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Triarylmethane Dye Decolorization by Pellets of *Pycnoporus sanguineus*: Statistical Optimization and Effects of Novel Impeller Geometry

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INTRODUCTION

Synthetic dyes are commonly used in biomedical, foodstuff, plastic, and textile industries (Pointing and Vrijmoed 2000). Vaidya and Datye (1982) reported that more than 10,000 commercial dyes are available to the textile industries and approximately 10–15% of these were release into the environment due to losses during dying process. This effluent is either treated by biological process at the plant treatment site or treated together with the domestic wastewater in a municipal water treatment center. However, synthetic dye cannot be degraded easily (Morais et al. 1999) and concern arises, as most of these dye are toxic to flora and fauna or mutagenic and carcinogenic (Baughman and Perenich 1988; Nilsson et al. 1993). Furthermore, certain chromophores can have a negative impact on organisms living in water because of the light diffusion reduction (Banat et al. 1996).

Thermokinetics of an azo dye decolorization by pellets of *P. sanguineus* had been studied recently by Annuar et al. (2009). It was shown that the extent and rate of decolorization were directly proportional to the initial dye concentration and reaction temperature. This apparent first-order behavior of the decolorization reaction offers an inherent advantage in dealing with high-strength polluting dye concentration. Low activation energy at 23 kJ mol^{-1} for the studied biological decolorization allows for a less energy-intensive process. It was also determined that when the average diameter of the pellets were at 2.4 • } 0.5 mm, mass transfer processes within and outside the pellets did not limit dye degradation (Annuar et al. 2009).

An extended study on the use of *P. sanguineus* pellets for dye decolorization is presented in the current work. The objective of this study was to find out the optimum conditions for the decolorization of a triarylmethane dye, viz., crystal violet, by *P. sanguineus* pellets. The three parameters selected for investigation were initial dye concentration (ppm), agitation speed (rpm), and process time (days). The possible interactions that may exist among the three parameters during the dye decolorization process were examined using response surface analysis. Response surface analysis was also employed to determine the best operating parameters combination, which was then implemented in the subsequent studies using a stirred tank reactor (STR). This study also reported for the first time the effect of novel impeller geometry, viz., 180◦ curved blade and 60◦ angled blade impellers, on the decolorization process and power consumption in the STR.

MATERIALS AND METHODS

Microorganism and Growth Medium

P. sanguineus Linn. Ex Fr (Murrill) was maintained by weekly transfer to potato dextose agar (PDA), which was incubated at 27◦C for 7 days, after which it was stored at 4◦C until required. Glucose yeast extract malt extract peptone (GYMP) medium was used to grow the fungus in liquid suspensions. The composition of the medium (g L^{-1}) was as follows: 0.5 $MgSO_4$, 1.0 K_2HPO_4, 0.46 KH_2PO_4, 20.0 glucose, 2.0 peptone (Becton-Dickinson, Sparks, MD, USA), 2.0 yeast extract (Becton-Dickinson), and 2.0 malt extract (Becton-Dickinson). Glucose solution was prepared and autoclaved separately. The medium was autoclaved for 10 min at 121◦C, 15 psi, and cooled to room temperature (25 •} 1◦C) before use.

Growth and Preparation of Fungal Pellets

The fungal culture that had grown on PDA plates was used to prepare the biomass pellet in the liquid medium identified above. Mycelium suspension was prepared by pouring 20 ml of 1% Tween 80 into the plate containing the mycelia growth. The surface of the mycelia was scratch to release the mycelium and spores. A 2.0-ml aliquot of mycelia suspension was inoculated into GYMP medium in Erlenmeyer flasks, followed by incubation on a rotary shaker for 4 days where a number of flasks were started in parallel. Suspension of pellets produced was filtered using 1.5-mm^2 sieve to recover the spherical pellets.

Preparation of Dye, Laccase Activity

and Dye Decolorization Assays

Crystal violet ($C_{25}H_{30}ClN_3$; molar mass of 407.98 g mol^{-1}) was purchased from BDH Chemicals (Poole, UK). This commercial triphenylmethane dye was chosen as the model compound for decolorization. The stock solution of crystal violet was prepared at the concentration 80 mg L^{-1} and stored in an amber bottle to protect it from direct sunlight. Spectral analysis of the crystal violet dye was initially done. This was to determine the light wavelength at which the crystal violet absorbs the strongest. Standard calibration of dye concentration and absorbance was constructed (Annuar et al. 2009). Absorbance was measured at the wavelength of 590 nm based on spectral analysis. The standard calibration fitted Equation 1: