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Short Communication

Biodegradation of Orange G by wood-rot fungi *Phanerochaete sordida* TXJ-1302A and *Tyromyces lauteus* TXJ-1302B

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Abstract

Two strains isolated from the organic layers of forests on Zijin Mountain have indicated a strong capability of decolorization for Orange G on the solid plates. They were identified as *Phanerochaete sordida* and *Tyromyces lauteus* according to phenotypic and molecular techniques. Through this study, we try to find the suitable condition and cheapest way for decolorization by two strains. The result shows that malt extract and ammonium sulfate are the best N source for *P. sordida* and *T. lauteus*, respectively; 0.95 g per L glucose + 0.05 g per L ethonal are the best C source both for *P. sordida* and *T. lauteus*. Oxalate plays an important role as the organic acid chelator which can also enhance the decolorized capability of fungi.

Keywords: White-rot fungi; Brown-rot fungi; Glucose; Chelator; Ethanol

1. Introduction

According to the statistics, India, the former USSR, Eastern Europe, China, South Korea and Taiwan consume approximately thousand tons (kt) of dyes annually (Ishikawa et al., 2000). During processing, up to 15% of the used dyestuff is released into the processed water (Vaidya and Datye, 1982). The dyestuff mills have become a great concern of wastewater treatment, for the dyes are synthesized to resist fading upon exposure to sweat, light, water, many chemicals including oxidizing agents, and microbial attack (Dirk et al., 2003). Organic dyes include diarylmethine dyes, triarylmethine dyes, nitro and nitroso dyes, anthraquinonic dyes, etc. Orange G is a kind of azo dye which is hard to be reduced by abiotic means.

Wood-rot fungi are main degraders of the major plant polymers lignin, cellulose, and hemicellulose in the biosphere (Boominathan and Reddy, 1992; Kirk and Rarrell, 1987; Reddy and D'Souza, 1994). They secret the non-specific substrate extracellular enzyme: laccases, lignin peroxidases (LiP) and manganese-dependent peroxidases (MnP) which are capable of degrade xenobiotic compounds. *Phanerochaete sordida* is a well-known decolorization white-rot fungus (Glenn and Gold, 1993). *Tyromyces lauteus* belongs to the group of brown-rot fungus.

In the practical process of treatment, the requirement for some chemicals that is unlikely to be present in a wastewater. In this work, the cheap and simple materials were studied for application. The ammonium salts were often used as the nitrogen source for decolorization, whereas in the treatment of waste water, ammonium salts is unrealistic for its non-cheap price. The comparison of efficiency among the ammonium salts and between the ammonium salts and other nitrogen were tested this time. Glucose is the common carbon source for decolorization, there were fewer studies focusing on the co-metabolized carbon for decolorization. It has been pointed out oxalate is an organic acid chelator produced by both white-rot and brown-rot fungi (Popp et al., 1990; Punja and Jenkins, 1984; Traquair, 1987) and the media for decolorization to examine the role of chelators for these two strains.

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A series of α-hydroxy acids as the chelator were added into the media for decolorization to examine the role of chelators for these two strains. In light of look for a simple and efficient way without adding too many varieties of agents for decolorization, this experiment is designed to study the effect of nitrogen resource, co-metabolic carbon-resource, the H_2O_2 and chelator on the decolorization of orange G and the incubation method was improved for decolorization by fungi which achieved a better effect than using the plugs cut from the actively growing part of a colony (Ivana and Ladislay, 2005). Through the strongest capability of these two strains and the factors for decolorization was studied, the application of these two strains for the wastewater treatment especially the dyestuff emission can be considered. More effective decolorization methods would be introduced to industry.

2. Methods

2.1. Fungal culture

P. sordida TXJ-1302A and the *T. lautus* TXJ-1302B were isolated from the Zijin Mountain with pure culture. The fungi were grown on potato-dextrose agar plates for 5–6 days and then preserved at 4 °C.

Fungal mycelial (2%) were inoculated into 9 cm diameter plate containing 10 ml potato liquid media, which enable a 9 cm diameter mycelial biofilm can be formed, and this mycelial biofilm can degrade the dyestuff efficiently. It was prepared for the further decolorization experiment.

2.2. Media

The composition for the decolorization media is: NaCl, 0.5 g per L; K_2HPO_4, 1 g per L; $MgSO_4$, 0.2 g per L; KH_2PO_4, 0.5 g per L, $NaNO_3$, 0.5 g per L; $MnSO_4$, 0.5 g per L, Orange G, 200 mg per L and the different nitrogen source, carbon source or chelators separately added into the media. The pH of all media was adjusted to 4.5. After the 7 days cultivation for the mycelial biofilm, the mycelial biofilms were transferred into the 150 ml Erlenmeyer flasks under the shaking condition at 220 rpm, 30 °C. Control flasks contained only dyestuff and nutrients without fungi.

2.3. Analytical methods

Samples were withdrawn and centrifuged at 15000 rpm for 5 min to separate the fungal mycelial. The color measurements were carried out on the evaluation of decolorization. For every 12 h, absorbances of samples were measured photometrically at the maximum absorption wavelength 476 nm by using UNICO UV 2100. Samples were diluted by a factor of 1 per 4 with distilled water prior to measurement. The percent of color removal was calculated as the extent of the decrease from the initial value of $\sum$OD.

The fungal mycelia were harvest by paper filtration (mesh size 0.45 μm), washed with distilled water and dried at 80 °C to a constant weight. The experiment was carried out in the Plant Laboratory of Nanjing University, China.

3. Results and discussion

3.1. Effect of nitrogen source

Five different nitrogen sources (ammonium nitrate, malt extract, ammonium sulfate, urea and ammonium tartrate) with 1% concentration were tested to determine simple and suitable nitrogen for decolorization. Nearly 96% of color removal was obtained by *P. sordida* within 84 h of incubation added with malt extract. Higher performance (97.8% of color removal) was observed by *T. lauteus* with ammonium sulfate (Fig. 1a). No color removal was observed in the control flasks. Malt extract contains approximately 13% N, other sources such as protein and chloride can accelerate the metabolism of fungi (Liu, 2002). The percent of color removal by *T. lauteus* was higher than result of Koichi, which 92% of color removal was achieved by *P. sordida* with malt extract (Koichi and

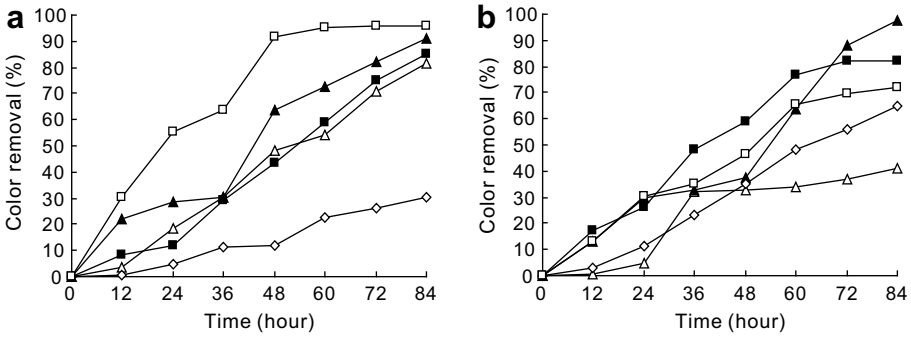

Fig. 1. Effect of different nitrogen source on decolorization for Orange G by fungi, "a" represents *P. sordida*; "b" represents *T. lauteus*. The maximum absorbance wavelength of Orange G is 476 nm. Symbols: (■) ammonium nitrate; (□) malt extract; (▲) ammonium sulfate; (△) urea; (◇) ammonium tartarate.