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Enzyme degradation mechanism of white rot fungi and its research progress on Refractory Wastewater

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Abstract. The lignin-degrading enzyme system of white rot fungi is highly efficient and non-specific, and can degrade a variety of pollutants, including dyes, phenolic compounds and pesticides.The article presents an overview of the mechanism of enzymatic degradation of white rot fungi and its research status in several refractory wastewater were described.

1 Introduction

The development of printing and dyeing, tanning, synthetic resin and other chemical industries has produced a class of refractory wastewater, which has the characteristics of complex composition, refractory and high biotoxicity.Pollutants discharged into the water environment will cause great harm to the ecological environment and human health.Traditional treatment methods are far to reach ideal treatment effect, so it is urgent to explore new treatment technologies for this kind of wastewater.

White rot fungi(WRF) is a kind of filamentous fungus which grows on wood. Its hyphae can penetrate into wood, release lignin degrading enzyme to degrade lignin into white sponge substance, and completely oxidize lignin to CO_2 and H_2O[1].The oxidability of ligninolytic enzymes has attracted much attention in wastewater treatment.Its ability to oxidize organics in liquid media has been verified and recognized, and has been widely used in the treatment of refractory organic pollutants[2].The catalytic mechanism of ligninase degradation system and the application of WRF in several refractory wastewater were reviewed.

2 Enzyme degradation mechanism

The degradation of pollutants by white rot fungi is mainly completed by the lignin degrading enzyme system,including lignin peroxidase (LiP), manganese peroxidase (MnP) and Laccase (Lac).They can attach to the cell wall or secreted outside to form a synergistic degradation system.

2.1 Catalytic Mechanism of LiP&MnP

LiP has a high redox potential, and can oxidize many substances directly or indirectly with the aid of free radical catalysis. The degradation mechanism of the direct oxidation of lignin peroxidase is shown in the figure[3]: LiP(Fe^{3+}) is excited by H_2O_2 and loses electrons, generating compound I ($[Fe^{4+}]^{+}\bullet$); compound I gains electrons and transforms into compound II(Fe^{4+}), and a free radical (R•) is generated at the same time; Compound II continues to lose electrons to form Fe^{3+} and a free radical (R•) is generated. The free radical is unstable and easy to undergo a reduction reaction. Indirect oxidation of lignin peroxidase can be assisted by substances that are easily oxidized to generate free radicals.

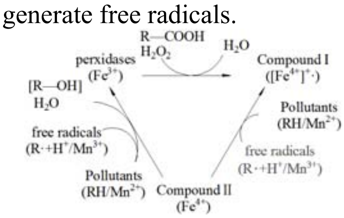

Fig1. Generic scheme of the catalytic cycle of perxidases

2.2 Catalytic mechanism of Lac

Laccase is an oxidase protease containing copper ions. Its catalytic mechanism mainly depends on the synergistic action of four copper ions in laccase molecules and the production of free radicals.The oxidative degradation mechanism of laccase is shown in the figure[4]:The white-rot fungal laccase type I Cu^{+} reacts with O_2 to form the intermediate oxide type II Cu^{+}. It reacts with H_2O to form type Ⅲ Cu^{2+}, which can continue to produce inactivated laccase type III Cu^{2+}.Inactivated laccase type III Cu^{2+} loses 4 electron reduce to laccase type I Cu^{+}, which converts the substrate molecule into free radicals and simultaneously generates water. Substrate free radicals are generally unstable and prone to redox.

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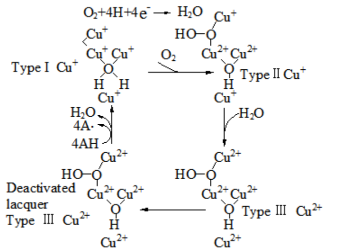

Fig2. The catalytic cycle of laccases

3 Research on dyeing wastewater

Dyeing wastewater has high concentration, high COD, high biotoxicity, poor biodegradability and other characteristics, is an important kind of refractory wastewater.Nowadays, dyes are developing towards good light stability, hard oxidation and high temperature resistance, and conventional treatment methods cannot meet the requirements of degradation[5].The degradation of pollutants by the extracellular enzymes of WRF is nonspecific and can transform substrates without distinction.Its degradation characteristics can adapt to the water quality characteristics of printing and dyeing wastewater.

Si Jing et al.[6]studied the decolorization effect of the azo dye Congo red by Trametes pubescens under non-nutrient conditions.The removal was up to 80.52%(pH2.0,30℃,dye concentration 80mg/L).The phytotoxicity test of the treated dye solution showed that the toxicity of degradation products and dyes was greatly reduced.This study shows that white rot fungi can degrade and detoxify dyes, and have great application potential in dye wastewater treatment.Bugra et al.[7]studied the decolorization effect of Trametes versicolor on RB220 and methyl red Dyes in soil medium.The degradation of methyl red and RB220 were 91%-50%,80% -49%. The removal decreases with increasing concentration, and the decolorization rate is high at low concentration, which may be caused by dye toxicity.Wu Yi et al.[8] used Porodaedalea laricis as the research object to deal with a variety of dyes, including reactive bright blue X-BR, rema bright blue R, Congo red,etc.The decolorization rate reached 95.64%, 97.21%, 91.63% after 7 days. Experimental results show that this strain has a good degradation effect on synthetic dyes.

According to the above, it can be recognized that WRF have a degrading and detoxifying effect on dyes.However,the poor biodegradability of dye wastewater is difficult to ensure the rapid propagation of fungi and the secretion of lignin extracellular enzymes, resulting in low treatment efficiency.This problem is the first to be solved before white rot fungi are applied in actual wastewater treatment.

4 Research on heavy metal wastewater

Unlike organic pollutants, heavy metal ions are non-biodegradable and easy to accumulate through the biological chain.The accumulation of heavy metals in organisms can cause many kinds of damages through thiol binding, protein denaturation, essential metal displacement and oxidative stress and other side effects, which is extremely harmful to human health and ecological environment safety.The fungal cell wall has a high content of polysaccharides and proteins, and contains a large number of functional groups that can be combined with heavy metal ions. In recent years, the removal of heavy metals by biosorption has attracted the attention of many scholars.

Muhammad et al.[9]studied the potential of immobilized Pleutrotus sajor-caju, Agaricus bitorquis, and Ganoderma lucidum to repair wastewater containing Cu(II), Pb(II), Cr(III) and Cr(VI) under the influence of strain dosage, initial concentration, time and other factors.Adsorption experiments were carried out at a pH of 4.5, 30℃ and 120r/min. The results showed that the maximum potential of immobilized fungi to repair Cu(II), Pb(II), Cr(VI) and Cr(III) was 205.1, 208.5, 226.6 and 207.3mg/g, respectively.Chen et al.[10] found that Ganoderma Sinensis could enrich Cd(II) in aqueous solution, and the removal rate of Cd(II) by this strain ranged from 21.9% to 6.78% when the concentration of Cd(II) increased (10~30mg/L).Anwei Chen et al.[11] removed Cd(II) from aqueous solution with Phanerochaete chrysosporium.At a pH of 6.5, the removal rate reached the maximum of 83.93%.The highest removal rate of Cd(II) at different initial concentrations (2, 5, 10, 15, 20, 30 mg/L) is 63.62%(5mg/L).

In conclusion, WRF can adsorb and enrich heavy metals to a certain extent. However, the adsorption capacity of the same strain to different heavy metal ions and different strains to the same heavy metal ion is significantly different, and high concentrations of heavy metal ions can inhibit the biological activity of WRF.

5 Research on wastewater containing phenol

Phenolic substances are highly toxic, and even at very low concentrations, they will pose a huge threat to environmental safety and human health.Due to the cell wall rich in chitin and the production of non-specific extracellular enzymes, WRF are more resistant to toxic compounds and have the potential to treat high-concentration phenolic wastewater.

Fan et al.[12]used different carriers to immobilize Trametes to treat phenol-containing wastewater under different factors(pH, concentration and temperature), and analyzed its treatment effect and optimal treatment conditions.The treatment effect is best when wood chips are used as the carrier.Under the conditions of pH 6.5, temperature 30℃, and initial concentration of phenolic compounds 250mg/L, the removal rate of phenolic compounds by the fungus was up to 89.97%.Kietkwanboot et al.[13] investigated the ability of Trametes Hirsuta AK04 to utilize phenolic compounds as a single and mixed substrate in mineral media and palm oil effluent.The initial concentrations of phenolic compounds tested were 100, 200, 500, and 1,000 mg/L for the single substrate systems. The removal ranged from 86-95% for phenol, 78-98% for benzoic acid derivatives, 78-92% for cinnamic acid derivatives, and 87-90% for phenylacetic acid derivatives after 7 days.The mixed substrate medium was prepared to have equal concentration of each phenolic compound at 100, 200, 400, and 600 mg/L to be used in the mixed substrate experiment.The results showed that the removal rate of phenolic substances reached more than 87% after 7 days of treatment(100 and 200mg/L), and the biodegradation efficiency of phenolic substances exceeded 200mg/L decreased.When the concentration was 400 and 600mg/L, the removal rates were 72-83% and 51-69%, respectively.Bernats et al.[14] studied the degradation of phenol by a single white-rot fungus under different factors (biological inoculation amount, pH and temperature). The degradation rate of total phenol in batch flasks by four fungal monocultures of Trametes versicolor, Phanerochaete chrysosporium, Gloeophyllum trabeum and Irpex lacteus in synthetic medium was compared.The batch study of four fungi showed that the degradation effect of Ganoderma lucidum was the best. The optimum conditions were pH 5~6, temperature 25℃, biomass 10%(V/V), and the total phenol degradation rate reached 93% after 7 days.

In summary, WRF can effectively degrade phenolic compounds, immobilization can improve the degradation efficiency.Relatively speaking, WRF have higher tolerance to single phenolic substances than mixed phenolic substances.

6 Conclusion and prospect

The research on white rot fungi is still at the basic experimental level.The application research of white rot fungi needs the following research: a) To conduct in-depth research on the secretion conditions and degradation mechanism of lignin-degrading enzyme systems, so as to provide theoretical guidance for actual wastewater treatment. b) Free microorganisms are not resistant to impact load, and changes in water quality have a greater impact on microbial activity and affect the solid-liquid separation effect. Immobilization can solve the above problems.It is necessary to develop new immobilized carrier with high economy, high mechanical strength and good mass transfer performance. c)Research on inactivated white rot fungus body adsorption.The deactivated white rot fungus is not restricted by culture environment and water quality and can be used as adsorptive material in heavy metal wastewater treatment.

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