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Oxidative removal of phenol by HRP-immobilized beads and its environmental toxicology assessment

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ABSTRACT

Horseradish peroxidase shows potential biological and environmental applications on the removal of phenolic compounds. In the present study, the HRP-immobilized beads were synthesized to detect the efficiency of the removal of phenol at optimum pH and H_2O_2 concentration. Comparative in vitro cytotoxicity of phenol/treated solutions were evaluated in HeLa, HepG2 and mcf-7 cells by using MTT method along with flow cytometry study for cell viability and cell cycle distributions. The results showed that the toxicity of phenol solutions were greatly reduced after treated by HRP-immobilized beads, and phenol could lead to deactivate of cells in the S phase and preventing them from going into the G2/M checkpoint. In addition, molecular docking study showed that phenol was a valid inhibitor for the cyclin E in the cell cycle and cell metabolism. Thereby, we established a suitable strategy with promising application for efficient harmless removal of phenol, which significantly decreased the cytotoxic impacts of phenol.

1. Introduction

Environmental pollution, being a serious problem, has attracted more and more researchers' attentions. Many toxic contaminants, such as heavy metals, dyes, inorganic and organic compounds, are generated in the production processes of pesticides, pharmaceuticals, coal gasification, et al. (Michałowicz and Duda, 2007). For lacking of the awareness of healthy environmental protection, large amounts of toxic contaminants are poured into the rivers, lakes and oceans without sufficient treatment. These pollutants not only affect the environment causing great damages to plants and animals, but also impose toxic and carcinogenic effects upon humans through the drinking water and food chain. Therefore, how to effectively govern and manage the toxic contaminants in wastewater has grown into a fascinating field of research and has been explored by researchers worldwide.

Among different kinds of toxic pollutants, phenol and its derivatives are regarded as the most representative organic pollutants in wastewater. Studies reveal that phenol can inhibit the growth of renal epithelial cells and muscle fibers, as well as its mutagenic effects (Michałowicz and Duda, 2007; Mittal et al., 2009). Accordingly, the removal of those toxic pollutants is becoming an important environmental issue. In the past decades, some methods such as adsorption, distillation, oxidation and photocatalytic degradation have already been established as useful methods for wastewater treatments (Spiridon et al., 2013; Kiai et al., 2014; Szabados et al., 2015; Pirila et al., 2015). However, biocatalysis, as an alternative approach, has emerged overwhelming attentions for the treatment of phenolic contaminants in industrial wastewater by the enzyme-catalysed polymerization (Ghioureliotis and Nicell, 2000). Horseradish peroxidase (HRP) is a promising enzyme which can be used for the removal of phenolic compounds from wastewater and provides high stability over a broad pH and temperature range (Karam and Nicell, 1997). It is used as a catalyst for the oxidation process of phenolic compounds in the presence of H_2O_2, and the residuals can be easily removed by sedimentation of filtration (Wagner and Nicell, 2002). However, the free enzymes in the solution are unstable and non-reusable, and the high cost further leads to limited applications in the industry. In contrast, immobilized enzymes show better stability and reusability (Akgol et al., 2009; Mateo et al., 2007). It is very easy to recover both the enzyme and product, and can facilitate rapid termination of the reaction (Katuri et al., 2009). The enzymatic properties of immobilized enzyme were determined by the physical and chemical characteristics of support materials. Therefore, the choice of the support materials is very important. It has been reported that some materials such as glass beads, ion exchange

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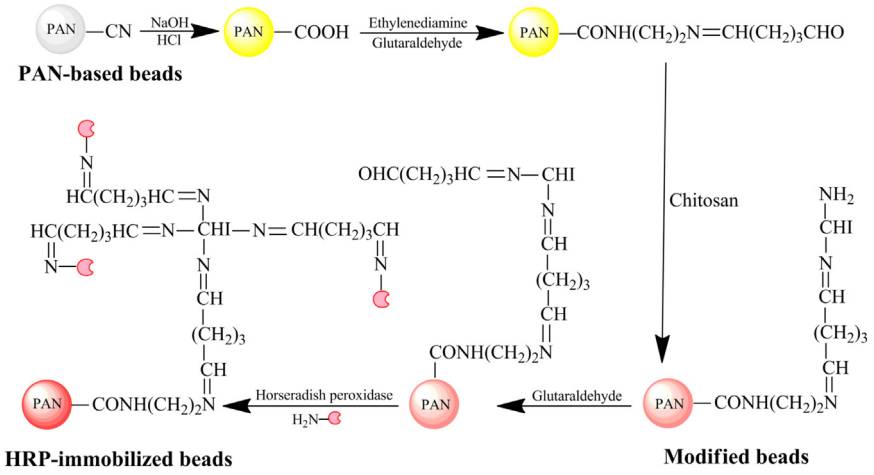

Fig. 1. Modification and enzyme immobilization of PAN-based beads.

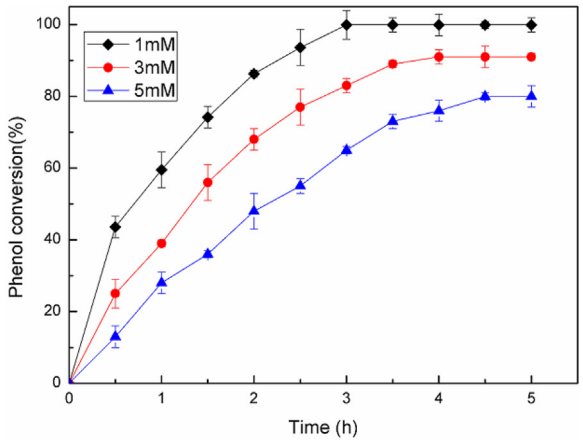

Fig. 2. Time course of phenol remove with the HRP-immobilized beads for different phenol concentrations (1 mM, 3 mM, 5 mM). Data are presented as the mean values ± standard deviation of triplicate experiments.

resins, magnetite, polymers and aluminum-pillared clay have been used for the immobilization of horseradish peroxidase (Tatsumi et al., 1996; Azevedo et al., 2004; Fernandes et al., 2003; Cheng et al., 2006; Caramori and Fernandes, 2004). Recently, synthetic polymers are widely used as enzyme immobilization carriers since they have good mechanical stability, less susceptible to bacterial attacks, and can be easy prepared in various geometrical configurations (Marinov et al., 2009). Polyacrylonitrile(PAN), as a synthetic polymer, has been widely used for enzyme immobilization due to its good performances (Gabrovska et al., 2008a). To immobilize enzymes on the polyacrylonitrile material, functional groups are introduced into the polymer backbone of PAN. Firstly, to form amino groups, the PAN-based beads were modified with NaOH, HCl, ethylenediamine, chitosan and glutaraldehyde, respectively. Then, the enzyme was introduced on the beads through crosslinking with glutaraldehyde.

This paper is aim to detect the efficiency of the removal of phenol by HRP-immobilized beads, as well as the environmental toxicology assessment. Briefly, the HRP-immobilized beads were used for the removal of phenol at optimum pH and H_2O_2 concentration, respectively. And the corresponding optimum conditions have been demonstrated in our previous work (Wang et al., 2015). The relevant toxicological analyses were also studied in this paper. In general, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] is a yellow water-soluble salt, which can be used for detecting the cell viability and large scale mycotoxins screening testing (Mosmann, 1983; Laville et al., 2004). And this soluble tetrazolium salt can be converted to water insoluble and dark blue formazan derivative by reductive cleavage of the tetrazolium ring with metabolically active cells (Sundaram et al., 2013). Formazan crystals can be dissolved in an organic solvent such as dimethyl sulfoxide (DMSO) or isopropanol and quantified by measuring the absorbance of the solution at 570 nm (Xu et al., 2015). Here, HeLa, HepG2 and mcf-7 cells were selected to detect the toxicity of phenol using MTT method. Moreover, HeLa cells were chosen to further explore the mechanism of effects of phenol toxicity by using flow cytometry. We also analyzed the protein-ligand interaction with phenol by using molecular modelling studies. So far, there are few reports concerning with such a strategy. From the results obtained in this work, we could establish a suitable strategy with promising application for efficient harmless removal of phenol.

2. Materials and methods

2.1. Materials

Horseradish peroxidase(HRP) ((EC 1.11.1. 7), >250 U/mg) was purchased from Shanghai SANGON Biological Engineering Co., Ltd., China. Polyacrylonitrile beads(Polyacrylonitrile-8%, dimethylformamide-92%) were prepared by extrusion using a simple one step process similar to that described in our previous paper (Wang et al., 2015), which is a product of Heowns Biochem Technologies LLC, China. All other chemicals were of analytical grade and were used without further purification. Solutions were prepared with distilled water (supplied by Fuzhou University).

2.2. Preparation of HRP-immobolized beads

The modification of PAN-based beads were carried out by sequential processing of initial beads with NaOH, ethylenediamine, chitosan and glutaraldehyde, respectively, which had been described by Gabrovska et al. (2008b), Wang et al. (2015) and Nicolucci et al. (2011). The modified beads were immersed in 10% water solution of glutaraldehyde for 1 h at 4 °C. Then, the beads were washed thoroughly by water. After that, the beads were kept in the buffer solution of horseradish peroxidase(1 mg/ml in PBS, pH 7.4) for 20 h at 4 °C (Wang et al., 2016). Finally, the HRP-immobilized beads were stored in phosphate buffer (0.1 M, pH 7.0) at 4 °C.