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A hierarchical assembly of flower-like hybrid Turkish black radish peroxidase-Cu^{2+} nanobiocatalyst and its effective use in dye decolorization

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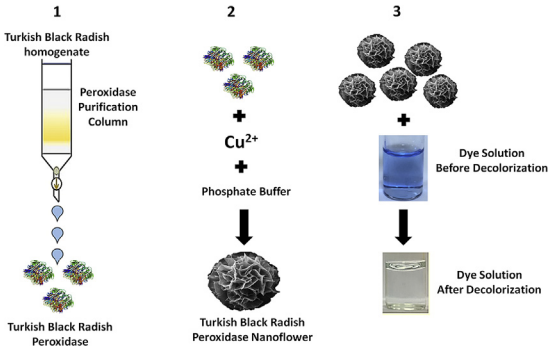
HIGHLIGHTS

- Turkish black radish peroxidase-Cu2+ hybrid nanoflowers.
- Enhanced Enzyme activity and stability.
- Dye decolorization.

GRAPHICAL ABSTRACT



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ABSTRACT

Effective dye decolorization in wastewater still shows a big challenge. Although the biological methods, especially using enzymes, offer alternative and effective process for dye degradation and overcome the limitations of chemical and physical methods such as the instability, lack of reusability and high cost of free enzymes strictly, which limit their use in many scientific and technical applications. Enzymes rapidly lose their activities in aqueous solutions and against environmental changes due to their very susceptibility and unfavorable conformations. Herein, we report preparation of the enzyme-inorganic hybrid nanostructures with flower-like shape consisting of Turkish black radish peroxidase and Cu^{2+} metal ions using an encouraging enzyme immobilization approach. The peroxidase-Cu^{2+} hybrid nanoflowers (NFs) exhibited enhanced stability and activity towards various pH values and provided excellent dye decolorization efficiency for Victoria blue (VB) dye with more than 90% within 1 h. The NFs were also repeatedly used in efficient and caused 77% VB decolorization efficiency even at tenth cycles. However, to the best of our knowledge, for the first time, we prepared peroxidase enzyme isolated from Turkish black

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Enzyme activity and stability
Dye decolorization

radish incorporated NFs and used them for dye decolorization. We believe that the NFs can be promising materials for dye decolorization in real wastewater treatment.

1. Introduction

Many factors, especially industrialization, rapid population growth, the use of chemical fertilizers and pesticides, cause environmental and water pollution. In particular, the textile industry is considered as the most polluting industry due to discharge volume and composition of wastewater (Dos Santos et al., 2007). The dye based colors are the indication of the water pollution and are first contaminants identified in the wastewater. It is worthy to mention that pollution from some dyes even at very low concentrations (0.005 ppm) can be visually seen with naked eye (O'Neill et al., 1999; Robinson et al., 2001; Seesuriyachan et al., 2007; Tilli et al., 2011). It has been reported that 10–15% of dye used in the textile industry during the dyeing process passed to wastewater (Gomez et al., 2007). In this case, the dyes can threaten aquatic life and have also toxic, mutagenic and carcinogenic effects towards living organisms (Robinson et al., 2001; Seesuriyachan et al., 2007; Tilli et al., 2011). Several methods such as physical, chemical, and biological, have been in used for decolorization of waste water. (Banat et al., 1996; Robinson et al., 2001; Hu et al., 2001; Saratale et al., 2011; Zeng et al., 2012; Si et al., 2013). However, no single method with an international validity has been recommended for wastewater treatment. The methods are varied and employed separately or together based on the type of wastewaters and pollution for treatment. The physical and chemical methods have many disadvantages such as high cost, excessive use of chemicals and energy, and concentrated sludge formation etc.(Robinson et al., 2001; Stolz, 2001; Si et al., 2013; Jin et al., 2013; Abdel-Aty et al., 2013).

Biological treatment of wastewater is considered to be the least harmful method to the environment and using enzymes in which has potential to effectively and economically decolor large volume of wastewater (Rai et al., 2005). Many studies have focused on use of biological methods for decolorization and have indicated that oxidative enzymes like lignin peroxidase, horseradish peroxidase and laccase play a major role in the removal of dyes (Rodriguez et al., 1999; Nyanhongo et al., 2002; Rodriguez et al., 2004; Unyayar et al., 2005; Eichlerova et al., 2006; Gou et al., 2009; Zeng et al., 2012). Horse radish, white radish, lignin and bitter gourd peroxidase enzymes produced from different sources have been performed for decolorization of various synthetic dyes (Nath et al., 2004; Satar et al., 2009; Matto et al., 2009; Abdel-Aty et al., 2013; Ambatkar et al., 2015). Enzymes can be functioned in a wide range of pH, temperature and salt concentrations without the leading of any biomass production. Although enzymes in free form have short lifetime and lack of recovery from reaction mixture and reusability (Kulshrestha et al., 2006; Kim et al., 2006; Mohamed et al., 2013), they currently have been used in industries with various forms due to their specific and high catalytic activities. The activity, stability and reusability of enzymes should be increased in order to effectively use them as industrial biocatalysts in practice. The enzymes have been chemically and physically immobilized onto/into micro and nano sized supports using several different immobilization strategies in recent years. (Arica et al., 1998; Oh et al., 2000; Kheirolomoom et al., 2002; Godjevargova et al., 2006; Sheldon, 2007; Mateo et al., 2007; Lee et al., 2009; Rana et al., 2010; Garcia-Galan et al., 2011; Gokhale et al., 2013; Netto et al., 2013; Wang et al., 2015).

Generally, immobilized enzymes have higher stability than free enzymes presumably due to reduction in mobility. However, enzymatic activity either remains constant or decreases after immobilization owing to mass transfer limitations between the enzyme and substrate and unfavorable conformational changes of enzymes. (Arica et al., 1998; Oh et al., 2000; Kheirolomoom et al., 2002; Godjevargova et al., 2006; Lee et al., 2009; Netto et al., 2013). Recently, Zare and coworkers discovered an encouraging method for preparing flower-like shaped protein–inorganic hybrid nanostructures, which have shown much great stabilities and activities compared to free enzymes (Ge et al., 2012; Zhu et al., 2013; Wang et al., 2013). This advancement inspired several researchers to design new hybrid nanostructures with flower-like shapes for a variety of applications (Wang et al., 2013; Lin et al., 2014; Somturk et al., 2015, 2016; Ocsoy et al., 2015; Altinkaynak et al., 2016a,b; Yilmaz et al., 2016; Ildiz et al., 2017). Peroxidase (EC 1.11.1.x; donor: hydrogen-peroxide oxidoreductase) is a member of oxidoreductases and catalyzes the oxidation of substrates in the presence of H_2O_2. Peroxidases play a vital role in many biological processes such as controlling plant growth. They have been widely used in medicine, chemistry and food industries and also acted as component of biosensor for visual detection of H_2O_2 and aromatic compounds. The peroxidases are widely existing in microorganisms, plants and animals and purified and characterized from them. Plant peroxidases can perform oxidation of phenolic molecules such as guaiacol, catechin, chlorogenic acid, and catechol. Turkish black radish (Raphanus sativus L.) is cultivated in all regions of Turkey (Şişecioğlu et al., 2010). Şişecioğlu and co-workers have successfully purified and characterized the peroxidase enzyme from Turkish black radish. The purified enzyme showed better thermal stability.

In this study, flower-like shaped hybrid flowers (NFs) were prepared using Turkish black radish peroxidase and copper ions (Cu^{2+}) and they exhibited highly enhanced enzymatic activity and stability. To best of our knowledge, the Turkish black radish peroxidase incorporated NFs are produced and used, for the first time, for decolorization of a representative Victoria blue dye (VB). The morphology of NFs is investigated as functions of the peroxidase concentration and reaction temperature. Dye decolorization efficiency of the NFs is employed at different times and pH values to determine the optimal conditions for efficient decolorization. Additionally, the reusability of NFs was also studied.

2. Experimental

2.1. Chemicals and materials

Peroxidase enzyme was purified from Turkish black radish (*Raphanus sativus L.*). Bovine serum albumin (BSA), hydrogen peroxide (H_2O_2), guaiacol, copper sulfate pentahydrate, methanol, phosphoric acid, victoria blue dye (Acros Organics, code: 190190250) and other chemicals were purchased from Sigma-Aldrich. NaCl, KCl, Na_2HPO_4, KH_2PO_4, HCl, NaOH and Coomassie brilliant blue G-250 were used for buffer solution and prepared using ultrapure water.