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Efficient immobilization of mushroom tyrosinase utilizing whole cells from *Agaricus bisporus* and its application for degradation of bisphenol A

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ABSTRACT

A simple and efficient procedure for preparation and immobilization of tyrosinase enzyme was developed utilizing whole cells from the edible mushroom *Agaricus bisporus*, without the need for enzyme purification. Tyrosinase activity in the cell preparation remained constant during storage at 21 °C for at least six months. The cells were entrapped in chitosan and alginate matrix capsules and characterized with respect to their resulting tyrosinase activity. A modification of the alginate with colloidal silica enhanced the activity due to retention of both cells and tyrosinase from fractured cells, which otherwise leached from matrix capsules. The observed activity was similar to the activity that was obtained with immobilized isolated tyrosinase in the same material. Mushroom cells in water were susceptible to rapid inactivation, whereas the immobilized cells maintained 73% of their initial activity after 30 days of storage in water. Application in repeated batch experiments resulted in almost 100% conversion of endocrine disrupting bisphenol A (BPA) for 11 days, under stirring conditions, and 50–60% conversion after 20 days, without stirring under continuous usage. The results represent the longest yet reported application of immobilized tyrosinase for degradation of BPA in environmental water samples.

1. Introduction

Bisphenol A (BPA) is an important bulk chemical that is mainly used for fabrication of polycarbonate plastics and epoxy resins, which are common constituents of many household plastic products. BPA is also used, to a lesser extent, in the production of thermal paper. Due to its endocrine disrupting activity, BPA has received considerable attention (Alonso-Magdalena et al., 2006; Deutschmann et al., 2013; Howdeshell et al., 2003; Jobling et al., 2004; Kawai et al., 2003; Kubo et al., 2003; Markey et al., 2001; Oehlmann et al., 2006; Tarafder et al., 2013; vom Saal and Hughes, 2005), since it has been found in waste waters (Fürhacker et al., 2000;

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Lagana et al., 2004; Lee and Peart, 2000; Rigol et al., 2002), surface waters (Bolz et al., 2001; Heemken et al., 2001; Stachel et al., 2003), food (Ballesteros-Gómez et al., 2009; Biles et al., 1998) and mineral water (Toyo'oka and Oshige, 2000), as well as in human blood and urine (Dekant and Völkel, 2008; Völkel et al., 2008; Zhou et al., 2013). BPA may not be completely degraded in sewage treatment plants (Lagana et al., 2004; Lee and Peart, 2000; Rigol et al., 2002; Spring et al., 2007), hence there is a great demand for its removal from water bodies, for example, in waste water treatment (Kang et al., 2007).

The enzymatic oxidation of BPA with tyrosinase has been suggested as a method for the degradation of this anthropogenic contaminant (Ispas et al., 2010; Yoshida et al., 2001). Tyrosinase is able to utilize molecular oxygen to oxidize phenolic compounds to *o*-diphenols and further to *o*-quinones. The *o*-quinones are colored and often toxic compounds, which can be removed via adsorption or binding to chitosan (Ispas et al., 2010; Tamura et al., 2010; Wada et al., 1993; Yamada et al., 2006). It has been shown that treatment of phenol solutions with tyrosinase and chitosan resulted in detoxified and colorless solutions (Ikehata and Nicell, 2000).

Pure tyrosinase is expensive to produce on the scales required to be used for catalytic BPA degradation in waste water streams, therefore, cost reduction plays an important role with respect to an industrial application. Tyrosinase is present in the fruiting body of the edible mushroom *Agaricus bisporus*, which is produced in large amounts for human consumption, inexpensive, and readily available throughout the year. Some efforts have been made using semipurified tyrosinase preparations (Burton et al., 1993; Ensuncho et al., 2005; Labus et al., 2011; Marín-Zamora et al., 2006; Munjal and Sawhney, 2002) or whole mushroom tissue (Kameda et al., 2006; Silva et al., 2010). Since some enzyme activity may be lost during purification, and even simple purification strategies contribute significantly to overall process costs, it is a promising prospect to completely avoid enzyme purification prior to desired application. However, direct use of mushroom tissue may have disadvantages due to an inherent small surface to volume ratio, decreasing enzymatic reaction rate, as well as issues with respect to stability of the mushroom cells. These disadvantages may be mitigated by immobilization techniques.

Immobilization of biocatalysts offers the possibility to protect these substances against deactivation as well as to facilitate their handling, separation, and reutilization. Nevertheless there are, to date, few reports regarding the immobilization of whole cells from *A. bisporus* in scientific literature (Friel and McLoughlin, 1999). In particular, information regarding immobilization of cells from the fruiting body of *A. bisporus* is currently non-existent. Immobilization of cells can be accomplished by entrapment in biopolymer materials, such as alginate or chitosan, both are inexpensive and commercially available, exhibit high biocompatibility, and have simple as well as mild immobilization methods (Smidsrød and Skjåk-Bræk, 1990; Kaya and Picard, 1996).

Immobilization has been demonstrated for purified tyrosinase (Ispas et al., 2010; Munjal and Sawhney, 2002). However, leaching of isolated enzyme from the biopolymer matrix capsules, including during their fabrication, is an issue which lowers immobilization efficiency, leading to high process costs.

Modification of alginate matrix capsules with colloidal silica allows manipulation of capsule permeability (Pachariyanon et al., 2011) and can be utilized for more efficient immobilization, including better retention of enzyme.

In this report, a simple procedure for preparation and immobilization of whole cells from the fruiting body of *A. bisporus* in alginate and chitosan matrix capsules is presented. The procedure is evaluated in terms of resulting tyrosinase activity. In order to reduce loss of tyrosinase due to release from fractured cells, a modification of this system with colloidal silica is also presented, demonstrating an efficient modification of the system for quantitative immobilization of mushroom cells, which maintain tyrosinase activity without the need for purification. These matrix capsules are described with respect to some of their characteristics as well as their application for degradation of BPA. Since most reports deal with BPA solutions prepared with laboratory water with relatively short reaction cycles (Ispas et al., 2010; Nicolucci et al., 2011; Yoshida et al., 2001), this report deals with real environmental water samples spiked with BPA and application of matrix capsules for several days in order to better simulate possible application in an industrial process.

2. Materials and methods

2.1. Materials

Mushrooms (*Agaricus bisporus*) at developmental stages 2–3 (Hammond and Nichols, 1976) (velum still closed) were acquired from a local supermarket and were used on the day of purchase.

Tyrosinase from mushroom (product number T3824), alginic acid sodium salt from brown algae (suitable for immobilization of micro-organisms), chitosan from crab shells (highly viscous), Ludox® HS-30 colloidal silica 30% (w/w), sodium triphosphate pentabasic (NaTPP, ≥98% purity) and BPA (≥99% purity) were purchased from Sigma–Aldrich GmbH, Steinheim, Germany. Acetonitrile (≥99.9% purity), $CaCl_2 \cdot 2H_2O$ (≥99% purity), HCl (37%) and NaOH (≥99% purity) were obtained from Carl Roth GmbH & Co KG, Karlsruhe, Germany, acetic acid (glacial) from Merck KGaA, Darmstadt, Germany, 3,4-dihydroxy-L-phenylalanine (L-DOPA, 98% + purity) from Alfa Aesar GmbH & Co KG, Karlsruhe, Germany and 2-morpholinoethanesulfonic acid (MES, molecular biology grade) from AppliChem GmbH, Darmstadt, Germany.

Double distilled deionized water (ddH_2O) was used for all solutions except BPA solutions, which were prepared with environmental water samples. Tyrosinase stock solution of 235 U/ml (according to the assay described in Section 2.5) was stored at −20 °C and further diluted prior to use.

2.2. Preparation of mushroom cells

The mushrooms were cut into small pieces and subsequently treated according to one of the following procedures.

Procedure 1: Mushroom pieces were added to ddH_2O (0.5 g/ml) and crushed with a Philips HR2096 blender.