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Encapsulation of oxygen carriers and glucose oxidase for higher gluconate yield

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INTRODUCTION AND OBJECTIVES

Efficient enzymatic production of D-gluconic acid via biooxidation from D-glucose is catalyzed by the glucose oxidase-catalase (GOD-CAT) system and requires permanent saturation by oxygen as a cosubstrate. It was shown that the encapsulation of GOD in the polyelectrolyte complex capsules composed of sodium alginate (SA), cellulose sulfate (CS), poly(methylene-co-guanidine) (PMCG), $CaCl_2$ and NaCl (SA-CS/PMCG capsules) (Vikartovská 2007) is made possible when the pore size of the capsular membrane is lower than the size of the encapsulated enzyme. This feature is a prerequisite for a successful enzyme encapsulation without additional preimmobilization steps. The focus of this study was a preparation of SA-CS/PMCG capsules with a GOD biocatalyst co-immobilized with oxygen carriers, which is an extension of our previous study with GOD immobilized within SA-CS/PMCG capsules (Vikartovská 2007). The main aim was to increase the oxygen level within the capsules to improve the space-time yield of produced D-gluconic acid and δ-gluconolactone (Bučko 2010).

MATERIALS AND METHODS

Materials. Enzyme glucose oxidase (GOD) from *Aspergillus niger*, encapsulation materials, water-insoluble oxygen carriers perfluorodecaline (PFD), *n*-dodecane (DOD) and all other chemicals were purchased from commercial suppliers. Water-soluble oxygen carrier poly(dimethylsiloxane) (PDMS) was a gift from Dr. E. S. Dey (Lund University, Sweden) (Bučko 2010).

Encapsulation. GOD encapsulation and coencapsulation of GOD and oxygen carriers within SA-CS/PMCG capsules was performed using a custom-made coaxial air-stripping extrusion device fitted with a multiloop reactor as reported previously (Bučko 2010).

Biooxidations. D-glucose solutions of different concentrations in sodium phosphate buffer (0.05M, pH 6) supplemented with oxygen (0.625 mM) were added to 800 mg of wet GOD-SA-CS/PMCG capsules in a model bioreactor of 24 ml. The double-jacketed reactor was thermostated (30°C) and equipped with a cylindrical oxygen sparger for oxygenation and mixing, to keep capsules in fluidized bed-like conditions. Buffer aliquots (60 µl) were periodically withdrawn and analyzed by the HPLC method as described previously (Bučko 2010). The biooxidation reactions were carried out for 5 hours.

Determination of GOD activity in an immobilized form. The activity of immobilized GOD was determined spectrophotometrically by the method based on horseradish peroxidase-coupled oxidation of *o*-dianisidine as previously described (Vikartovská 2007). One unit of GOD activity corresponds to the oxidation of 1 µmol of o-dianisidine (measured as the change in the absorbance at 460 nm) in 1 min under conditions used.

Glucose monitoring by a flow calorimetry. A flow microcalorimeter (FC) (3300 Thermal Assay Probe, Advanced Biosensor Technology, AB, Lund, Sweden) was used for monitoring of glucose consumption as described previously (Štefuca 1997, Bučko 2010). The concentrations of glucose were calculated from the thermometric signal according to the autocalibration method, as described previously (Štefuca 1997). Simultaneously, the glucose concentration in samples withdrawn from the stirred reservoir was analyzed by HPLC described elsewhere (Bučko 2010).

Measurement of dissolved oxygen concentration was described elsewhere (Bučko 2010).

RESULTS AND DISCUSSION

Coencapsulation of oxygen carriers with GOD. It was found out, that production of functional capsules is possible with PDMS concentration up to 4% (w/w). It was also noted, that the mixing of PDMS with the polyanion (PA) solution for production of capsules (SA, CS, NaCl) containing GOD and water-insoluble PFD or DOD in proper ratios resulted in the production of homogenous and stable emulsions that could be used for a reproducible production of stable and functional GOD-SA-CS/PMCG capsules. Despite rather high concentrations of PDMS (4%) and DOD (10%) or PFD (10%) used, the capsules were mechanically and chemically stable even when exposed to biooxidative conditions in a batch-wise reactor or when they were stored in 0.9% (w/v) NaCl at 4°C. Figure 2 shows representative images of GOD-SA-CS/PMCG capsules with coencapsulated oxygen carriers. Statistical analysis revealed reproducible preparation of the capsules with SD up to 5% for capsule size or 14% for membrane thickness. Batch to batch variation of the capsule size up to 8% is another advantageous feature of the encapsulation protocol used, which enables a reliable comparison of GOD catalytic performance using these different oxygen-carrying systems (Bučko 2010).

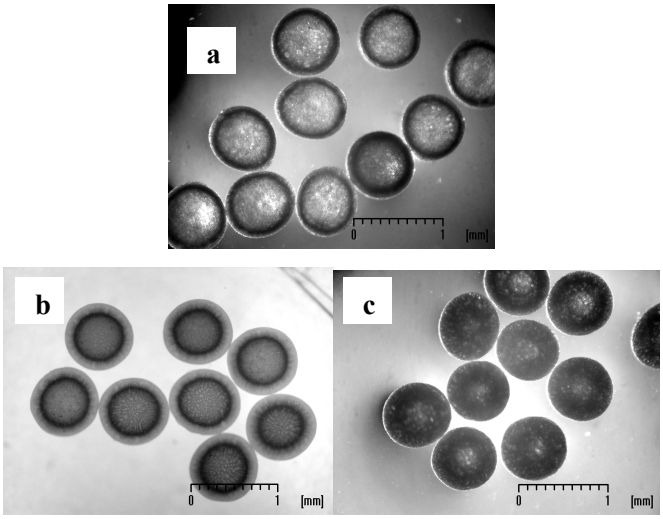

Figure 2: GOD-SA-CS/PMCG capsules: a) capsules with PDMS 4% and PFD 10% , b) capsules with PDMS 4% , c) capsules with PDMS 4% and DOD 10% (Bučko 2010).

Catalytic efficiency of coencapsulated GOD with oxygen carriers. The set of data demonstrating the effect of coencapsulated oxygen carriers with GOD is shown in Table 2. Enzyme activities (A), measured spectrophotometrically and space-time yields (STY) of the product (mixture of δ-D-gluconolactone and D-gluconic acid), measured by HPLC, were significantly higher in all preparations containing oxygen carriers compared to the reference sample without carriers. In this regard, the highest values of enzyme activity of A = 38.4 ± 2.0 $U \cdot g^{-1}$ and STY of 83.4 ± 3.4 $g \cdot l^{-1} \cdot day^{-1}$ was observed using the capsules with emulsified DOD and PDMS, which was roughly twofold compared to the reference sample in the absence of oxygen carriers (A = 21.0 ± 1.1 $U \cdot g^{-1}$ and STY = 44.3 ± 2.0 $g \cdot l^{-1} \cdot day^{-1}$).

Table 2: Comparison of enzyme activity (A) and space-time yield (STY) of the product for GOD-SA-CS/PMCG capsules for the following concentrations of coencapsulated oxygen carriers: 4% PDMS, 10% DOD and 10% PFD. Reference : GOD-SA-CS/PMCG capsules without oxygen carriers (Bučko 2010).

Preparative	A/ $U \cdot g^{-1}$	STY/ $g \cdot l^{-1} \cdot day^{-1}$
reference*	21.0 ± 1.1	44.3 ± 2.0
DOD-PDMS	38.4 ± 2.0	83.4 ± 3.4
PFD-PDMS	36.5 ± 1.5	79.6 ± 3.7
PDMS	35.2 ± 2.1	77.9 ± 3.1

Figure 4 shows the effect of oxygen carriers on product formation in time (mixture of δ-D-gluconolactone and D-gluconic acid, both measured by HPLC) during glucose oxidation. An increase in the product concentration during oxidation (Figure 4) is proportional to glucose consumption as monitored by HPLC and on-line by a flow microcalorimeter (Figure not shown), which represents an important consistency test (Bučko 2010).

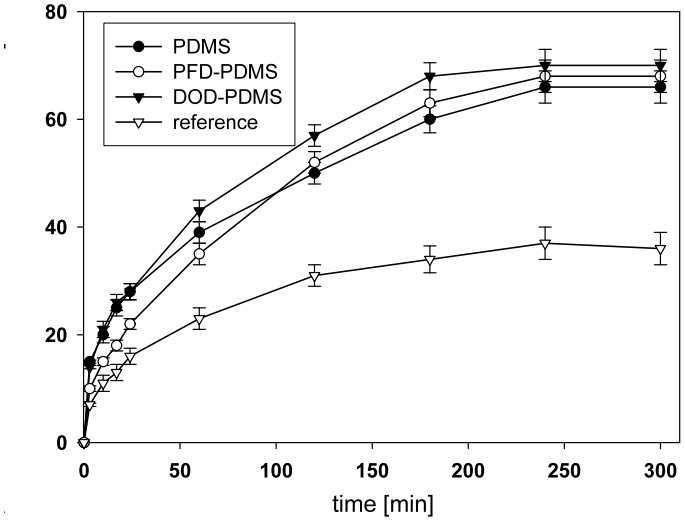

Figure 4: Effect of coencapsulated oxygen carriers within the GOD-SA-CS/PMCG capsules expressed as the time evolution of the product concentration during glucose oxidation. The oxygen carrier concentrations in PA solution were PDMS 4%, PFD 10%, DOD 10%. GOD-SA-CS/PMCG capsules formed in the absence of oxygen carriers were used as the reference.

CONCLUSIONS

Coencapsulation of oxygen carriers – PDMS, PDMS with DOD and PDMS with PFD − together with GOD enhanced the activity of encapsulated GOD and product yield of mixture of δ-D-gluconolactone and D-gluconic acid as compared to capsules without oxygen carriers. Coencapsulation of GOD and oxygen carriers is recommended as a feasible way for improving the GOD catalytic efficiency.

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ACKNOWLEDGEMENT

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