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Decolorization of Direct Black 22 by *Aspergillus ficuum*

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Abstract: The decolorization of Direct Black 22 by *Aspergillus ficuum* has been studied. It was found that *Aspergillus ficuum* could effectively decolorize Direct Black 22 especially when grown as pelleted mycelia. Results showed that the media containing Direct Black 22 at 50 mg/L could be decolorized by 98.05% of the initial color in 24 h. The optimum pH and temperature of decolorization are 4.0 and 33 ℃ respectively. Aeration was quite beneficial to decolorization. Medium composition and the concentration of Direct Black 22 could affect the rate of decolorization. The dye degraded products assayed by UV-visible spectrophotometer and macroscopic observation showed that the decolorization of Direct Black 22 by mycelial pellets includes two important processes: bioadsorption and biodegradation. The degradation experiment agree with the Michaelis-Menten kinetics equation.

Keywords: *Aspergillus ficuum*; mycelial pellet; decolorization condition; degradation; kinetics

Introduction

Synthetic dyes are widely used in the field of printing and dyeing of textiles, paper printing, leather and as additives in petroleum products. With the large output of dyeing waste water, that dyes are accumulated in the environment lead to serious environment pollution. Because of the higher chemical stability, lower dissolvability in water and darker color in the environment, together with the carcinogenicity and hard degradation of most of the dyes, how to deal with dyeing waste water has become the difficult points and hot spots worldwide. Some physical and chemical techniques are available for the treatment of coloured effluents including : coagulation and sedimentation, adsorptoin, bleaching by ozone or chlorine, ion-exchange on synthetic adsorbentresins, reverse osmosis, etc. Unfortunately, such processes have high operating costs and are of limited applicability(Whaters, 1984; Cooper, 1993). An effective and inexpensive biological treatment system would be of great value. The basis and key of biological treatment method are to find high efficiency decoloring strains of dyes. At present, many bacteria and algae are found effective to dye decolorization (Jia, 1998, Dai, 1999; Chen, 1998; Zhang, 1998). However, little is reported about fungi(Li, 1997; Cripps, 1990), studies on dye decolorization focus on adding dominant strains or immobilized cells(Song, 1999; Zheng, 1998; Ling, 1998), the decolorization of dyes by mycelial pellets has not be seen in the literature in China. Through long-term research work, the authors isolated a dye decolorization strain of fungus, which is identified as *Aspergillus ficuum*. This paper reports the decolorization of the azo dye Direct Black 22 by mycelial pellets, *Aspergillus ficuum*.

1 Materials and methods

1.1 Organisms and material

Aspergillus ficuum was isolated from sludge of Wenzhou Printing Plant. It was maintained on plates of Sabouraud Dextrose Agar and stored at 4℃. Mycelial pellets growth media was Sabouraud dextrose agar broth (1L) consisting glucose 40g, peptone 10g, pH 4.0. Media of decolorizing tests (1L) consisting ammonium tartrate 0.22g, Glucose 10.0g, KH_2PO_4 2.0g, $MgSO_4$ 0.5g, $CaCl_2$ 0.1g, α-ketoglutaric acid 1.08g, vitamin B1 1 mg, Tween 80 0.1% 50 ml, pH 4.0.

The decolorization tests were done with azo dye Direct Black 22 (Fig.1), Dye: C.I. Direct Black 22 was from Tianjin No.3 Factory of Dye Chemicals, Tianjin, China.

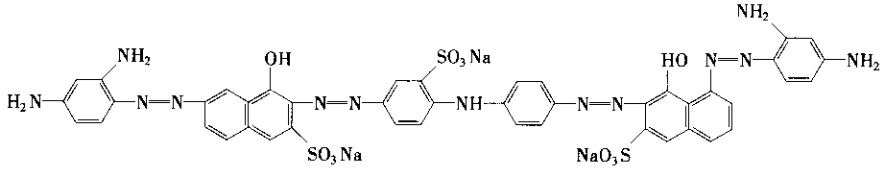

Fig.1 The chemical structure of Direct Black 22

1.2 Cultivation of mycelial pellets

The mycelial inoculum was prepared by homogenising fungal mycelium aseptically. Erlenmeyer flasks (250 ml) containing 100 ml of Sabouraud Dextrose Agar were inoculated with 1 ml of the mycelial suspension and incubated at 33℃ on a rotary shaker (150 r/min) for 2 days. The mycelial pellets were harvested after cultivation and then used in the decolorization tests.

1.3 Decolorization of dye

Mycelial pellets were separated from the growth media and inoculated to media of decolorizing tests in Erlenmeyer flasks. Media of decolorizing tests and the dye solution were autoclaved separately at 121℃ for 15 min. Within any one experiment the initial biomass of *Aspergillus ficuum* was always very similar in all the cultures. In typically most experiments, 50 ml

media containing Direct Black 22 (the final dye concentration was 50 mg/L) was inoculated with 2.5g wet mycelia and incubated at 33℃ on a rotary shaker for 1 day.

The optical density (OD) of samples before and after decolorization was measured (at λ_{max}-618 nm) spectrophotometrically using 751 GW UV-visible spectrophotometer. The rate of decolorization was calculated according to the following formulation. All experiments were repeated three times.

Rate of decolorization(%) = $[OD_{(before\ decolorization)} - OD_{(after\ decolorization)}]/OD_{(before\ decolorization)} \times 100\%$.

2 Results and analysis

2.1 Effect of culture condition on the decolorization of Direct Black 22

To determine the effect of aeration, c. 2.5g wet mycelial were inoculated to 50 ml media which contains Direct Black 22 (the final dye concentration was 50 mg/L). The inoculated medium was separated into two parts. One part was cultivated statically at 33℃ for 24h. The other part was cultivated at 33℃, 150 r/min for 24h. The rate of decolorization was 45.00 and 96.47%, respectively. This result suggests that the decolorization of Direct Black 22 by Aspergillus ficuum is an aerobic process.

To determine the affect of pH and to establish the optimum pH for decolorization, a series of media differing only in pH was tested. The results(Fig.2) showed that Direct Black 22 was easily decolorized by mycelial pellets of *Aspergillus ficuum* when the initial pH values in the range pH 2.0—9.0. The optimum pH is 4.0. Decolorization of Direct Black 22 by *Aspergillus ficuum* was carried out at various temperatures (15—50℃). Decolorization ability was stable at the range of 22—40℃ and was maximal at 33℃, while at 50℃ it was relatively low(Fig.3)

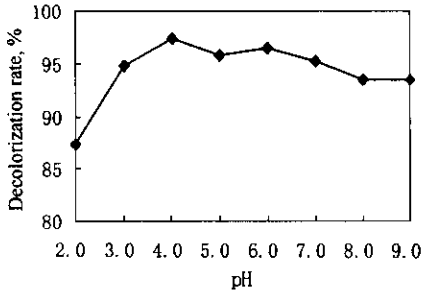

Fig. 2 Effect of pH on decolorization rate

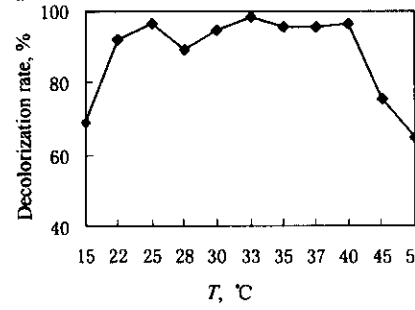

Fig.3 Effect of temperature on decolorization rate

Direct Black 22 with different initial concentrations (50 mg/L, 100 mg/L, 200 mg/L) were also tested, the rate of decolorization was 98.05%, 92.98% and 46.33%, respectively. The rate of decolorization decreased sharply with dye concentration. It suggests that the decolorization ability of *Aspergillus ficuum* can be strongly inhibited by Direct Black 22.

2.2 Effect of medium composition on Direct Black 22 decolorization

The effect of glucose concentration (0—20 g/L) in media on the decolorization of Direct Black 22 was investigated. Changes in decolorization ability are shown in Table 1, the decolorization ability was very similar irrespective of glucose concentration.

The effect of nitrogen source concentration on decolorization was investigated with different amounts of ammonium tartrate being added to media of decolorizing test(Table 1). When the amounts of ammonium tartrate is between 0 and 2.0 g/L. The decolorization rate increased with increasing ammonium tartrate concentration. However, at 3.0, 4.0 g/L ammonium tartrate the decolorization rate was 80.16 and 69.39%, respectively. The results showed that nitrogen enriched condition is not beneficial to dye decolorization. The optimum concentration of ammonium tartrate for *Aspergillus ficuum* was about 2.0 g/L.

Table 1 Effect of medium composition on dye decolorization ability

Medium composition	Rate of decolorization, %
Ammonium tartrate, g	
0	92.27
0.25	96.23
0.5	95.70
1.0	97.73
2.0	98.23
3.0	80.16
4.0	69.39
Glucose, g	
0	61.63
5	97.80
10	97.87
20	98.17

2.3 Bioadsorption and biodegradation

In this experiment, we observed that the color of mycelia pellets changed from white to black in 4h after they were inoculated to media containing Direct Black 22, while it changed from black to blue after 20h, just then the color of media also changed from black to coffee. The macroscopic observation showed that the decolorization of Direct Black 22 by mycelial pellets includes two important processes: bioadsorption and biodegradation.

After decolorization with mycelia pellets of *Aspergillus ficuum* absorption spectra show that the specific peak (at 618nm) of Direct Black 22 had completely disappeared and there was a sharp increase in low UV light absorption. This result showed that the molecular structure of Direct Black 22 has been changed after decolorization and that biodegradation really happened.

2.4 Kinetics of Direct Black biodegradation

In optimum conditions, mycelial pellets were tested for their ability

to degradation Direct Black 22 at different initial concentrations. The result is shown in Table 2.

Table 2 Reaction kinetics of Direct Black 22 biodegradation

Initial concentration, mg/L	Final concentration, mg/L	t, h	V, mg/(L·h)
50	6.33	8	5.42
75	20.33	8	6.83
100	25.33	8	9.33
150	61.67	8	11.04
200	75.33	8	15.58
250	143.00	8	13.92
300	176.67	8	15.42
350	239.33	8	13.83
400	275.33	8	16.83
450	309.67	8	17.54
500	360.67	8	17.34

Due to the complex of bioreaction, many models have been proposed to describe bioreaction. As biodegradation of Direct Black 22 is due to decolorizing enzyme, Michaelis-Menten equation could be used to describe the reaction kinetics. Michaelis-Menten equation:

$$V = V_{max} \times S/(K_m + S).$$

Where V_{max} is the maximum substrate (i.e., Direct Black 22) consumption rate, mg/(L·h); V is the substrate consumption rate, mg/(L·h); S is the substrae concentration, mg/L; K_m is the Michaelis-Menten constant, mg/L. The result is shown in Fig. 4. Fig. 4 shows that experimental data can be fitted quite well with Michaelis-Menten equation. By calculating sum of the squared deviations, and let it be minimum, the equation coefficients can be obtained. The results are K_m = 146.03 mg/L, V_{max} = 22.55 mg/(L·h). Another way to obtain K_m and V_{max} is by using Lineweaver-Burk plot. Michaelis-Menten equation is transformed by a double reciprocal approach to Lineweaver-Burk equation as follows: $1/V = K_m/(V_{max}S) + 1/V_{max}$.

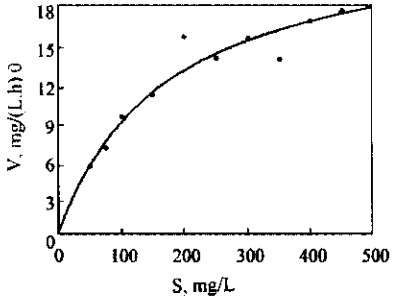

Fig. 4 Degradation rate curve of Direct Black 22 by *Aspergillus ficuum*

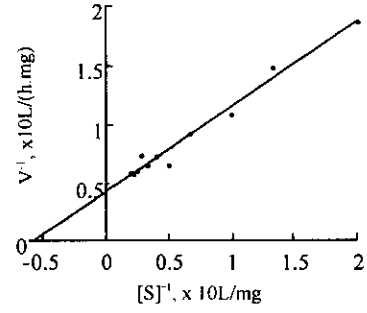

Fig.5 Double-reciprocal plot of Direct Black 22 by *Aspergillus ficuum*

Table 3 The results and analysis

Aspergillus ficuum	$V-S$		$1/V-1/[S]$	
	V_{max}, mg/(L·h)	K_m, mg/L	V_{max}, mg/(L·h)	K_m, mg/L
	22.55	146.03	24.03	173.12

A plot of $1/V$ versus $1/S$ gives $1/V_{max}$ as intercept on the ordinate when $1/S$ approaches zero, and an intercept of $-1/K_m$ on the abscissa for V approaches zero. The result is shown in Fig.5. By calculating sum of the squared deviations, and let it be minimum, another set of K_m and V_{max} can be obtained. These are K_m = 173.12 mg/L, V_{max} = 24.03 mg/(L.h). A comparison for K_m and V_{max} obtained from above two approaches is shown in Table 3. It shows that these results are similar. This suggests that the biodegradation of Direct Black 22 by *Aspergillus ficuum* is first order irreversible process.

2.5 Decolorization tests of other dyes

Besides of above decolorization tests of Direct Black 22, the authors also examined the decolorization ability of *Aspergillus ficuum* to direct yellow 12, reactive brilliant red KD-8B, and weak acid scarlet GF. The rate of decolorization was all more than 90%. This shows the wide dye decolorization spectra of *Aspergillus ficuum*.

3 Conclusions

Aspergillus ficuum used in this study degrades the dye direct black 22 at (1) a certain dye concentration (50 mg/L), (2) a fast rate of decolourisation, (3) a low glucose concentration, and (4) a temperature close to indoor ambient temperature. The method of dye decolorization by mycelial pellets is simple and practical, which may avoid the inconvenience of dye decolorization by immobilized cells. These are all required conditions for an economical and practical industrial microbiological process.

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