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Decolorization of reactive brilliant red K-2BP by white rot fungus under sterile and non-sterile conditions

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Abstract: Almost all the studies both domestic and international using white rot fungus for dye wastewater treatment are performed under sterile conditions. However, it is obviously unpractical that wastewater with dyes is treated under sterile conditions. A feasible study was made for using white rot fungus *Phanerochaete chrysosporium* to degrade reactive brilliant red K-2BP dye under non-sterile conditions. The results showed that there was no decolorizing effect under non-sterile condition if white rot fungus was incubated under non-sterile condition, and the decolorization was always near to 0% during decolorizing test for 3 d; in the meantime, a lot of yeast funguses were found in liquid medium when white rot fungus was incubated under non-sterile conditions; however, if white rot fungus was incubated under sterile condition firstly, its decolorization was above 90% under non-sterile condition, which was similar to that of sterile condition. So we point out that the treating process for wastewater with dyes should be divided into two stages. The first stage is that white rot fungus should be incubated under sterile conditions, and the second stage is that reactive brilliant red K-2BP is decolorized under non-sterile conditions. The method not only save the operation cost which decolorizing reactive brilliant red K-2BP under sterile condition, but also provide the feasibility for using white rot fungus to degrade wastewater with dyes under non-sterile conditions.

Keywords: white rot fungus; *Phanerochaete chrysosporium*; reactive brilliant red K-2BP; decolorization; non-sterile condition

Introduction

Synthetic dyestuffs are extensively used for textile dyeing as well as other industrial applications. According to chemical structure of the chromophoric group, dyestuffs are classified as azo dyes, anthraquinone dyes, triarylmethine dyes, phthalocyanine dyes etc. (Heinfling *et al.*, 1997). Azo dyes are commercially the largest group. And the majority of synthetic dyestuffs are hardly removed from textile wastewater by conventional wastewater treatment such as activated sludge (Shaul *et al.*, 1991). Existing physical and chemical technologies are expensive (e.g., membrane technologies) or often produce large amounts of solid waste (e.g., coagulation and flocculation; Borchert *et al.*, 2001).

Under anaerobic condition, bacteria can decolorize azo dyes. However, the metabolites produced are seldom mineralized and may be carcinogenic because of the formation of aryl amine derivatives (Cerniglia *et al.*, 1982; Delclos *et al.*, 1984; Chung and Cerniglia, 1992).

In recent years, many researches have demonstrated that white rot fungus have the capability to degrade or transform a broad spectrum of xenobiotic organo-pollutants including synthetic dyestuffs (Leidig *et al.*, 1999; Novotny *et al.*, 2001; Choi *et al.*, 2002; Zouari *et al.*, 2002; Shim and Kawamoto, 2002). The basidiomycete *Phanerochaete chrysosporium* is the most extensively studied and discussed dye-decolorizing white rot fungus (Bakshi *et al.*, 1999; Assadi *et al.*, 2001; Selvam *et al.*, 2003). And some significant mineralization (20%—48%) was observed during degradation of azo dyes (Spadaro *et al.*, 1992).

However, almost all the studies both domestic and international using white rot fungus for dye wastewater treatment are performed under sterile conditions. As we can know, it is obviously unpractical that wastewater with dyes is treated under sterile conditions. So the study on the methods of using white rot fungus under non-sterile conditions have an important significance and extensive value. A few of recent studies concerned the bacterial contamination under non-sterile conditions (Leidig *et al.*, 1999; Libra *et al.*, 2003).

The purpose of this work was to study the feasibility of using white rot fungus *Phanerochaete chrysosporium* to degrade reactive brilliant red K-2BP dye under non-sterile conditions. Previous research found that nitrogen limited liquid medium has the potential to suppress bacterial growth under non-sterile conditions (Gao *et al.*, 2004), and the maximum amount of manganese peroxidase and laccase were achieved under nitrogen limitation when the ratio of carbon and nitrogen in the medium was 56:8.7 (Gao *et al.*, 2004). So nitrogen-limited liquid medium (C/N=56/8.7) was chosen in this study. In order to find the methods of using white rot fungus under non-sterile conditions, except for incubating white rot fungus and adding dyes under sterile conditions, two kinds of operation modes were

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applied, one was both incubating and decolorizing under non-sterile conditions, the other was incubating under sterile conditions, and then decolorizing was under non-sterile conditions.

1 Materials and method

1.1 Fungal strains

Phanerochaete chrysosporium BKM-F-1767 was obtained from Guangzhou Institute of Chemistry, Chinese Academy of Sciences. The fungus was maintained on plates of Potato Dextrose Agar at 4℃.

1.2 Medium

1.2.1 Solid medium

Solid medium used contains potato lixiviums 200 g/L, glucose 20 g/L and agar 20 g/L.

1.2.2 Liquor medium

The growth medium was prepared according to Tien and Kirk (1988), and medium compositions are as follows: glucose 10 g/L; ammonium tartrate 0.8 g/L; KH_2PO_4 2.0 g/L; $MgSO_4$ 0.5 g/L; $CaCl_2$ 0.1 g/L; 20 mmol/L acetate buffer (pH 4.4); 1.5 mmol/L veratryl alcohol; trace elements 70.0 ml/L. This trace elements solution contained: $MgSO_4$ 0.21 g/L; $MnSO_4$ 35 mg/L; NaCl 70 mg/L; $FeSO_4 \cdot 7H_2O$ 7 mg/L; $CoCl_2$ 7 mg/L; $ZnSO_4 \cdot 7H_2O$ 7 mg/L; $CuSO_4$ 7 mg/L; $AlK(SO_4)_2 \cdot 12H_2O$ 0.7 mg/L; H_3BO_3 0.7 mg/L; $Na_2MoO_4 \cdot 2H_2O$ 0.7 mg/L; nitrilotriacetate 0.105 g/L. Before inoculation vitamin B1 was injected under sterile condition, and its concentration was 1 mg/L.

1.3 Dyestuff

Reactive brilliant red K-2BP was selected for this study because of its widespread use in textile dyeing plants. It is a monoazo reactive dye, and has a maximum absorbance wavelength at 533 nm. Fig.1 is its chemical structure.

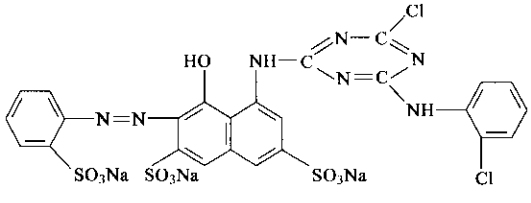


Fig.1 Chemical structure of the reactive brilliant red K-2BP

1.4 Cultivation condition

The white rot fungus was inoculated on PDA medium for 5—7 d at 32℃. Then, spores were harvested in sterile deionized water by gently scraping the surface of the culture with a sterile inoculating loop and filter-sterilized. The spore suspension was inoculated in 250 ml Erlenmeyer flasks with 100 ml of liquid medium. The inoculated spores concentration was adjusted to 10^5 spores/ml and used as an inoculum for further studies. The Erlenmeyer flasks were incubated under agitation (160 r/min) at 37℃.

1.5 Dye decolorization experiments

After incubating white rot fungus for 5 d, 2 ml reactive brilliant red K-2BP(1000 mg/L) was added to 100 ml of liquid medium. Experiments were carried out in triplicate for both sterile and non-sterile conditions, and samples were analysed in triplicate, and the results were expressed as the mean values. Light absorbance was measured daily using a SHIMADZU UVmini1240 Spectrometer for a period of 4 d.

2 Results and discussion

2.1 Decolorization of reactive brilliant red K-2BP by white rot fungus under sterile condition

In order to compare the decolorizing effect of reactive brilliant red K-2BP by white rot fungus under sterile and non-sterile condition, an experiment of decolorization of reactive brilliant red K-2BP by white rot fungus under sterile condition was studied. The incubation of white rot fungus and decolorization of reactive brilliant red K-2BP were all carried out under sterile condition in this experiment. The result is shown in Fig.2.

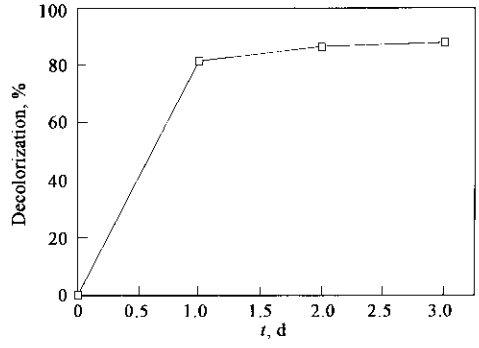


Fig.2 Decolorization of reactive brilliant red K-2BP under sterile condition after incubation of white rot fungus under sterile condition for 5 d

Fig.2 shows that there is a very good decolorization of reactive brilliant red K-2BP under sterile condition after white rot fungus was incubated under sterile condition for 5 d, and the decolorization was more than 82% after reactive brilliant red K-2BP was injected to liquid medium for 1 d. Subsequently, the decolorizing rate increased slowly. From 1 d to 3 d on which injecting reactive brilliant red K-2BP, the decolorization only increased 6%. The result showed that the degradation of reactive brilliant red K-2BP mainly depended on lignin-degrading enzymes secreted by white rot fungus during secondary metabolism. There have been some lignin-degrading enzymes in liquid medium after white rot fungus was incubated for 5 d. So the rate of decolorizing reactive brilliant red K-2BP in the first day is very fast.

2.2 Decolorization of reactive brilliant red K-2BP by white rot fungus under non-sterile condition

Decolorization of reactive brilliant red K-2BP by

white rot fungus under non-sterile condition means the operation of injecting reactive brilliant red K-2BP which is not treated under sterile condition to liquid medium and whole decolorizing process is performed under non-sterile condition. So, in order to achieve the optimal decolorizing effect under non-sterile condition, two operation modes were performed, i.e. white rot fungus was incubated under sterile condition and under non-sterile condition, respectively.

2.2.1 Decolorization after incubation of white rot fungus under sterile condition

After white rot fungus was incubated under sterile condition for 5 d, reactive brilliant red K-2BP was injected to liquid medium under non-sterile condition, and subsequent decolorization also was carried out under non-sterile condition. The result is shown in Fig.3.

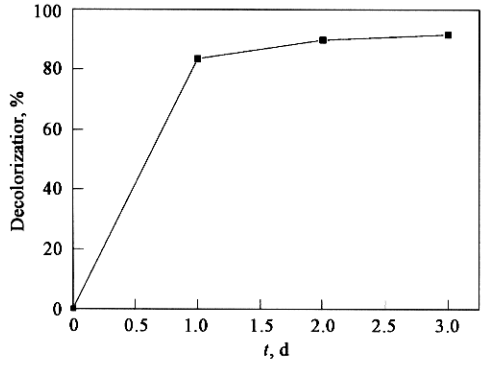


Fig.3 Decolorization of reactive brilliant red K-2BP under non-sterile condition after incubation of white rot fungus under sterile condition for 5 d

Fig.3 shows that good decolorizing effect was achieved under non-sterile condition when white rot fungus was incubated under sterile condition for 5 d. Decolorization was above 90% after injecting reactive brilliant red K-2BP for 3 d. In addition, similar to that of sterile condition, the decolorization was more than 83% after injecting reactive brilliant red K-2BP for one day, and then the decolorizing rate began to decrease. So the result indicates that if white rot fungus is incubated under sterile condition its decolorizing effect under non-sterile condition will be not influenced. The reason is that nutrient in liquid medium is very small when white rot fungus is incubated for 5 d, and the other microbial strains could not rapidly propagate owing to lacking nutrient. Thus, the method of incubation under sterile condition and decolorization under non-sterile condition might suppress the other microbial strains colonizing in reaction system.

2.2.2 Decolorization after incubation of white rot fungus under non-sterile condition

The whole experiment was done under non-sterile condition from inoculating spores to injecting reactive brilliant red K-2BP. The aim is to investigate the influence of natural situation (non-sterile condition) on the growth of white rot fungus and decolorization of reactive brilliant red K-2BP by it. The result is shown in Fig.4.

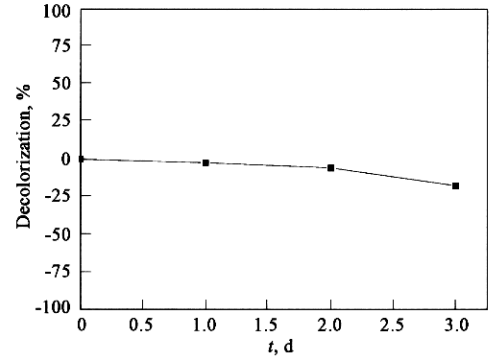


Fig.4 Decolorization of reactive brilliant red K-2BP under non-sterile condition after incubation of white rot fungus under non-sterile condition for 5 d

Fig.4 shows that there was no decolorizing effect under non-sterile condition if white rot fungus was incubated under non-sterile condition, and the decolorization is always near to 0% during decolorizing test for 3 d, and decolorization gradually decreased with time. Decolorization falling is because of the rise of the absorbance of liquid medium with reactive brilliant red K-2BP for water vaporizing during 3 d at 37℃. So if white rot fungus is incubated under non-sterile condition, it will not possess decolorizing ability. The reason is the incubation system might be contaminated by the other bacteria or fungus, and the propagation rate of these bacteria or fungus is faster than white rot fungus. Fig.5 shows incubation system under sterile and non-sterile conditions. The left Erlenmeyer flask expresses white rot fungus is incubated under sterile condition for 2 d, and the right Erlenmeyer flask expresses that it is incubated under non-sterile condition. The left liquid medium is clear, and the right liquid medium is turbid. This phenomenon also testifies that the incubation system under non-sterile condition is easy to be attacked by other bacteria or fungus.

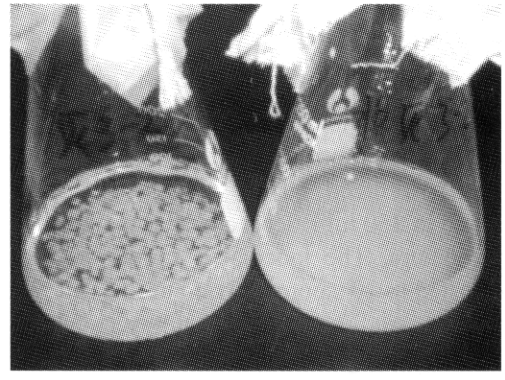
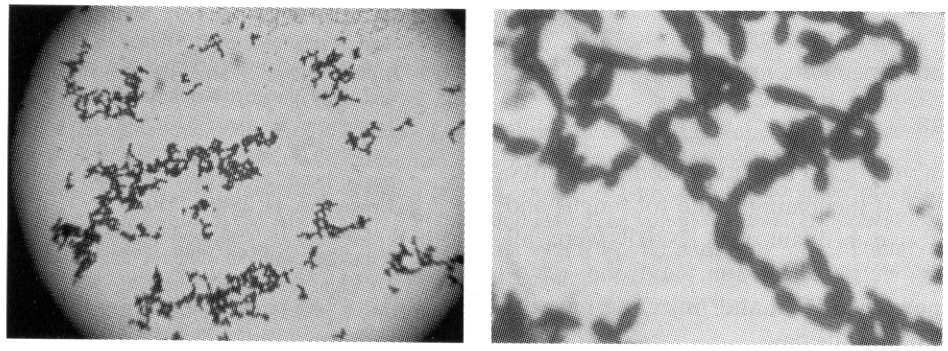


Fig.5 Comparison of incubation system under sterile (left) and non-sterile(right) conditions

In order to investigate the sorts of microbial community colonizing in nitrogen limited liquid medium, microbial shapes were observed by using microscope. The result is shown in Fig.6.

Fig.6 shows that the shapes of fungus contaminating liquid medium were in ellipse, and they clustered together. Subsequently, spread plate test was performed too, and its result was the same as the above. The fungus contaminating liquid medium is yeast fungus according to its microbial shapes.



a. Normal (40×10) b. Magnifying

Fig.6 Shapes of fungus in liquid medium under non-sterile condition

In general, pH value of liquid medium will change if the other microbial communities colonize in liquid medium. Because different microbial communities have different optimal pH value ranges for growing and propagating. Fig.6 shows that yeast fungus has become advantage fungus in liquid medium. So it could adjust pH value in medium to its optimal range for survival by itself physiological characteristic. Based on this, variation of pH value in medium during incubation under sterile and non-sterile conditions was investigated. Results are shown in Fig.7.

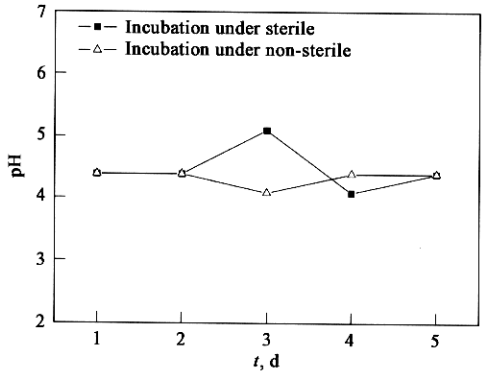


Fig.7 Variation of pH in medium during incubation

Fig.7 shows that there was a little variation of pH value in medium during incubation under sterile and non-sterile condition, and the pH were in the range of 4.1—5.1 during incubation from 1 d to 5 d. Especially under non-sterile condition, pH value was still maintained at 4.4. Yeast fungus could survive at different pH value (pH=2.2—8.0) with its different strains. So it is very easy to attack liquid medium with low pH value, and could grow and propagate rapidly under low pH value. In addition, only yeast fungus was found in nitrogen limited liquid medium, and there were not other bacteria in it. The result further testifies nitrogen limited liquid medium is prone to suppress bacteria.

2.3 Comparison of decolorizations of reactive brilliant red K-2BP by white rot fungus under sterile and non-sterile conditions

Although white rot fungus incubated under non-sterile condition have not decolorizing ability. If firstly incubate white rot fungus under sterile condition, and then decolorize reactive brilliant red K-2BP under non-sterile conditions. Its decolorization ability under non-sterile condition will be similar to that of sterile condition. The results are shown in Fig.8.

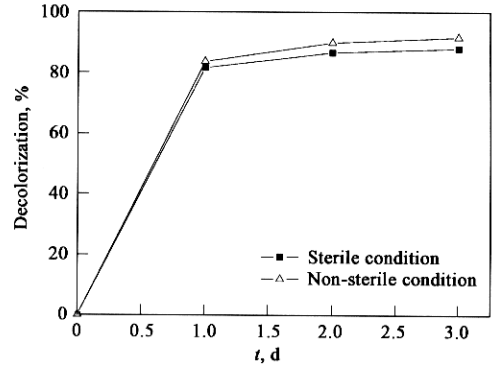


Fig.8 Comparison of decolorizations of reactive brilliant red K-2BP by white rot fungus under sterile

Fig.8 shows that the decolorizing rate under non-sterile condition almost equals to that of sterile condition, and both decolorizing efficiency are also similar. The decolorizations both under sterile and under non-sterile condition were 82% and 83% when reactive brilliant red K-2BP was decolorized for one day, respectively.

So, in order to obtain a better treatment effect, the treatment process for wastewater with dyes should be divided into two stages. Firstly, white rot fungus

should be incubated under sterile conditions. Then, after mycelium formed, white rot fungus begin to degrade wastewater with dyes under non-sterile condition. The method not only save the operation cost which degrading wastewater with dyes under sterile condition, but also provide the feasibility for using white rot fungus to degrade wastewater with dyes under non-sterile conditions.

3 Conclusions

There was a very good decolorization of reactive brilliant red K-2BP if incubating white rot fungus and decolorizing reactive brilliant red K-2BP were all performed under sterile condition, and the decolorization was more than 82% after injecting reactive brilliant red K-2BP for one day.

There was no decolorizing effect under non-sterile condition if white rot fungus was incubated under non-sterile condition, and the decolorization is always near to 0% during decolorizing test for 3 d. In addition, a lot of yeast funguses were found in liquid medium when white rot fungus was incubated under non-sterile conditions. So it is yeast fungus that influences and suppresses the growth of white rot fungus.

The better decolorizing effect was achieved under non-sterile condition if white rot fungus was incubated under sterile condition, and its decolorization was above 90% after injecting reactive brilliant red K-2BP for 3 d. Its decolorizing ability under non-sterile condition was similar to that of sterile condition. So if white rot fungus is incubated under sterile condition, its decolorizing effect under non-sterile condition will be not influenced.

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