Bioresource Technology 126 (2012) 117–122

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Functionalized magnetic mesoporous silica nanoparticles: Fabrication, laccase adsorption performance and direct laccase capture from *Trametes versicolor* fermentation broth

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HIGHLIGHTS

- Functional magnetic mesoporous silica nanoparticles (MMSNPs) were fabricated.
- Laccase was efficiently captured from fermentation broth by the MMSNPs.
- Magnetic separation provides a powerful means for laccase purification in practice.

ARTICLE INFO

Article history:
Received 19 July 2012
Received in revised form 3 September 2012
Accepted 4 September 2012
Available online 10 September 2012

Keywords:
Mesoporous
Size-selective
Purification
Laccase
Magnetic separation

ABSTRACT

A simple and highly efficient protocol using magnetic mesoporous silica nanoparticles (MMSNPs) with metal affinity ligands was developed to directly capture laccase from *Trametes versicolor* fermentation broth. The Cu^{2+}-chelated magnetic mesoporous silica nanoparticles (MMSNPs-Cu^{2+}) with pore sizes ranging from 3.6 to 27.1 nm exhibited size selectivity on laccase capture from the fermentation broth, and the MMSNPs-Cu^{2+} with an average pore size of 14.5 nm provided 60.6-fold purification of laccase and 114.6% recovery yield of enzyme activity. Both size selectivity of the MMSNPs and affinity of the chelated metal ion resulted in high laccase capture efficiency from the fermentation broth. The most efficient MMSNPs-Cu^{2+} demonstrated no significant loss in laccase capture effectiveness following 10 reuse cycles. This simple and efficient strategy has the potential to be used for the robust and inexpensive preparation of purified laccase at the industrial scale.

1. Introduction

Laccase (benzenediol: oxygen oxidoreductase; EC 1.10.3.2) belong to the multicopper oxidase family and oxidize phenolic and non-phenolic compounds with oxygen as the final electron acceptor. Laccase are useful in a variety of industrial applications including pulp processing, the synthesis of organic materials, dye decolorization, the removal of phenolic compounds from beverages, and wastewater bioremediation (Li et al., 2011). Certain applications, such as those in the fields of biosensors, diagnostics, and nanobiotechnology, require highly purified enzyme preparations (Freixo et al., 2008). Separation methods for laccase purification have been developed for different species of white rot fungi; however, these methods have been utilized primarily for enzyme characterization rather than for commercial implementation (Forootanfar et al., 2011). These methods involve a combination of conventional purification methods, such as ammonium sulphate precipitation, ultrafiltration, gel filtration, ion exchange, and affinity chromatography (Rajeeva and Lele, 2010). However, these multi-step protocols are time consuming, expensive, and often result in a recovery with low enzyme activity (Kumar et al., 2012). Recently, foam fractionation and three-phase partitioning have been evaluated for the development of a scalable method for the large-scale purification of laccase (Rajeeva and Lele, 2011; Kumar et al., 2011). Although a higher level of enzyme activity was retained, the level of purification was relatively low compared to conventional methods. Therefore, there is still considerable interest in the development of simple and highly efficient laccase purification methods that result in high levels of pure and active enzyme (Freixo et al., 2008).

Mesoporous materials have large pore volumes, a large surface area, tunable pore sizes, and a controllable framework composition (Deng et al., 2011). Magnetic mesoporous materials possess not only the unique features of mesoporous materials, but also magnetic properties that allow the mesoporous materials to be easily

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http://dx.doi.org/10.1016/j.biortech.2012.09.005