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Decolorization and biosorption for Congo red by system rice hull- *Schizophyllum* sp. F17 under solid-state condition in a continuous flow packed-bed bioreactor

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Abstract

Synthetic dyes are important chemical pollutants from various industries. This work developed an efficient and relatively simple continuous decolorization system rice hull-*Schizophyllum* sp. F17 under solid-state condition in a packed-bed bioreactor, for decolorizing Congo red. In the decolorization system, two decolorization mechanisms exist, one is decolorization by *Schizophyllum* sp. F17, the other is biosorption by rice hull. The decolorization efficiency was greatly affected by dye concentration and hydraulic retention time (HRT), which were quantificationally analyzed and optimized through response surface methodology (RSM). A 2^2 full factorial central composite design (CCD) was performed, and three second order polynomial models were generated to describe the effects of dye concentration and HRT on total decolorization ($R^2 = 0.902$), decolorization by *Schizophyllum* sp. F17 ($R^2 = 0.866$) and biosorption by rice hull ($R^2 = 0.890$). Response surface contour plots were constructed to show the individual and cumulative effects of dye concentration and HRT, and the optimum values. A maximum total decolorization 89.71% and maximum decolorization by *Schizophyllum* sp. F17 60.44% was achieved at dye concentration 142.63 mg/L, HRT 41 h, and dye concentration 110.7 mg/L, HRT 29.4 h, respectively. Meanwhile, the role of manganese peroxidase (MnP) in the decolorizaion process was investigated. This study proved the feasibility of continuous mode for decolorizing synthetic dyes by white-rot fungi in solid-state fermentation bioreactors.

Keywords: Continuous decolorization; Solid-state fermentation; Packed-bed bioreactor; Rice hull; *Schizophyllum* sp. F17

1. Introduction

The main characteristic which differentiates white-rot fungi from most other microorganisms is their ability to mineralize all compounds of lignin to carbon dioxide and water (Moreira et al., 1998). The lignin degradation capacity of white-rot fungi is attributed to extracellular oxidative enzymes, including lignin peroxidase (LiP), manganese peroxidase (MnP), manganese-independent peroxidase (MIP) and laccase (Moreira et al., 1999). The same non-specific mechanisms of these enzymes that confer on white-rot fungi the ability to degrade lignin also allow them to degrade a wide range of recalcirant compounds, including polycyclic aromatic gydrocarbons, chlorinated phenols, droxins, pesticides and dyes (Boer et al., 2006). This ability has opened new prospects for the development of biotechnological process aimed at the degradation of xenobiotic compounds and attracted increasing scientific attention on the use of white-rot fungi (Palmieri et al., 2005).

Solid-state processes are becoming more popular due to their advantages over to the liquid culture, such as higher productivity, simpler operation, and lower cost. In addition, solid-state fermentation reproduces the conditions under which the white-rot fungi grow in nature (Boer et al., 2006; Ürek and Pazarlioglu, 2004). Because of these reasons, solid-state fermentation of white-rot fungi is utilized not only to produce ligninolytic enzymes, but also

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to biodegrade pollutants. Synthetic dye is one class of the most commonly used compounds in the study of the ability of solid-state fermentation to degrade hazardous materials.

Synthetic dyes are extensively used for dyeing and printing in various industries. Over 7×10^5 t and approximately 10,000 different dyes and pigments are produced annually worldwide, about 10% of which may be found in wastewater (Deveci et al., 2004). Many dyes are believed to be toxic carcinogenic or to be prepared from known carcinogens such as benxidine or other aromatic compounds that might be reformed as a result of microbial metabolism (Kariminiaae-Hamedaani et al., 2007).

In previous reports, there were two main methods to decolorize dyes by solid-state fermentation of white-rot fungi. One method was decolorizing dyes utilizing extracted ligninolytic enzymes in vitro (Deveci et al., 2004; Ünyayar et al., 2005; Shrivastava et al., 2005; Cheng et al., 2007), another was decolorizing dye solution by solid-state mediums of fungi in flasks (Tychanowica et al., 2004; Boer et al., 2004). These two methods proved the high ability to decolorize dyes by solid-state fermentation of white-rot fungi, but their efficiency was very low and they could not be developed in application because of the large amount of effluent generated on a daily basis. An efficient application of dye decolorization at industrial scale required the performance of a continuous system technology, but there were very few papers that addressed continuous decolorization by white-rot fungi under solid-state fermentation.

This work tried to develop an efficient and relatively simple continuous process based on solid-state fermentation of white-rot fungi for decolorizing synthetic dyes. It is essential to select adequate bioreactor and substrate for performing continuous decolorization under solid-state fermentation, since the success of both the fermentation and continuous decolorization process depends on them. After comparison, packed-bed bioreactor and rice hull were selected in the experiment, because they are suitable not only for solid-state fermentation but also for continuous decolorization process. Packed-bed bioreactors successfully performed to produce some kinds of enzymes under solid-state condition (Pandey and Radhakrishnan, 1992; Aikat and Bhattacharyya, 2001; Almeida et al., 2005), and they were employed, filled with white-rot fungi immobilized on insert substrates, to decolorize synthetic dyes (Zhang et al., 1999; Pazarlioglu et al., 2005;). Rice hull is a byproduct obtained from milling process, it has the physical to serve as supporting material and can function as the nutrition source for white-rot fungi, in addition, its particle size and porosity that naturally forms make the solid-state medium colonized by white-rot fungi fully contact with the dye effluent. More important, rice hull is a good material for biosorption of dyes (Vadivelan and Kumar, 2005; Chuah et al., 2005). So, in the decolorization system rice hull-white-rot fungus, two kinds of decolorization mechanisms exist, one is the decolorization by white-rot fungus, the other is the biosorption by rice hull.

Dye concentration and hydraulic retention time (HRT) are two of the most important factors affecting the continuous decolorization efficiency, which were frequently studied (Mielgo et al., 2001; Chen et al., 2003; Wu et al., 2005). In order to evaluate the effects of these parameters and to optimize the decolorization process, response surface methodology (RSM) was proposed. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing process. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions (Chen et al., 2005; Karacan et al., 2007). *Schizophyllum* sp. F17, a local white-rot fungus, has previously shown potential for dye decolorization in liquid culture (Jia et al., 2004a). In this study, we selected a widely used azo dye Congo red as the indicator to analyze the ability of system rice hull-*Schizophyllum* sp. F17 to continuously decolorize synthetic dyes in packed-bed bioreactor under solid-state condition.

2. Methods

2.1. Microorganism and culture conditions

Schizophyllum sp. F17, isolated from a decayed wood chip pile in the vicinity of Hefei, was maintained on PDA (potato dextrose agar) slants at 4 °C.

2.2. Pack-bed bioreactor configuration

Packed-bed bioreactor was selected in this study. It consisted of a glass column with dimensions of 25 cm height and 7.6 cm in internal diameter (working volume of 1000 ml). Three sampling ports were equidistantly distributed in the body of the bioreactor for enzyme measure. The sampling ports were plugged by cotton in the fermentation process, while by rubber stopper in the decolorization process. Meanwhile, the packed-bed bioreactor had one inlet port at the bottom and one outlet port at the upper part for dye effluent, they were used only in the decolorization process.

2.3. Solid-state fermentation in packed-bed bioreactor

Schizophyllum sp. F17 was cultured on PDA slants for 7 days at 28 °C. The grown mycelium mat was washed with sterile water. The mycelia obtained were blended to mycelial suspension and 100 ml liquid medium in 250 ml flasks were inoculated with 10 ml of such suspension. The liquid medium contained 20 g potato, 2 g dextrose, 0.3 g KH_2PO_4, 0.15 g $MgSO_4$ and 1 mg thiamine. The liquid medium was sterilized at 121 °C for 20 min before inoculation. The inoculated liquid medium was cultured at 28 °C on a rotary shaker at 130 rpm. Mycelial pellets formed in the liquid medium, and the 3-days old mycelial pellets were blended to mycelial suspension, which (50 ml) was used to inoculate 250 g solid-state fermentation medium. The