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PRODUCTION OF GLUCONIC ACID FROM GLUCOSE CATALYZED BY CROSS-LINKED AGGREGATES OF GLUCOSE OXIDASE AND CATALASE IN A BUBBLE COLUMN REACTOR

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ABSTRACT

Cross-linked aggregates of glucose oxidase (GOD) from Aspergillus niger were prepared, evaluating the influence of the type of precipitant agent and cross-linker concentration, as well as bovine serum albumin as feeder protein on the GOD activity. The yield of immobilization was 56%. A combination of CLEAs of GOD and catalase was used in the gluconic acid production from glucose solution (26 g.L^{-1}, 40 ^{o}C, pH 6.0) in a pneumatic reactor, yielding around 80% conversion.

1. INTRODUCTION

The gluconic acid (GA) is an organic acid resulting from the oxidation of glucose. It has low corrosive capacity and good complexation with metal ions, which allows its application in the food, pharmaceutical, and textile industries (Ramachandran et al., 2006). Glucose oxidase (GOD) is an oxidoreductase (Bankar et al., 2009) that is capable to produce gluconic acid. The GOD catalyzes the oxidation reaction of β-D-glucose to β-D-glucolactone, using molecular oxygen as electron acceptor, which is spontaneously hydrolyzed (or catalyzed by gluconolactonase) to GA, releasing as by-product hydrogen peroxide (H_2O_2). Hydrogen peroxide is GOD inhibitor, being necessary its elimination of the reaction medium. The decomposition of H_2O_2 to water and oxygen can be catalyzed by the enzyme catalase (CAT, E.C.1.11.16) (Nakao et al., 1997).

The use of the multi-enzyme system in the immobilized form could contribute to reduce operational costs, because if the combined biocatalyst is thermal and mechanically stable it can be recovered and reused in batch processes or used by long time in continuous processes (Xue; Woodley, 2012). Carrier-free immobilized enzymes (Cao, 2006) by aggregation and crosslinking (CLEAs) has been studied in this work as a promising technique to immobilize the GOD from *Aspergillus niger*. The GOD-CLEAs were prepared by coprecipitation and crosslinking of GOD and bovine serum albumin (BSA). The effect of the concentration of cross linker (glutaraldehyde) and feeder protein (BSA) on the GOD activity was investigated. Additionally, a mixture of GOD-CLEAs

and CAT-BSA-CLEAs, previously optimized (MAFRA et al., 2016), was used in the production of GA in a bubble column reactor.

2. MATERIAL AND METHODS

2.1. Material

Aspergillus niger glucose oxidase (GOD, EC 1.1.3.4) (240 U/mg protein) was donated by Granotec, bovine liver catalase (CAT; EC:1.11.1.6) (30 U/mg protein) and bovine serum albumin (BSA) was obtained from Sigma-Aldrich. Other reagents were of analytical grade.

2.2. Methods

GOD assay: Activities of soluble GOD and GOD-CLEAs were determined by oxygen consumption (initial rates) in the oxidation of a 55 mM glucose solution at 30 °C and pH 6.0 (50 mM sodium phosphate buffer). A sterilizable amperometric electrode (Model InPro 6800, Mettler Toledo), linked to a silicone membrane (Model InProT96, Mettler Toledo) and installed on the middle of the circular section of the reactor, was used to measure the change in oxygen concentration throughout time. One GOD unit was defined as the initial rate of consumption of 1 µmol of O_2 per minute under the assayed conditions.

CAT assay: The initial velocity method was used, as described by Mafra et al (2016). Activities of soluble CAT and CAT-CLEAs were determined spectrophotometrically at 240 nm, following the decomposition of a H_2O_2 solution (0.35 mM H_2O_2 in 0.05 M phosphate buffer, pH 7.5) at 25 °C.

CLEAs preparation: The preparation of CLEAs followed the method described by Mafra et al. (2016).

GOD-CLEAs were prepared dissolving 10 mg of GOD and 20, 40 and 60 mg of BSA in 1 mL of sodium phosphate buffer (100 mM, pH 7.0). After, 1 mL of precipitant (saturate solution of ammonium sulfate - AS, tert-butyl alcohol – TBA or dimethoxyethane - DME) was added for protein aggregation. After 1 min of mixing in a vortexer, glutaraldehyde was slowly added to the final concentration of 25, 50 and 75mM.

CAT-BSA-CLEAs were prepared dissolving 20 mg of CAT powder and 60 mg of BSA in 1 mL of sodium phosphate buffer (100 mM, pH 7.0). Protein aggregation was induced by mixing 1 mL of the enzymatic solution and 1 mL of AS. After mixing, glutaraldehyde was slowly added to the final concentration of 50 mM.

After 3 h of crosslinking at 4 °C and 200 rpm shaking, all the CLEAs were centrifuged and washed twice with the same buffer. The activities of GOD and CAT were calculated as described above and the yield of immobilization (Y) was calculated as the ratio of the total activity of GOD or CAT in the CLEAs and the total GOD or CAT activity used for CLEAs production.

Gluconic acid production: Production of GA from glucose solution (26 $g.L^{-1}$) was performed as described by Mafra et al. (2015) in a bubble column reactor of 1 L volume at 40 °C and pH 6.0. A specific air flow rate of 10 vvm (10 $L.min^{-1}$) was used to ensure an excess of dissolved oxygen. Samples were withdrawn at 0.5-h intervals for the determination of the concentrations of glucose (Miller, 1959).

3. RESULTS AND DISCUSSION

Fig. 1 shows the influence of precipitants (AS, TBA and DME), feeder protein (BSA) and crosslinker concentration on the overall immobilization yield of GOD-CLEAs. It can be seen that the concentrations of glutaraldehyde and BSA of 25 mM and 20 mg BSA (2 mg BSA/mg GOD), respectively, using DME as precipitant yielded GOD CLEAs with the highest immobilization yield (56 ± 0.6%). Similar yields have been reported for other oxireductases immobilized by this technique (Touahar et al. 2014; Mafra et al, 2016).

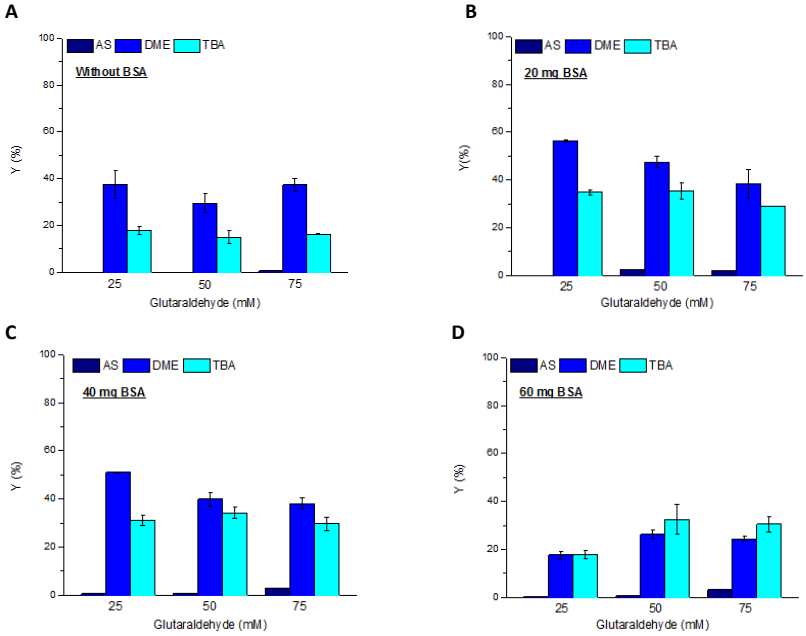

Figure 1. Immobilization yield (Y) of GOD by CLEAs method as function of glutaraldehyde concentration and type of precipitant agents. (A) Without BSA, (B) 20 mg BSA, (C) 40 mg BSA, (D) 60 mg BSA.

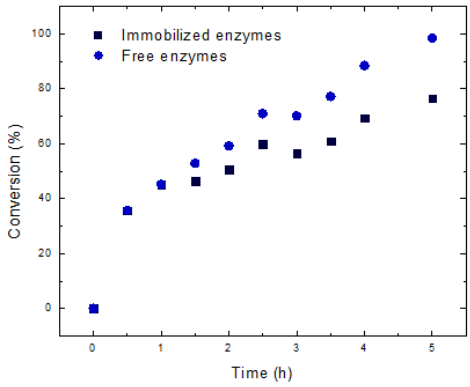

Figure 2. Gluconic acid production from 26 g.L^{-1} glucose solution catalyzed by GOD-BSA-CLEAs and CAT-BSA-CLEAs in a pneumatic reactor at 40 °C and pH 6.0.

Table 1. Gluconic acid productivity.

Immobilized biocatalyst	GA productivity (g.L^{-1}.h^{-1})	Reference
GOD immobilized within calcium alginate beads and palladium particles	3.84	Nakao et al. (1997)
Immobilized *Aspergillus niger*	8.2	Fiedurek (2001)
GOD immobilized on anion-exchange membrane	0.1	Hestekin et al.(2002)
GOD/CAT immobilized on anion-exchange membrane	0.18	Godjevargova et al. (2004)
Immobilized *Aspergillus niger*	1.28	Mukhopadhyay et al. (2005)
INV/GOD/CAT confined in an ultrafiltration membrane reactor	1.42	Silva et al. (2011)
GOD-BSA-CLEA and CAT-BSA-CLEA	4.5	This work

GA production was carried out in a pneumatic reactor with a volume of 1 L, operated at 10 vvm, 40 ^{o}C, and pH 6.0. The experiments were carried out with 95 mL of GOD-BSA-CLEAs per liter of reactor or 380,556 U and 50 µL of CAT-BSA-CLEAs per liter of reactor or 14.6 U. The conversion of glucose to GA obtained in the batch was ~ 80 %, as shown in Fig. 2. The GA productivity (4.5 $g.L^{-1}.h^{-1}$, Table 1) in comparison with previous findings showed that a combination of CLEAs of GOD and CAT has great potential to the multi-enzymatic conversion of glucose to gluconic acid.

4. CONCLUSIONS

Aspergillus niger GOD was immobilized by the CLEAs method, yielding a biocatalyst high activity retention (~ 60 %). The multi-enzyme system (GOD-BSA-CLEA/CAT-BSA-CLEA) showed to be promising in the production of gluconic acid from glucose (~ 80 % conversion, 4.5 $g.L^{-1}.h^{-1}$ productivity).

5. ACKNOWLEDGMENTS

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