidase and catalase enabled by a self-assembly approach: Matching between molecular dimensions and hierarchical pore sizes



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ABSTRACT

To achieve efficient one-step production of gluconic acid, cascade reactions of glucose oxidase (GOD) and catalase (CAT) have been advocated in the biocatalysis system. In this work, the methodology of co-immobilization of GOD and CAT was investigated in details for obtaining improved enzyme loading and activity. The maximum adsorption capability of GOD and CAT was 24.18 and 14.33 $mg\cdot g^{-1}$, respectively. The matching between dimensions of enzymes and hierarchical pore sizes of carriers are critical to the success of immobilization process. The simultaneous self-assembly on glutaraldehyde cross-linked mesoporous carriers exhibited favorable properties in comparison with sequential immobilization of GOD and CAT. The conversion of glucose under adequate air by co-localized GOD&CAT sustained the activity more than 90% after repeated utilization in the production of sodium gluconate and gluconic acid, suggesting that the co-immobilized GOD&CAT could be a promising catalyst for gluconate and gluconic acid production in some chemical and food industries.

1. Introduction

Biocatalysts are economic and environmentally friendly and widely used in the fields of science and industry due to their substrate specificity and stability under extreme conditions (Bornscheuer, 2016; Bornscheuer et al., 2012). As a matter of fact, many reactions are catalyzed by multi-enzymes to eliminate the side effect of intermediates. Cascade reaction is a highly effective way where highly ordered assembled enzymes are used (Zhang, Tsitkov, & Hess, 2016). Despite multi-enzyme systems have been studied extensively, the process still suffer from low space-time yield because the catalysis environment may do harsh to some kind of the enzyme and non-recyclability of the free enzymes (Bankar, Bule, Singhal, & Ananthanarayan, 2011; Liu et al., 2017). The immobilization can be one effective method to address above issues, which the intermediates are transported between the different active sites on enzyme subunits without leaving the immobilized enzyme (DiCosimo, McAuliffe, Poulose, & Bohlmann, 2013).

The common strategies of co-localizing multi-enzymes are based on the single enzyme immobilization techniques (Ozyilmaz & Tukel, 2007). Yet, the single immobilization methods do not involve or effectively address the relative position of multiple enzymes (Schoffelen & van Hest, 2012). To select a rational strategy in multi-enzyme co-localization, the structure and function of each enzyme need to be considered as the discrepancy of each individual enzyme in the resistance of environmental conditions (Fu et al., 2014; Sun et al., 2014). Since enzymes are typical large molecules, it tends to cause steric hindrance in the process of co-immobilization and influence the distribution and proximity of multiple enzymes. Moreover, enzyme-carrier interaction is also important for obtaining high loading capacity and catalytic activity simultaneously (Zhuang et al., 2017). Non-specific interaction would induce the conformational change of protein and

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reduce the enzyme activity (Hanefeld, Gardossi, & Magner, 2009). The common structural features of various enzymes would aggrandize difficulty in the selectivity of enzyme on carrier. Last but not least, to explore the efficient collaborative catalytic mechanism of co-localized enzymes, the relative position should be carefully controlled (Tian et al., 2016). Hence, the selection of appropriate carrier and immobilization method needs to be further investigated.

Porous materials with large surface areas can be suitable candidates, showing potentials to balance the enzyme loading efficiency and immobilized enzyme activity (Bankar et al., 2011). The trade-off of size between pores and enzymes has a significant influence on the stability of immobilized enzymes. Large molecular cannot enter into the small holes, while overlarge pores would result in enzyme leaching (Patterson, Schwarz, Waters, Gedeon, & Douglas, 2014). Consequently, to improve the loading of enzyme on carriers, it is effective to adjust the pore size and surface function group via modification of pore surface and highly ordered enzyme assembly (Bayne, Ulijn, & Halling, 2013). Weina Xu and coworkers have coated activated carbon with Concanavalin A appealing for high performance enzymatic catalysis with high yield of enzymatic activity and improved stability against pH and temperature (Xu et al., 2017).

Glucose oxidase (GOD) is a dimer flavoprotein with one ferrous ion and one FAD prosthetic group in each subunit and the two chains of glyprotein is linked with each other via disulfide bonds (Bankar, Bule, Singhal, & Ananthanarayan, 2009). With molecular oxygen as an electron acceptor, glucose oxidase can promote the oxidation of β-D-glucose to D-glucono-δ-lactone and H_2O_2 (Zhang et al., 2016). Subsequently, D-glucono-δ-lactone is non-enzymatically hydrolyzed to gluconic acid and the flavine adenine dinucleotide (FAD) ring of GOD is reduced to $FADH_2$. However, H_2O_2 is notorious for its excessive coverage of GOD active sites. In order to suppress the limitation, CAT is added for its outstanding capacity to decompose toxic H_2O_2 into valuable O_2 which can be used for another oxidation reaction of glucose (Rocker, Schmitt, Pasch, Ebert, & Grossmann, 2016). In the previous literatures, many applications of cascade reaction of GOD and CAT have been exploited. As we know, there is no report on the self-assembly of multi-enzymes for cascade bioreactions.

Gluconic acid ($C_6H_{12}O_7$) and its derivatives are exceptional intermediates with many uses in industrial applications, such as pharmaceutical, food, textile, detergent, leather, photographic and other biological industries (Canete-Rodriguez et al., 2016; Godjevargova, Dayal, & Turmanova, 2004). Gluconic acid can be obtained from oxidation/metabolism of glucose through chemical or microbial strategy (Bourdillon, Lortie, & Laval, 1988; Dowdells et al., 2010). Although fermentation has prevailed at the industrial scale, the biochemical process using glucose oxidase instead of cells has attracted much attention for its simplicity with no cell separation and little formation of by-products (Decamps et al., 2012; Godjevargova et al., 2004). Hence, for most large-scale industrial production procedure, GOD and CAT cascade reaction is used due to its mild operating conditions, simple separation process and lower energy consumption.

In this work, we fabricated a cascade GOD-CAT catalytic system on glutaraldehyde modified porous amino resin. Adsorption curves and Brunner – Emmet – Teller (BET) measurements were utilized to analyze the relationship between molecular dimensions of enzymes and hierarchical pore sizes of carriers during the self-assembled procedure of GOD and CAT. The investigation of the immobilization sequence of GOD and CAT was carried out for comparison. By varying the synthetic process, superior activation of desired reactions by GOD and CAT can be achieved. The leakage of enzymes could be mitigated via chemical bonding, and also amino groups on the carrier could prevent the enzyme deactivation in low pH conditions.

2. Materials and methods

2.1. Materials and chemicals

Glucose Oxidase (GOD, 22.43 mg·mL^{-1}, 1833 U·mL^{-1}) and catalase (CAT, 16.68 mg·mL^{-1}, 5533 U·mL^{-1}) were purchased from Novozymes (Tianjin, China). Glutaraldehyde (50% aqueous solution) was purchased from Aladdin Reagent (Shanghai, China). All chemicals were analytical grade. Porous support (HA) used throughout the study was made and stored in our laboratory (Cheng et al., 2017) and it was highly stable both chemically and mechanically in aqueous solution. The synthetic of HA-Glu was performed according to our previous protocol (He, Liu, Huang, Zhuang, & Ying, 2016; Huang et al., 2018; Zhuang, He, et al., 2016) and it was employed via chemical bonding between $-NH_2$ of resin and –CHO of glutaraldehyde.

2.2. Determination of enzymatic activities and protein concentration

The GOD activity was measured by the titration method (Bankar et al., 2009) using glucose as the substrate. The method was performed in the following manners: 1 g co-immobilized enzyme HA-Glu-CAT&GOD or 1 mL soluble CAT&GOD (U:U = 1:1) was added into 20 mL phosphate buffer (0.2 M, pH 6.0) containing 2.5% glucose independently. The reaction was conducted in air at 30 °C for 1 h in a rotary shaker at 200 rpm and then stopped by adding sodium hydroxide solution (30 mL, 0.1 M). The resulting mixture was titrated with standard hydrochloric acid (HCl) solution to calculate volume of added standard HCl with phenolphthalein as indicator. The blank assay (enzyme was not added) was performed under the same experimental condition. Thereby calculated equation GOD activity was described as follows:

$$\text{Enzyme}(\text{U}\cdot\text{g}^{-1}) = (V_0 V) \times 100 \div 60 \quad (1)$$

where V_0 is volume of added standard HCl solution in blank assay, mL; V is volume of added standard HCl solution in hydrolysis assay, mL; c is the concentration of standard HCl solution, M.

The protein concentration was essayed according to Bradford's methods (Bradford, 1976) using Coomassie Brilliant Blue-G 250 reagent with bovine serum albumin as a reference.

2.3. The adsorption properties of HA-Glu for GOD and CAT

The adsorption behaviors of GOD/CAT on the HA-Glu resin at room temperature was investigated in terms of Langmuir isotherm models. 1 g HA-Glu was suspended into 30 mL GOD or CAT with different concentration ranging from 0 to 0.9 mg·mL^{-1} to detect the maximum adsorption capacity of GOD and CAT.

2.4. Preparation of co-immobilization enzymes

2.4.1. Immobilization sequences

Parameters of the immobilization process and amounts of immobilized GOD and CAT was shown in Table S1. The co-immobilization was performed with three various sequences. (1) HA-Glu-CG: the activated carrier HA-Glu was first used to absorb CAT. Afterwards, HA-Glu-CAT was taken out and added into fresh GOD solution. (2) HA-Glu-G + C: HA-Glu was first immersed into GOD solution following by fresh CAT solution. (3) HA-Glu-CAT&GOD: first of all, CAT and GOD solution were mixed together and the support HA-Glu was utilized to absorb CAT and GOD at the same time.

The amount of supports (HA), CAT and GOD used in each immobilization method was 100 g, 336.45 mg and 83.40 mg. And all assays were carried out in 500 mL phosphate buffer (0.2 M and pH 6.4) under shaking condition (150 r·min^{-1}) at room temperature for 10 h and the carriers were washed with phosphate buffer (0.2 M and pH 6.4) to remove unbounded or loosely bounded enzymes before further use

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