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Time regulation researches on degrading reactive dyes by immobilized white rot fungi under non-sterile condition

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ABSTRACT

The effects and mechanisms on the decolorization of reactive dyes K-2BP at the different dye-added time were investigated in this study under non-sterile conditions. The results showed that based on the optimal bacteriostasis incubation strategy for immobilization, the decolorization efficiency achieved 94.69% and 93.65% with 2 and 3 days cultivation after adding dye. The cultivation and degradation cycle were shortened from the original 10 days to 4-5 days. However the maximum decolorization efficiency was only 57.56% in the 5th day dye-added system. The lower decolorization efficiency was due to the peak of enzyme production was missed by adding dye after 5 days cultivation, instead of the disturbance of other bacterias. Therefore, the optimal bacteriostasis incubation strategy for immobilization under non-sterile conditions could successfully restrain the growth of other bacterias. Furthermore accompanied with the appropriate dye-added time, the degradation cycle on the reactive dyes can be shortened.

KEYWORDS

White rot fungi; Non-sterile conditions; Time regulation; Decolorization; Reactive dyes.

INTRODUCTION

The dyes wastewater, released from dyestuff industry, printing industry or dyeing industry, is one of the most difficult industrial wastewater to treat. Dye has high stability and is hard to be biodegraded, so it can still exist in the environment after a long time. Traditional treatments of dyes wastewater cost too much and are hard to achieve desirable effects. In recent years, an innovative environmental biotechnology—fungus technology, becomes a promising approach to the dyes and dyeing industrial wastewater pollution control.

The degradation of persistent organic pollutants by white rot fungi *Phanerochaete chrysosporium* was firstly reported by John in 1985[1]. Subsequently, Kirk[2] found out the nonspecificity of the lignin peroxidase system produced by white rot fungi at the secondary metabolism stage. White rot fungi can degrade lignin and many organic pollutants, such as polycyclic aromatic hydrocarbons, chloro-aromatics, dyes, dynamites, pesticides and so on[3–8]. Moreover it also has a strong degradation of the synthetic organic pollutants. In recent years extensive researches on white rot fungi have been widely carried out. Most researches are focused on the biological characteristics, degradation law, biochemistry mechanisms, enzymology, molecular biology, commercial process, application in the environmental engineering and etc.

However almost all the studies using white rot fungi for the treatment of dye wastewater are of laboratory-scale and researches have realized that the bacterial contamination is a bottle-neck problem for the development of technology of degrading dyes by white rot fungi in practical application[9-12]. The main reason is that white rot fungi is low-grade eukaryotic micro-organisms and grow slowly compared with bacteria. Once bacteria which grow and reproduce much faster than white rot fungi invaded into the reaction system, they could compete for nutrients with the white rot fungi and predominate in the reaction system rapidly. As a result, white rot fungi would stop growing further and lose the degradation ability for dyestuffs in the entire processing system and the invading bacteria exerted much impact on the secretion of extracellular degradation enzymes. Therefore, it is obviously unpractical to use the sterilization means in the laboratory to prevent a full-scale reactor system from bacterial contamination due to high operational cost[13].

In recent years, many researchers have tried to work on the bacterial contamination problem in practical application for white rot fungi. Currently there are two ways to solve this problem. One is to study bacteriostasis medium and optimization of the incubation conditions. Libra et al.[14] and our group's previous research[15] indicated that a nitrogen-limited medium could effectively suppress bacterial growth in different white rot fungi systems during the degradation of dyes. Bacterial growth can be effectively restrained by pH[16], incubation method[17], injecting time of dye[18], and the levels of trace element Fe[19]. The other is to study the bacteriostatic carrier and immobilized culture technology. Leidig et al.[20] used PVAL hydrogel to embed the mycelia of *Trametes versicolor* in order to avoid the fungi and extracellular enzyme being attacked by bacteria under non-sterile conditions. Our group's previous research[13] also determined that selection of appropriate carriers could suppress the interference of other bacterias to white rot fungi, increase the enzyme-production and improve the degradation effeciency of dyes. However, the enzyme activity produced by white rot fungi declined sharply under non-sterile conditions. Once missing the appropriate dye-added time, the degradation cycle would prolong and the degradation efficiency would obviously decline. Therefore, the research of dye-added time control has great importance in improving the degradation efficiency, shortening the operational cycle and reducing the operational cost.

The purpose of this paper is to investigate the time regulation on decolorizing reactive dyes by immobilized white rot fungi under non-sterile conditions, in order to figure out a better way to optimize

the immobilized incubation strategy for dyes degradation and shortening the dyes degradation cycle and the white rot fungi pure cultivation cycle.

MATERIALS AND METHODS

Micro-organism

P. chrysosporium BKM-F-1767 was obtained from ESPC State Key Joint Laboratory, Department of Environment Science and Engineering, Tsinghua University, Beijing, China. The culture was maintained on potato–glucose–agar slants at 4°C.

Culture media

Solid medium

(1) PDA medium: lixivium of potato 200 g/L, glucose 20 g/L, and agar 20 g/L.
(2) Beef lotion and peptone medium: beef lotion 5.0 g/L, peptone 10.0 g/L, Na_2Cl 5.0 g/L, and agar 15 g/L.

Liquid medium

The medium used for the growth of *P. chrysosporium* and decolorization was prepared according to Tien and Kirk[21] with modification. The medium contained glucose, 10 g/L; ammonium tartrate, 0.8 g/L[22]; KH_2PO_4, 2 g/L; $MgSO_4$, 0.5 g/L; $CaCl_2$, 0.1 g/L; veratryl alcohol, 0.22 mL[23]. Vitamin B1 was added under sterile condition to obtain a concentration of 1 mg/L.

Carriers

The polyurethane foam (aperture 0.031 cm, density 0.039 g/cm^3) was used as carriers in the experiments, the size of the carrier of 1.0 cm×1.0 cm×1.0 cm, the weight of carrier of 1.2g, and three prism-shaped carrier. These carriers were washed three times with distilled water, then dried at 60°C and autoclaved at 121°C for 20 min prior to use.

Dyes

The dye used in this research was Reactive brilliant red K-2BP, a disperse dye which belongs to the class of diazo dyes. The initial concentration of the dye added to the medium was 30 mg/L.

Culture condition

Inoculum was obtained on PDA slants at 37°C for 6 days. The growth of cultures (suspended cells and immobilized cells) was carried out in 250 mL Erlenmeyer flasks containing 100 mL of liquid medium in a reciprocating shaker (model TZ-2EH) at 160 rpm and under 37°C. The inoculum level was 2.5×10^6 spores.

Experiment design

Control experiments

The control experiments were conducted under three process conditions: (a) incubation of *P. chrysosporium* and decolorization under sterile conditions, (b) incubation under sterile conditions but decolorization under non-sterile conditions, and (c) incubation and decolorization under non-sterile conditions.

Adding dyes experiments

The adding dye experiments were conducted with the optimal incubation strategy for immobilization[13], adding 3 mL sterile dyes (the final concentration was 30 mg/L) into every sample of the above three systems (i.e. incubation of *P. chrysosporium* and decolorization under sterile conditions,

incubation under sterile conditions but decolorization under non-sterile conditions, and incubation and decolorization under non-sterile conditions) on the 2nd, 3rd, 4 th and 5th day.

All experiments were carried out in triplicate and the results were expressed as the mean values. The decolorization efficiency and other measurement indexes were analyzed daily. The *P. chrysosporium* and other microorganisms were observed daily by a microscope. The optimal decolorization system with high decolorization efficiency and short decolorization time was screened out under non-sterile conditions through the comprehensive comparisions, as a result the optimal control scheme of the adding dye time was determined.

Analytical methods

Decolorization efficiency

The dye concentration was measured spectrophotometrically at 533 nm (SHIMADZU UVmini 1240, Japan). Decolorization is reported as % decolorization = $((A_0 - A_t)/A_0) \times 100$, where A_0 is the initial dye absorbance and A_t is the dye absorbance at time t.

Manganese peroxidase (MnP) activity[24] and Laccase (Lac) activity[25]

Biomass

Carriers were washed and dried (105°C, 24 h) until the unchanged weight (m_1) in a conical flask before fermentation cultivation. After the experiment carriers with mycelia were taken out from the flask, filtered, then dried (105°C, 24 h) to get the unchanged dry weight (m_2). The biomass is calculated as $m = m_2 - m_1$.

RESULTS AND DISCUSSION

chrysoporium growth and systematic bacterial contamination at the different dyes-added time

Growth of white rot fungi at the different dye-added time

The bacteriostatic effects were relatively good and the anti-interference ability of bacterias was correspondingly strong during the entire reaction process due to the optimal bacteriostatic incubation strategy for immobilization[13]. Therefore, the growth of white rot fungi was in good condition without any significant difference under both sterile and non-sterile conditions. The growth of white rot fungi was apparently affected by the dyes in different dye-added time systems, consequently the growth of white rot fungi was obviously distincted because of the different dyes-added time. The growth of *P. chrysosporium* at the different dye-added time was showed in TABLE 1.

TABLE 1 : Growth of *P. chrysosporium* at the different dye-added time

Adding time (d)	Injecting dyes			No dyes	
	Incubation and decolorization were under sterile conditions	Incubation was under sterile conditions and whereafter decolorization was under non-sterile conditions	Incubation and decolorization were under non-sterile conditions	Incubation under sterile conditions	Incubation under non-sterile conditions
2	+	+	+		
3	+	+	+	+++	+++

4	++	++	++
5	++	++	++

"+" — growth in general; "++" — growth relatively well; "+++" — growth well.

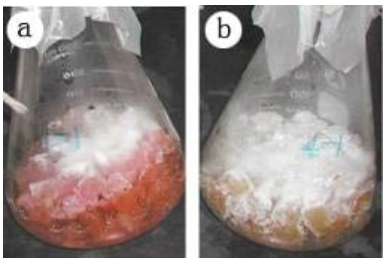

Figure 1 : Shape of white rot fungi in immobilized culture with polyurethane foam under dye (a) and no dye (b) conditions

From the apparent observation, the growth of white rot fungi followed two rules in our experiments.

Firstly, the growth of white rot fungi in the no-adding dye system was better than in the adding dye system (Figure 1). Until the 2nd cultivation day, the growth of white rot fungi in each system was almost the same with a small quantity of white powder-like thalli on the carrier surface. The liquid culture medium was clarified and there was slightly acidic odor. Until the 3rd cultivation day, there was a thick layer of white rot fungi covering on the polyurethane foam carrier surface in no-adding dye system. White rot fungi thrived and was similar to the natural state. While the mycelium of the foam surface in the system that adding dye after 2 days cultivation was obviously less than that in no-adding dye system (Figure 1-a). Moreover the mycelium in adding dye system (Figure 1-a) was more than that in no-adding dyes system, but the fastness between the carriers and the mycelium was worse (Figure 1-b). It turned out that dye had certain negative impacts on the growth of white rot fungi and the combination degree between the thalli and the carriers.

Secondly, the later the dye was added, the better the growth of white rot fungi was (TABLE 1). The growth of white rot fungi was all in good condition and basically similar during the 1st and 2nd cultivation day without adding dyes. As the dyes gradually added, the thalli in the former adding dyes system, to some extent, was restrained by the dyes. The quantity of white mycelium on the carrier surface in former adding dye system was more than the later one. Meanwhile many spores and mycelium were detached from the carriers to the culture medium. In a word, adding dye time had certain impacts on the growth of white rot fungi and the combination degree between the thalli and the carriers.

Systemic bacterial contamination at the different dye-added time

The adding dyes did have some impacts on the growth of white rot fungi based on the conditions of thalli growth mentioned above. The slight influence, as a whole, did not affect the predominate growth status of white rot fungi due to the optimal bacteriostatic incubation strategy for immobilization[13]. Therefore, the bacteriostatic capacity of every culture system under different conditions was strong. Figure 2 presented that the bacterial contamination was not found in the different dye-added time systems, except for little coccobacillus contamination in the 2nd day dye-added system.

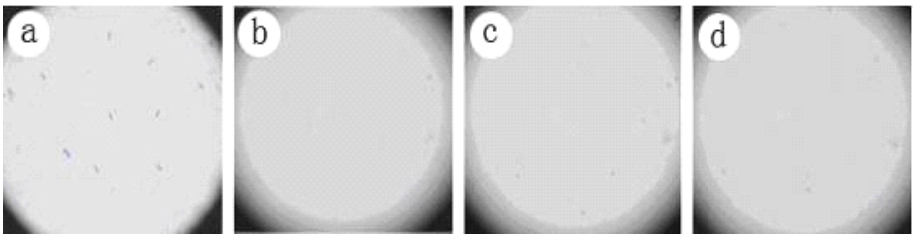

Figure 2 : Microscope photograph (1000 times) by liquid culture medium in immobilized culture with polyurethane foam of different dyes-added time under non-sterile condition.

Consequently, the premature dye adding objectively had negative impacts on the bacteriostatic effect, which was consistent with the previous researches[18]. Therefore, the optimal bacteriostatic incubation strategy for immobilization could apparently suppress the growth of other bacterias, ensure the predominated growth status of white rot fungi in the culture system and promote the enzyme production and the decolorization efficiency.

Decolorization of dye

Impact on the degradation the reactive dyes K-2BP by *P. chrysosporium* at the different dye-added time

At the different dye-added time, the decolorization performance of the degrading dye systems based on the optimal bacteriostatic incubation strategy for immobilization was shown as Figure 3.

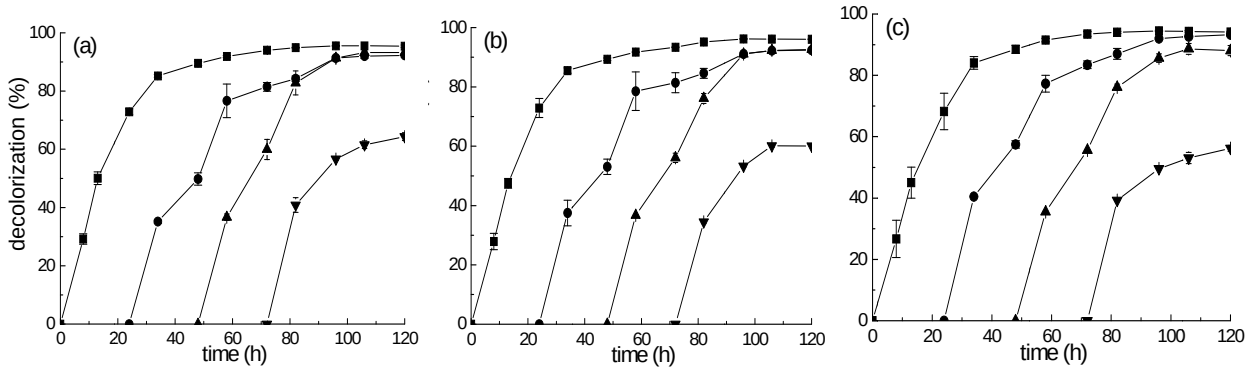

Figure 3 : The performance of the decolorization under different conditions based on the optimal suppressing bacteria immobilized culture; (a)decolorization under sterile condition after incubation under sterile condition;(b)decolorization under sterile condition after incubation under non-sterile condition;(c)decolorization under non-sterile condition after incubation under non-sterile condition.

(■) Dye- added on the 2nd day; (●) Dye-added on the 3rd day; (▲) Dye-added on the 4th day; (▼) Dye-adding on the 5th day

The decolorization of reactive dyes K-2BP by using white rot fungi showed a similar law under the three conditions i.e. the sterile culture with sterilization degradation dye, the sterile culture with non-sterilization degradation dye, and non-sterilization culture with non-sterilization degradation dye. The decolorization efficiencies in the systems adding dyes on the 2nd, 3rd, 4th day were overall high and the average maximum decolorization efficiencies were 95.39%, 92.92% and 91.58%, respectively. While the average maximum decolorization efficiency in the system adding dyes on the 5th day was only 61.41 %. The results showed that the dye-added time actually had obvious effects on the degradation of the reactive dyes K-2BP by using white rot fungi.

MnP and Lac production

In this study, we tested the enzyme activities of MnP and Lac in the sterilization culture and sterilization degrading dye system and the non-sterilization culture and non-sterilization degrading dye system at the different dye-added time (Figure 4).

The optimal bacteriostatic incubation strategy for immobilization could suppress the growth of other bacterias under the non-sterile condition, so the MnP production was basically unanimous under the sterile or non-sterile condition (Figure 4). And there were three characteristics as follow.

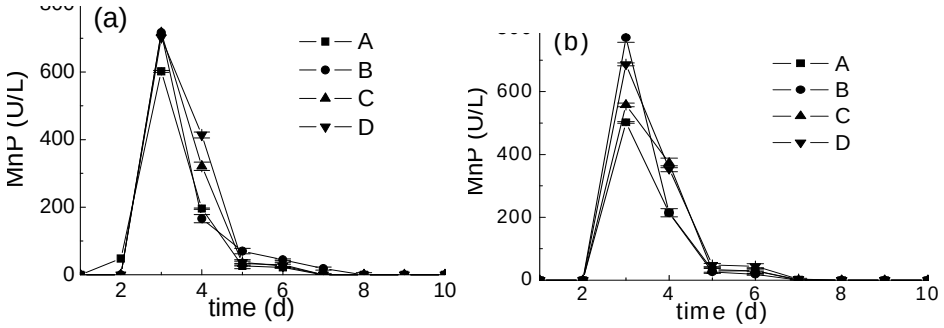

Figure 4 : MnP excreted in decolorization system under sterile (a) and non-sterile (b) conditions based on the optimal suppressing bacteria immobilized culture project; (A) Dye-added on the 2nd day; (B) Dye-added on the 3rd day; (C) Dye-added on the 4th day; (D) Dye-added on the 5th day

Firstly, the peak of MnP came out on the 3rd day in all the systems at the different dye-added time, which well duplicated the results of former studies[13].

Secondly, the dye-added time had an apparent influence on the peak of the enzymatic activity. The peak value of MnP was obviously lower in the system adding dye before the peak of the enzymatic activity than that adding dye after the peak of the enzymatic activity. Figure 4-a indicated that the maximum level of MnP was 601.8 U/L in the dye-added system when adding dye on the 2nd day. Correspondingly, the maximum levels of MnP were 716.25 U/L, 716.25 U/L, 701.48 U/L separately in the dye-added systems when adding dyes on the 3rd, 4th, 5th day, which were 100 U/L higher than that on the 2nd day. The dyes were added after the peak of the enzymatic activity in the 3rd, 4th, 5th day dye-added systems and the dyes did not affect the peak of the MnP activity, that was why their maximum levels of MnP were high and similar (Figure 4-b). Consequently it had obvious negative effect on the maximum level of enzymatic activity in dye-added system when adding dye before the peak of the enzymatic activity.

Thirdly, the size and rate of the decline of the enzymatic activity were affected by the dye-added time during the period after the peak of MnP. The decline of enzymatic activity from the 3rd to 4th day was observed in Figure 4-a and 4-b. The enzymatic activity in the 3rd day dye-added system was affected extremely and decreased fast. The peak levels of the enzymatic activity dropped from 716.25 U/L and 771.63 U/L down to 166.14 U/L and 214.14 U/L, which declined over 500 U/L in one day. The peak levels of the enzymatic activity also decreased sharply from the 3rd to 4th day in the 2nd day dye-added system, which was analogous to the 3rd day dye-added system. The rapid decline of enzymatic activity in a short time was due to the dye effects and the own unsteadiness of the enzyme causing the nature inactivation. The sizes and rates of the decline of the enzymatic activity were comparatively normal from the 3rd to 4th day in the 4th and 5th day dye-added systems without dye infection, which were mainly caused by the natural enzyme inactivation. Therefore, the decrease of the enzymatic activity after the peak of the enzymatic activity was partly because of the own unsteadiness of the enzyme causing the

nature inactivation, more importantly due to the dyes depredation causing the inactivation of the enzymatic reaction. Moreover the size and speed of the inactivation of the enzymatic reaction were obviously influenced by the dye-added time.

The performance of the Lac production and variation, similar to the MnP production, had three characteristics (showed in Figure 5). However, the peak period of the enzymatic activity and the maximum level of the enzymatic activity were different. The three characteristics listed as follow: Firstly, the peak of MnP came out on the 4th day under sterile or non-sterile condition at the different dye-added time, which well duplicated the results of former studies[13]. Secondly, the dye-added time had an apparent influence on the peak value of the enzymatic activity. The maximum levels of the enzymatic activity were affected by the dyes in the 2nd, 3rd, 4th day dye-added systems, generally lower than that in the 5th day dye-added system, for the dyes were added to the systems before the peak of the enzymatic activity. The more earlier time to add dyes, the greater impact on the enzymatic activity. The dye-added time was after the peak of the enzymatic activity in the 5th day dye-added system, as a result the maximum levels of the enzymatic activity were not affected by the dyes, the levels were high and up to 56.67 U/L and 46 U/L under the sterile and non-sterile condition. Thirdly, the size and rate of the decline of the enzymatic activity were still affected by the dye-added time during the period after the peak of MnP. The decline of the enzymatic activity from the 4th day to the 5th day was showed in the Figure 5-a and 5-b. The more earlier to add the dyes, the greater decline on the size and rate of the enzymatic activity.

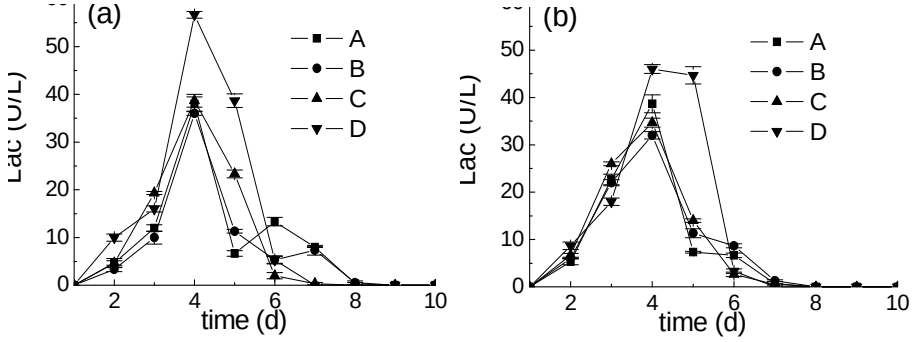

Figure 5 : Lac excreted in decolorization system under sterile (a) and non-sterile (b) conditions based on the optimal suppressing bacteria immobilized culture project.

Relationship between the enzyme activity and the decolorization efficiency at the different dye-added time

It existed an obvious corresponding relationship between the enzyme activity and the decolorization efficiency of the optimal bacteriostatic incubation strategy for immobilization. The higher the enzyme activity (MnP) was, the higher the decolorization efficiency was, and vice versa the lower. Based on the early study on the enzyme activity and the synthesized analysis of Figure 3, there were two kinds of corresponding relationship between the enzyme activity and the decolorization efficiency at the different dye-added time.

Firstly, the decolorization efficiency was high when the dye was added before the peak of enzyme activity. The peak of the enzyme production came in the 3rd day in this experiment, which was adopted the optimal bacteriostatic incubation strategy for immobilization. Figure 3 illustrated the maximum decolorization efficiencies were over 90% when adding the dyes before the peak of the enzyme activity under the three conditions, i.e. the sterile culture with sterilization degradation dye, the sterile culture with non-sterilization degradation dye, and non-sterilization culture with non-sterilization degradation dye (when to add dyes in the 2nd, 3 rd day). While the decolorization efficiency was

obviously lower in the 5th day dye-added system, with the highest decolorization efficiency was only around 60%, when dyes were added after the peak of the enzyme activity. The majority of dye decolorization was due to the absorption of the carriers and the thalli. Although the dye was added in the 4th day after the peak of the enzyme activity, the highest decolorization efficiency was still as high as 90%. Figure 4 showed that more than 300 U/L of MnP could be detected in the 4th day after the peak of the enzyme activity. 300 U/L of enzyme activity was enough for the system to degrade 30 mg/L the reactive dyes K-2BP according to the previous study[13].

Secondly, from the trend of the decolorization efficiency changing with the time, the more closer to the peak of enzyme production in the dye-added systems, the relatively higher the 24 h decolorization efficiencies and the highest decolorization efficiencies were. While the more away from the peak of enzyme production, the more decrease on the 24 h decolorization efficiencies and the highest decolorization efficiencies. For example, under the non-sterilization culture degradation and non-sterilization degradation condition, the 24 h decolorization efficiencies of the 2nd, 3rd, 4th, 5th day dye-added systems were 68.21%, 57.47%, 55.60% and 49.57% respectively. And the maximum decolorization efficiencies were 94.69%, 93.65%, 88.64% and 57.56%. The decolorization efficiency decreased with the postponed dye-added time. The results were opposite to the early researches[5] that the 24 h decolorization efficiency increased with postponing the dye-added time in the suspended culture program. The reason why drew out completely contrary results with the same method was the peak of the enzyme production under the suspended culture took place in the 5th day. Whereas the peak of MnP was advanced to the 3rd day by using the optimal bacteriostatic incubation strategy. In general the jump value of 24 h decolorization efficiencies is primarily consist of two parts, one is the adsorption rate of the mycelium and the carriers, the other is the degradation rate of the ligninolytic degradation enzyme system. The proportion of the two parts is different due to the specific circumstances. For instance, the 24h decolorization efficiency in the 2nd day dye-added system was up to 68.21%, which was partially attributed to the dye adsorption of the mycelium and the carriers and most due to the dye degradation by MnP. The 24h degradation period was just the period of the MnP enzyme activity ranging from generation to the peak (Figure 4). The same law worked in the 3rd day and 4th day dye-added systems as well. Although the 24 h decolorization efficiency was still high in the 5th day dye-added system, it mainly relied on the adsorption function of the mycelium and the carriers with less of enzyme degradation. Because the enzyme activity had almost disappeared during the 24 hours from the 5th day to the 6th day.

The results revealed that the high decolorization efficiency could be obtained by controlling the dye-added time before the peak of the enzyme activity. From the trend of the decolorization efficiency changing with time, the sudden change of the decolorization efficiency mostly concentrated in the 24 hours after the dyes adding. Then the decolorization efficiency slowly increased and basically reached the maximum until 48 hours. Following the principles of shortening the culture time and operating cycles, saving operational cost, not affecting the growth of thalli and enhancing the decolorization efficiency as far as possible, eventually we came to a conclusion. The desirable decolorization efficiency can be attained by adding dyes in the 2nd or the 3rd culture day on the basis of the optimal bacteriostatic incubation strategy for immobilization. Together with the 48h to degrade the dyes, the operational time of the dye degradation system of the immobilized white rot fungi was shortened from the original 10 days to 4 or 5 days.

Impact on the decolorization efficiency by the culture and degradation environment at the different dye-added time

The differences of the decolorization effects were slightly small under the different dye-added time conditions, the different culture conditions and the different degrading conditions showed in Figure6. According to the standard deviation analysis, it was illustrated in Figure6-a, b, c, d that the maximum standard deviations were 2.70, 3.84, 3.85 and 4.56 among the dye degradation curve A, B and C under three different culture and degrading conditions. Meantime the general discrete degrees of the

curve A, B and C were relatively small. Consequently, if the optimal bacteriostatic incubation strategy for immobilization was adopted, the culture condition and the degrading dye condition had not significant impact on the decolorization effects at the different dye-added time.

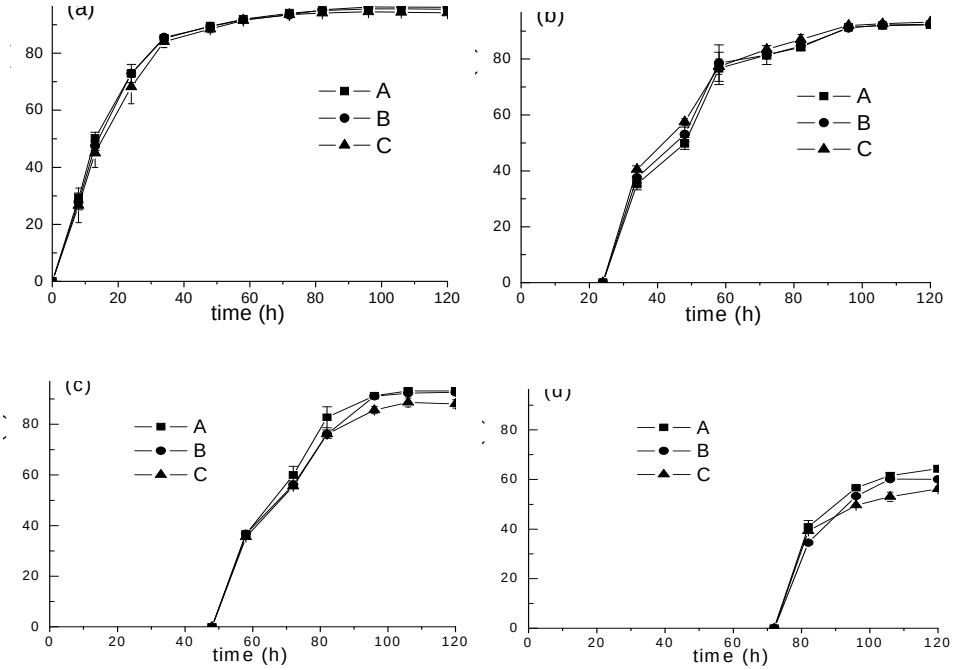

Figure 6 : Comparison of decolorization under different conditions based on the optimal suppressing bacteria immobilized culture project; (a) Dye-added on the 2nd day; (b) Dye-added on the 3rd day; (c) Dye-added on the 4th day; (d) Dye-added on the 5th day

(A) decolorization under sterile condition after incubation under sterile condition; (B) decolorization under sterile condition after incubation under non-sterile condition; (C) decolorization under non-sterile condition after incubation under non-sterile condition.

Biomass

In order to accurately assess the influences on the growth of the white rot fungi under the conditions of dye-added or not, different dye-added time and different culture systems, the biomasses of different dye-added time systems were investigated on the 10th day.

TABLE 2 : Biomass of reaction system at different dyes-added time

Adding time (d)	Injecting dyes (10^{-2} g/mL)			No dyes (10^{-2} g/mL)	
	Incubation and decolorization were under sterile conditions	Incubation was under sterile conditions and whereafter decolorization was under non-sterile conditions	Incubation and decolorization were under non-sterile conditions	Incubation under sterile conditions	Incubation under non-sterile conditions
2	0.1959±0.0105	0.1875±0.0297	0.1865±0.0150	0.2262±0.0010	0.2228±0.0018
3	0.1971±0.0117	0.1883±0.0109	0.1854±0.0179		
4	0.2013±0.0013	0.1995±0.0228	0.1986±0.0209		
5	0.2094±0.0098	0.2011±0.0216	0.2008±0.0196		

The results were illustrated in Table 2. The biomasses in the different dye-added time systems had two characteristics as follow.

Firstly, the dye definitely suppressed the growth of the white rot fungi, which was demonstrated by the growth of the mycelium in the different dye-added time systems (Table 1). Moreover, the vaviations of the biomass of the white rot fungi in the different dye-added time systems had further certified the restraint on the growth of the white rot fungi by adding dyes (Table 2). The quantity of the white rot fungi mycelium in the no-adding dye system was significantly more than that in the adding dye system under the sterile or non-sterile conditions.

Secondly, the dye-added time played an influential role on the growth of the white rot fungi. Under the sterile or non-sterile conditions, the later the dye-added time was, the greater the biomasses of the white rot fungi were (Table 2). However, this gap gradually became small as the culture time extended. During the middle culture period, the white rot fungi had gradually completed the logarithmic growth phase and further its biomass remained relatively stable. Then the influence on the white rot fungi by the dyes was restricted, that was why the biomass in the 4^{th} day dye-added system was almost the same as that in the 5^{th} day dye-added system.

CONCLUSIONS

Under the non-sterile condition, the dye-added time had significant influence on the decolorization efficiency. The closer to the peak of enzyme production in the dye-added systems, the higer the 24h decolorization efficiency and its maximum were (i.e. The 24h decolorization efficiencies were 68.21%, 57.47%, 55.60% and their maximums were 94.69%, 93.65% and 88.64% respectively in the 2^{nd}, 3^{rd}, 4^{th} day dye-added systems). the operational time of the dye degradation system of the immobilized white rot fungi was shortened from the original 10 days to 4 or 5 days.

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