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Short Communication

Univariate Degradation of Indigo Carmine in Aqueous Solution by Inactivated Biomass in *Heterobasidion Insulare*: Preliminary Studies

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Abstract

The role of white rot fungus in the treatment of dye wastewater has been widely researched. Numerous genera of fungi have assumed responsibility for dye decolorization, either in living or dead form. This study looks at the degradation of an acidic dye, indigo carmine (IC), as the medium in an aqueous solution by means of biological degradation in dead fungus of *Heterobasidion Insulare*. The dye decomposition of reaction time relies on the primary dye concentration, mortality quantity of biomass, churn rate, and primary pH. Experimental results show that an increase in the mortality quantity of biomass significantly affects dye degradation. The highest degradation rate of dye was achieved at 125-150 rpm. Slightly reduced biological activity was found when we reduced the stirring rates. The pH of the reaction system is a slight variation in the 4-8 range, when dye degradation efficiency was not affected so obviously. The dye of color discoloration was observed to occur rapidly within 60 minutes. The degradation of dye by inactivated biomass of *H. Insulare* definitely depended on original dye-wastewater concentration in the aqueous solution. Dye degradation was reduced from 64% to 93% as the original contents were enhanced from 50 to 500 mg/l. This study was desirable in that it shows it is possible to degrade textile dyes by inactivated biomass of *H. Insulare*.

Keywords: indigo carmine, decolorization, *Heterobasidion Insulare*, inactivated biomass

Introduction

Textile industries generate huge volumes of wastewater that contain large amounts of dye. The removal of dye from textile effluents is one of the most significant environmental problems in that dye wastewater is highly visible and undesirable and also reduces light penetration and photosynthesis [1]. Due to the stable chemical structure of the dye molecule, in conventional conditions dyes in chemical synthetic have stable three-dimensional space molecules and are resistant to the decomposition of biological enzymes. At present, it has numerous means of eliminating dyes, such as membrane-separating

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technology, biological treatments, chemical flocculation, electrochemical oxidation, physical coagulation, ionic reverse osmosis, ozone oxidation, and physical adsorption [2-3].

Degradation of wastewater in a routine treatment process in biological methods is not a perfect method. It is unacceptable to use traditional methods of biological degradation of dye wastewater due of the low biodegradability of dye wastewater in cost and efficiency [4]. The method of using activated carbon as an absorbent to absorb the dye wastewater is exciting. However, it is prohibitively expensive and has low adsorption capacities activated in carbon [5]. Therefore, it is necessary to explore new technologies and new processes and efficient for degrading dye wastewater. Bio-sorption can be a defined organism, an organism's province metabolites, and debris on the grounds in which to absorb the organism derivatives, metal closure, and dyes causing odor [6-7]. In the current study focuses on the active microbial cell degradation capacity of the dye. However, few studies have focused on the dried fungal biomass degradation of dye wastewater. Both living bio-mass and dead fungi bio-mass already have proven to be able to remove dyes due to the existence of various functional isozymes groups on the total biomass of organisms [8-9]. However, it has been proven that inactivate bio-mass cells may offer more merit than active cells. First of all, the active biomass process of microbial degradation of dye wastewater requires microbial growth conditions such as NPK, pH, and culture temperature requirements. Take advantage of microbial degradation of dead cell nutrients to avoid the need for a training environment, while avoiding the impact of wastewater on the growth of living cells. Furthermore, dried microbial cell mass as an adsorbent and its activity of the enzyme is satisfactory for long-term storage. Furthermore, there are studies to report that the dried microbial cells can be effectively adsorbed by dye in the traditional sense of variety, such as indigo and so on.

The dye in the water-soluble system can be used as an industrial precursor material in the chemical process. Its characteristics are more readily soluble in aqueous solution. For example, using sulfuric acid to generate indigo carmine (Acid Blue 74, Fig. 1) it is a kind of common dye that may also be used as food additives of a microscopic tracer in biology, and as a tracer in analytical chemistry [10]. In this study, inactivated biomass of *H. Insulare* was used as a biological degradation of indigo carmine. The aim of this research was to develop effective adsorbents for dye-removal technology. Therefore, it is indispensable for researching the effect of respective operating parameters with degradation of dye, such as concentrations of dye initial pH and absorbent amount.

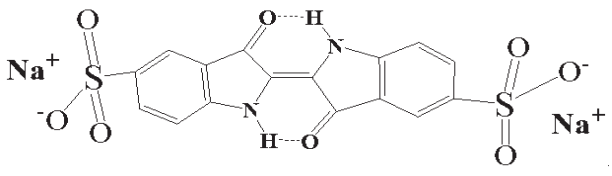

Fig. 1. Chemical structure of indigo carmine.

Materials and Methods

Fungal Biomass Preparation

The white rot fungus *H. Insulare* was cultured at 30ºC on potato dextrose agar (S-PDA). After 1-2 weeks [11], the mycelium suspensions were preparatory and used for nurturing the inoculums. The mycelia suspension was transferred into 250 ml flasks in 100 ml potato dextrose agar (S-PDA). The proliferation medium consisted of $CaCl_2$•$2H_2O$, 0.1 g; $FeSO_4$•$7H_2O$, 0.40 g; KH_2PO_4, 0.2 g; $MgSO_4$•$7H_2O$, 0.1 g; $(NH_4)H_2PO_4$, 0.5 g; glucose, 2.0 g; and potato extract 100 ml. All of them were dissolved and mixed in distilled water. The fungal mycelium balls were formed in 2-5 days under 30°C within 150 rpm. When the average diameter of mycelium ball diameters attain 3-5 mm, it is time to gain pellets.

After centrifuging at 3,000 rpm for 15 mins and rinsing in triplicate, the granules were dried at 30ºC for 24h and ground in a mortar to granularity of less than 100 μm of dried powdered mycelium balls (called inactivated biomass). Inactivated biomass was used for dye degradation studies. The experiments were replicated in triplicate. The statistical analyses were performed in the SPSS 16.0.

Analysis

The concentration of the dye wastewater from the plant was 0.50 g/L. Accurately diluted dye stock solution as 0.1, 0.2, 0.3, and 0.4 g/l, configured with different concentration gradients, got prepared test solution.

For setting different extensive inactivated biomasses with experimental design we transferred them to a

Table 1. Effect of time and agitation rate on the degradation (%) of indigo carmine by dried powdered mycelium balls of *H. Insulare*.

Time (minutes)	Indigo carmine removal (%)±Sd	Agitation rate (rpm)	Indio carmine removal (%) Sd
30	88±0.57	Static	92±0.57
60	93±0.57	50	93±0.57
90	94±0.00	100	94±1.00
120	94±0.57	150	95±0.57
180	95±1.00	200	93±2.51
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SD: standard deviation; the results shown are averages of duplicate shake cultures and triplicate assays with SD within 10% of the mean; results are averages of three independent assays with SD within 10% of the mean.

100 ml flask containing 2 mg/l of dye accommodated with distilled water. The impact of transfer was studies at different stirring rates of 10, 40, 80, 120, 160, and 200 rpm. The influences of the numerous dried powdered mycelium balls were investigated at different quantities, namely 0.1, 0.2, 0.3, 0.4, and 0.5 g. In 20 ml test tubes, the reaction of contact time was determined at 30, 60, 90, 120, and 180 minutes. For the sake of our study on the effect of pH on dye decomposition, the pH of the solutions was different from 2 to 8, by adding 0.1 M NaOH and 0.1 M HCl solutions. The effect of the original dye concentration on decomposition was studied between 150-500 mg/L at 30ºC. The concentration of residual dye was determined at their visible maxima for using absorbance values measured with a spectrophotometer comparing before and after the degrading treatment. All the experimental samples of the bio-sorption experimental system constructed three parallel samples. The same as the reference standard without adsorbent was the same tests and percentage decolonization was calculated as follows:

$$\text{Decolorization}(\%) = \frac{(\text{Initial absorbance} - \text{Observed absorbance})}{\text{Initial absorbance}} \times 100$$

Results and Discussion

Effect of Adsorbent Amount on Decolorization

The dye degradation of IC by dried powdered mycelium balls of *H. Insulare* was studied in changing the quantity of dried powdered mycelium balls (0.1, 0.2, 0.3, 0.4, and 0.5 g/20 ml) in the sample and repeating three times. For the original concentration of the dye the reaction temperature and time were 50 mg/L, 30ºC, 60 minutes, and 120 minutes. Unless otherwise indicated, the experiment was maintained without the variety of the pH of the dye solution. As expressed in Fig. 2, the percentage of discoloration was proportional to the adsorbent dose. In accordance with the above test, 0.2 g/20 ml of dried powdered mycelium balls were picked as the sorbent dose for further experiments.

The degradation effectiveness of gradient of amount of biomass was equivalent of 0.2 g and 0.5g, but more than 0.1 g. It is more inclined to use smaller amounts of adsorbent in order to reduce operating costs on the scale of industrial applications. The plurality targets of adsorption position in dye degradation adsorbent dose increase can be attributed to increased adsorption surface area and availability. Discoloration of similar outcomes were reported for discoloration of astra-zone blue by inactivated biomass of H.Insulare [12]. The degradation mechanism to enhance the discoloration of malachite green was possible by raising the sorbent dosage of the agro product waste [13]. A similar study was in the news for degradation of quinoline blue and the analogous anionic dyes, separately [14].

Effect of Initial pH on Decolorization

The impact of original pH on the dye-waste degradation capacity of inactivated biomass H.Insulare was researched in the pH extent 3.0-8.0 to consider the influence of pH on discoloration capacity of inactivated biomass. The studies were implemented at 3 mg/L original dye waste concentration with 0.2 g 20/mL adsorbent mass of dried powdered mycelium balls 100 mesh at 30ºC for 60 and 120 minutes and 150 rpm (Fig. 3).

Variation pH on the degradation of dye wastewater adsorbent may be in two kinds of mechanisms. The electrostatic interaction between the carbon chain active with acidic dyes ionizable groups superimposed the reaction in chemicals among the adsorbents [15]. The interaction in dye molecules and the functional group biomass of the dead is inexplicable. Furthermore, there are oligo electronic interactions acting on site wastewater dye and dried inactivated biomass adsorbent on the surface. However, in this study the death of raw materials for dye

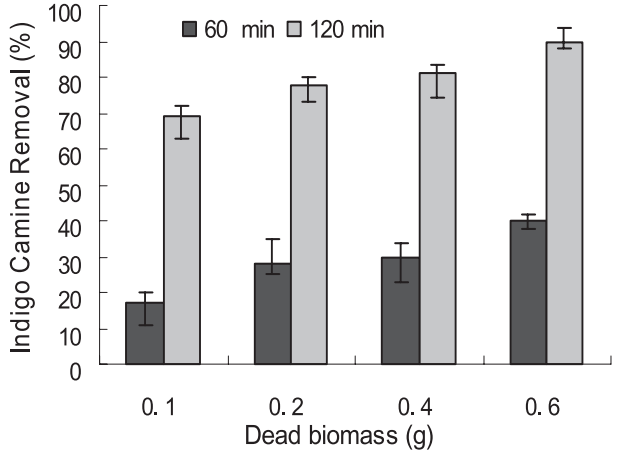

Fig. 2. Effect of dead biomass amount of *H. Insulare* on indigo carmine dye degradation.

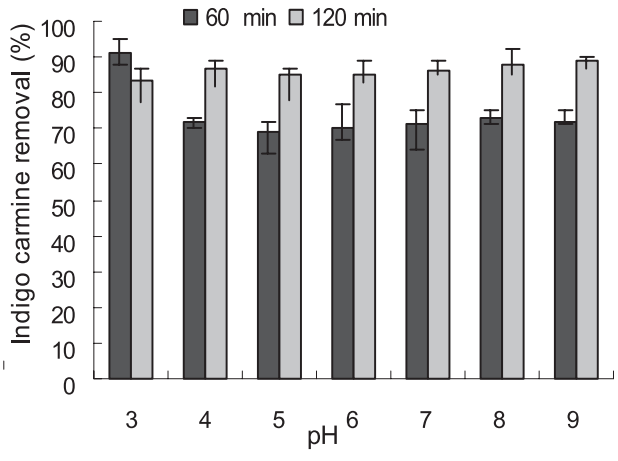

Fig. 3. Effect of pH on indigo carmine dye degradation.

degradation capacity remained at 4.0-8.0, illustrating that dried biomass enzyme activity cannot be ignored. On the other hand, such action by a weak electrostatic attraction effect shows that mutual interaction strength cannot be neglected [16-17].

Degradation of Indigo Carmine by Dried Powdered Mycelium Balls at Different Time Intervals

The inactivated biomass of *H. Insulare* was tested for IC degradation capacity at different time intervals in this section of our research. To research the degradation reaction time on decolorization capacity of inactivated biomass, the experimental conditions are at 0.2 g/20 ml adsorbent mass (100 mesh) at 30°C of the tests, which were conducted at 50 mg/L original concentration of dye, pH 2.0, and 150 rpm. We observed that the color of dye degradation obviously happens within 60 min (Table 1). The color of dye degradation was higher than the start of absorption at the original period in 260 min, and following this cycle the colorimetric of absorption in dyes did not undergo significant alterations.

Effect of Stirring Degradation of Indigo Carmine Dried Powdered Mycelium Balls of *H. Insulare*

To test the impact of stirring rate on the degradation of IC we used 0-250 rpm. To test the influence of the agitation rate of decolorization capacity of dried powdered mycelium balls in the experiment, the experimental conditions were at 0.2 g/20 ml adsorbent mass 100 mesh at 30ºC for 60 minutes and pH of the experiments, which were implemented at 50 mg/L original dye concentration 2.0. The dried powdered mycelium balls high performance dye degradation was at the entire stirring rate (Table 1). As Table 1 shows, the agitation values of optimized operating conditions for decolorization were ranged from 150 to 250 rpm. As the stirring speed was reduced slightly, the degree of decolorization of dye activities was found to be lower. Maybe the reason is that the biomass particles agglomerate at lower agitation speeds [18]. Experimental data suggest that the discoloration of orange II attains 44% after 24 h blended incubation without agitation conditions and 95% with stirring conditions. This means that high decolorization yield offers many advantages in development of practical processes when in lower or static agitated conditions [19].

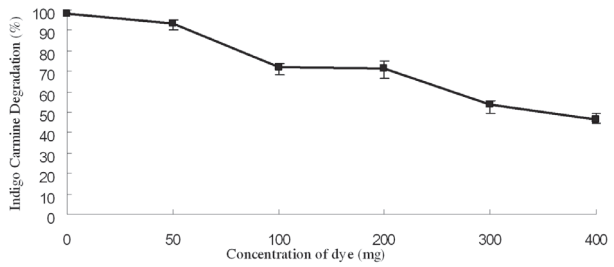

Fig. 4. Influence of original dye concentration (mg/L) on degradation of indigo carmine.

Effect of Original Dye Concentration on Decolorization

The influence of initial dye concentration of indigo carmine on decolorization reaction system biomass degradation processes was studied. Design of the experimental parameters is dry biomass dose was set to 0.2 g/20 ml solution at 30ºC, pH 2.0, and fixed agitation 100-150 rpm for 60 minutes at different experimental initial concentrations of sample dye 50, 100, 200, 300, 400, and 500 mg/l. The degradation of dye by dried powdered mycelium balls of *H. Insulare* obviously relied on the initial dye concentration of the solution (Fig. 4). Dye degradation was reduced from 93% to 64% as increment concentration gradients from 100 to 1,500 mg/l indigo carmine. Under normal circumstances in the textile industry – due to the restrictions process – the threshold value of its wastewater discharge is not more than 500 mg/l, and conventional dye concentration is about 10-50 mg/l. As a result, it is fairly significant to take precautions against environmental pollution.

Conclusions

During the last decade, research has focused on the development of new degradation technologies that result in complete decomposition of dye molecules. In the present study, dried powdered mycelium balls of *H. Insulare* were applied successfully for the sorption of indigo carmine. The operating conditions may lead to a negative effect on discoloration with living cells regarding concentration of dye, reaction system pH, and temperature. However, it has many advantages compared with live biomass inactivated biomass. Inactivated biomass may be stored or prolonged in application and the operation is not difficult and its regeneration is not complicated. Inactivated biomass may be produced with industrial and agricultural by-products. Therefore, it can be used as inexpensive and effective bio-sorbent.

By inactivated biomass of *H. Insulare* with the experimental conditions at 30ºC and pH 2.0 of the experiments whose exploration of was at fixed a sorbent dose (0.2 g/20 ml) in the experimental system of solution. The results obtained for the principle as it relates to dye degradation can be regarded as a prophase procedure for the representativeness of the experimental behavior and for developing technological design.

Acknowledgements

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