Water Qual. Res. J. Can. 2010 · Volume 45, No. 1, 91–98

Research on the Application of Laccase to the Treatment of Oily Wastewater

Zhi Lin Li,[1] Wei Liu,[1]* Xin Fang Chen,[1] He Sheng Li,[1,2] Yun Miao Tian[1]

[1] *College of Chemical Engineering, Beijing University of Chemical Technology, Box 137,15 Beisanhuan East Road, Chaoyang District, Beijing 100029, China*
[2] *Research Institute of Yanshan Petrochemical Co., Ltd., SINOPEC, Beijing 102500, China*

The feasibility of using laccase to treat oily wastewater was examined. When only laccase was added to the synthetic oily wastewater, the suitable technological conditions were laccase at 3 U/mL, pH at 6.0, a temperature of 30°C, and a reaction time of 6 h for the initial oil concentration of 120 mg/L. Under those conditions, the rate of oil removal was as high as 69%. The effects of Mg^{2+}, Mn^{2+}, Cu^{2+}, and Fe^{2+} ions in wastewater on the rate of oil removal using laccase were investigated. The results showed that $Cu^{2+}$and Fe^{2+} ions obviously inhibited the catalytic performance of laccase under the studied concentration. On the other hand, Mg^{2+} and Mn^{2+} ions only had slight effects on the rate of oil removal for the range of concentrations studied. A 95% oil removal rate could be obtained when actual wastewaters were treated using laccase with the additive chitosan under the suitable technological conditions.

Key words: **laccase, oily wastewater, polyethylene glycol, chitosan, enzyme catalytic treatment**

Introduction

Oil that is leaked during the processes of petroleum refining, storage, transportation, and production of petrochemical products as well as the accidental release of oil products may result in contamination of the water environment. Some ingredients of petroleum are organic mixtures containing low boiling-point aromatic hydrocarbon compounds that are toxic to aquatic plants. For example, such mixtures can decrease the content of oxygen in oily wastewater, thereby inhibiting photosynthesis by water plants (Galvão et el. 2006). Some petroleum compounds contain polycyclic aromatic hydrocarbons which are carcinogenic and can severely threaten the health of humans and aquatic animals. Therefore, oily wastewater should not be discharged directly without treatment.

Oil in water can exist as free or suspended oil, dispersed oil, emulsified oil, and dissolved oil. Suspended or dispersed oil can be readily separated from wastewater by simple physical processes. However, emulsified or dissolved oil is more difficult to remove from wastewater. At present, there are several methods for removing emulsified oil and dissolved oil from wastewater, including ozone oxidation, ultraviolet- (UV-) catalyzed oxidation, Fenton reagent, biological degradation, ultrasonic wave oxidation, etc. (Andreozzi et al. 2000; Chang et al. 2001; Badawy and Ali 2006; Galvão et al. 2006; Tezcan et al. 2006). These have some limitations, such as restricted application conditions, high operation costs, corrosion, and recontamination (Braun and Oliveros 1997; Zouboulis and Avranas 2000). Furthermore, these methods are not always effective in removing dilute emulsified oil or dissolved oil. Enzymatic degradation has the advantages of convenient operation, mild reaction conditions, high efficiency, a wide operating range, and the elimination of recontamination, so it may be a good solution to the above problems in the treatment of oily wastewater. Furthermore, because of the high efficacy of immobilized enzyme and the additive enhancement of enzymatic activity, the enzymatic degradation may be a promising economical procedure for industrial applications compared with the conventional physicochemical methods in treating emulsified oily and dissolved oily wastewaters (Durán et al. 2002; Kurian et al. 2006).

Laccase is one of the potential biocatalysts (Srebotnik and Hammel 2000; Mayer and Staples 2002a). It has been reported that laccase can catalyze more than 250 substrates; therefore, it has a broad substrate spectrum and may be especially suitable for the treatment of oily wastewater of complex composition (Setti et al. 1999; Claus 2004). In other words, laccase is different from enzymes that can only catalyze specific substrates, so it may be able to simultaneously catalyze various kinds of compounds in mixtures of oily wastewater. In the past the applications of laccase were mainly concentrated on the pulp and paper manufacturing industry, especially on aspects of lignin degradation and pulp biobleaching (Ishihara 1980; Li et al. 1999; Wong and Yu 1999; Mayer and Staples 2002b; Barreca et al. 2003). In recent years the laccase-catalyzed polymerization and precipitation processes were explored for the treatment of various aromatic compounds, such as phenols and anilines (Durán et al. 2002), but they were usually limited to the sole substrate (Ganjidoust et al. 1996; Tsioulpas et al.

*Corresponding author; liuw@mail.buct.edu.cn

2002). As far as we know, there are no reports regarding the application of laccase to the treatment of oily wastewater.

Laccases are multicopper-containing enzymes that can catalyze the oxidative conversion of a variety of chemicals. Laccase can couple four one-electron oxidations of the substrates. The various copper centres of laccases drive electrons (Claus 2004). The electrons can be further transferred and attack the substrates (emulsified oil and dissolved oil in the wastewater). The attack may generate radicals that react with each other to form insoluble products, such as dimers, oligomers, and polymers. The insoluble products can be removed from wastewater via precipitation during further treatment processes.

In this paper, laccase was applied to catalyze the oxidation of the oil in wastewater, and the suitable conditions of the laccase-catalyzed oxidation of the oil in wastewater were researched in terms of reaction time, temperature, pH, and laccase concentration. The effects of metal ions on the rate of oil removal were also evaluated. The effects of additives on the laccase-catalyzed oxidation of oil were explored. The rates of oil removal were compared between synthetic oily wastewater and actual oily wastewater.

Materials and Methods

Materials

Laccase (EC 1.10.3.2) was produced by Fluka Co. (Buchs, Switzerland). The laccase activity was quoted as 7.44 U/mg of dry solid. Laccase stock solutions were prepared by dissolving solid laccase in 0.1 mol/L of acetic buffer (pH 4.0), and were stored at a temperature of 4°C. Twice-distilled diesel oil was provided by Yanshan Petrochemical Co., Ltd. (Beijing, China). The synthetic oily wastewater was prepared by emulsifying diesel oil in a 0.2 mol/L phosphate buffer. Characteristics of the synthetic oily wastewater are listed in Table 1.

Petroleum ether (boiling point range: 60 to 90°C), acquired from Changhai Chemical Co. (Beijing, China), had a transmittance of light greater than 80% after being purified. Chitosan (viscosity of 420 centipoises) was produced by Sigma Chemical Co. (St. Louis, Mo.). A chitosan solution of 10 g/L was prepared by dissolving the polymers in a 1% by weight acetic acid solution, and any undissolvable particles were removed from the solution by vacuum filtration. The solution was stored at a temperature of 4°C after preparation. Polyaluminum chloride (PAC) of technical grade was obtained from Fisher Chemical (Fair Lawn, N.J.). The polyethylene glycol (PEG) (average molecular weight 8,000), 3-ethylthiazoline-6-sulfonate (ABTS), and other materials were all of analytical grade.

Analytical Methods

Laccase activity was measured with a colourimetric assay (Beckman Coulter DU530, Fullerton, Calif., U.S.A.) containing 1 mM ABTS substrate, 100 mM citrate buffer (pH 4.0), and a suitable amount of enzyme (0.05 to 0.25 U/mL) (Wolfenden and Wilson 1982; Eggert et al. 1996; Gianfreda et al. 1998; Wong and Yu 1999). Prior to significant substrate depletion, the enzyme activity was proportional to the rate of formation of a coloured product that absorbed light at the wavelength of 600 nm. The unit activity (U) of laccase in this assay mixture was expressed as 1 μmol of the coloured product formed from the ABTS oxidation per minute at a temperature of 25°C.

There are many analytical methods used to determine oil concentrations, such as the extractive-gravimetric method and instrumental methods (e.g., refractometry index determination and UV spectrophotometry). Among them, UV spectrophotometry is the most precise (Zhao et al. 2006; Rajakovic et al. 2007); this is because oil has characteristic absorbance in the ultraviolet range (Verner et al. 2000), and ultraviolet spectrophotometry provides superior precision and reliability. Therefore, oil concentrations in the wastewater were determined using UV spectrophotometry according to a Chinese national standard (Water and Wastewater Monitoring Analysis Committee of Chinese National Environment Bureau 1989). The oil concentration was measured with a UV spectrophotometer (WFZ800-D_3A, Beijing Optical Apparatus Factory, Beijing, China) at the wavelength of 225 nm which proved to have the maximum absorption for the oil used. The measurement was taken after the oil in the reaction mixtures or wastewater was extracted with petroleum ether. The rate of oil removal was expressed as the percentage of oil removed in relation to the initial oil amount.

Experimental Procedures

The overall experimental procedures included: preparation of the synthetic wastewater, reaction initiation, reaction

TABLE 1. Characteristics of synthetic oily wastewater

Main ingredients	*Oil concentration (mg/L)*	*COD [a] (mg/L)*	*Particle diameter of oil (μm)*	*SS [b] (mg/L)*
Alkane+cyclane+aromatic compounds	120	2,520–3,240	0.4–80	0.001

[a] COD = chemical oxygen demand.
[b] SS = suspended solids.

interruption, flocculation of reaction mixture, and the analysis of oil concentration.

Batch reactions were carried out in glass vials of 250-mL capacity at pH 6.0 (except for the tests on the effects of pH), an oil concentration in the synthetic wastewater of 120 mg/L, different concentrations of chitosan or PEG (which was added before the addition of laccase), and a suitable amount of laccase. The reaction mixtures of the synthetic wastewater, chitosan, and laccase were incubated in a shake bed (HYG-III shake bed, Shanghai Automatic Control Apparatus Factory, Shanghai, China) at 30°C (or at 27 to 75°C to determine the effects of temperature on the rate of oil removal). The pH of the reaction mixtures was adjusted to the desired level (from 2.7 to 9.0) using common buffers, i.e., a 25 mM acetate buffer at a pH of 2.7 to 4.9, and a 25 mM phosphate buffer at a pH of 5 to 9. For experiments involving reactions in the presence of metal ions, stock solutions of Mg^{2+}, Mn^{2+}, Cu^{2+}, and Fe^{2+} were prepared using the corresponding sulfate salts. The metal ion concentrations in the reaction mixtures were from 0 to 70 mg/L. For experiments involving PEG, chitosan, $Al_2(SO_4)_3$, and PAC, the solutions were prepared using deionized water. The concentrations of both PEG and chitosan in the reaction mixtures ranged from 20 to 160 mg/L. The concentrations of both $Al_2(SO_4)_3$ and PAC in the reaction mixtures were 80 mg/L.

The reaction was initiated by the addition of laccase into the reaction mixtures. The reaction mixture was capped and continuously stirred with a magnetic stirrer at a speed of approximately 140 rpm during the reaction. Our results demonstrated that the oxygen in the reaction mixture and the headspace in the reaction vial did not affect the transformation of the oil (data not shown here).

By adding sulfuric acid into the reaction mixture to adjust the pH of the reaction mixture to 2 or lower, the enzymatic reaction was stopped to test the reaction effectiveness at the required reaction time.

Prior to the analysis of oil concentration, the floc in the reaction mixtures was flocculated with the flocculating agents $Al_2(SO_4)_3$ (80 mg/L) and PAC (80 mg/L) at a pH of 8.0. Subsequently, the mixture was centrifuged for 30 min at approximately 3,000 rpm. Immediately after the supernatant liquid was poured out, it was analyzed for residual oil concentration with the colourimetric method described above.

Results and Discussion

Effect of pH

Generally, all enzymes have a suitable working pH value. It was reported that the suitable working pH value of free laccase was from 3.0 to 9.0 (Srebotnik and Hammel 2000). In order to determine the effect of pH on the removal of oil in the wastewater, three sets of reactions (with 3.0 U/mL of laccase, 4.0 U/mL of laccase, and 3.0 U/mL of laccase with 80mg/L of chitosan) were conducted in which the pH of water sample was varied from 3.0 to 9.0. The results are shown in Fig.1 and indicate that the rate of oil removal was a function of pH. The pH ranges suitable for achieving significant oil removal (reduction of the remaining oil concentrations to 50mg/L, equivalent to an oil removal rate of 58%) were from 4.5 to 7.2 for laccase at 3 U/mL, from 3.5 to 8.0 for laccase at 4 U/mL, and at all pH levels tested with laccase at 3 U/mL plus chitosan at 80mg/L. All three sets of reactions reached the highest rate of oil removal at pH 6, which was slightly higher than the reported isoelectric point of laccase (Yaropalov et al. 1994). Thus, all subsequent experiments were conducted at a pH of 6.0.

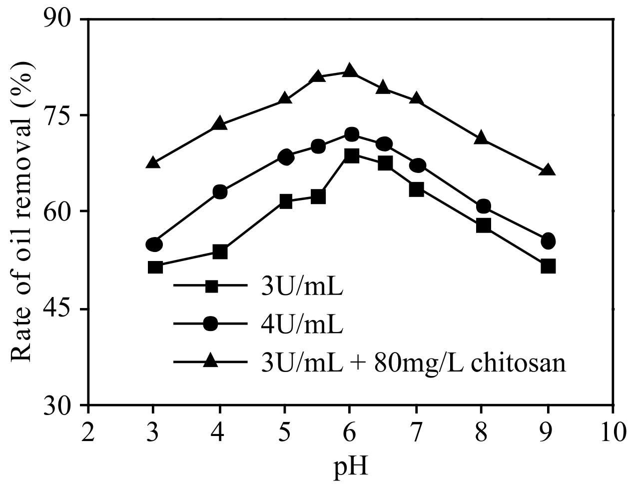

Fig. 1. Effect of pH on the rate of oil removal. The reactions were conducted for 6 h with an initial oil concentration of 120mg/L and temperature of 30°C.

It has been reported that the mechanism of laccase in catalyzing organic pollutants, such as aromatic compounds, is the conversion of the substrates into free radical products and the coupling reactions of the products leading to polymerization (Durán et al. 2002; Claus 2004). The mechanism of laccase catalyzing oil in wastewater may be similar to that process. Thus, the effects of the pH of the water sample on the rate of oil removal may be attributed to its influence on the state of the functional groups of laccase, and the consequent influence on its ability to attack substrates. Therefore, the pH value changed the action efficiency of laccase on substrates.

Effect of Laccase Concentration

The commercially available laccases are very expensive. Therefore, it is important to determine the minimum suitable amount of laccase for achieving a relatively high rate of oil removal from the wastewater.

In order to determine the suitable concentration of laccase, batch experiments were conducted in which the concentrations of laccase added to the water samples was varied from 0 to 4 U/mL. The results are shown in Fig. 2. As expected, the rate of oil removal increased

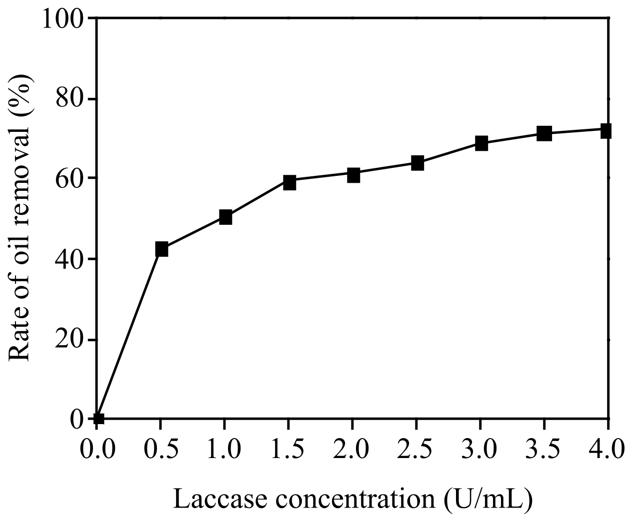

Fig. 2. Effect of laccase concentration on the rate of oil removal. The reactions were conducted for 6 h with an initial oil concentration of 120mg/L, pH of 6.0, and temperature of 30°C.

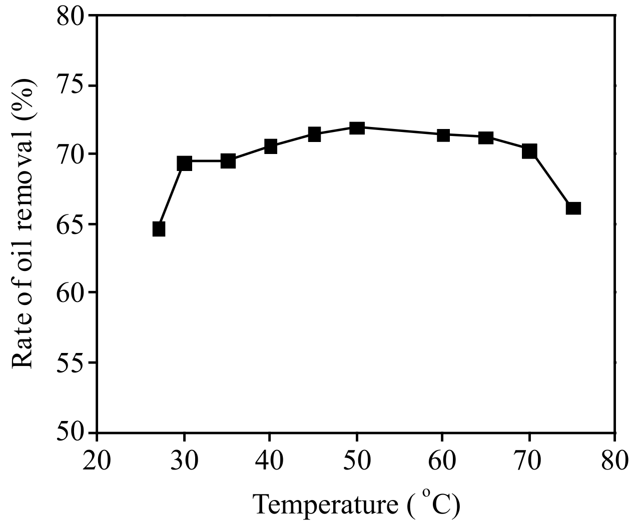

Fig. 3. Effect of temperature on the rate of oil removal. The reactions were conducted for 6 h with an initial oil concentration of 120mg/L, laccase concentration of 3 U/mL, and pH of 6.0.

with the increasing concentration of laccase. To achieve a reduction from 120 to 50 mg/L of oil remaining, the minimum laccase concentration required was 1.5 U/mL. To reduce the oil concentration remaining to 30 mg/L (equivalent to a rate of oil removal of 75%), the minimum laccase concentration required was 3.0 U/mL. To make a further reduction of the remaining oil concentration to 28 mg/L (equivalent to a rate of oil removal of 76%), the laccase concentration had to be increased to 4.0 U/mL or more.

The effects of laccase concentration on the rate of oil removal can be attributed to the fact that larger concentrations of laccase can cause the formation of greater amounts of the free radical products and the subsequent coupling-reaction polymers. When the laccase concentration was over 3.0 U/mL, its increase had little effect on the rate of oil removal. The reason could be that the products of polymers can inhibit the generation of free radical products and the laccase-catalyzing reaction. Therefore, the incremental rate of oil removal was inhibited when the concentration of laccase exceeded a certain amount. Therefore, the suitable concentration of laccase in this study was considered to be 3 U/mL.

Effect of Reaction Temperature

The effect of reaction temperature on the rate of oil removal from wastewater for a 6 h reaction time is shown in Fig.3. The results show that the rate of oil removal increased with an increase of temperature from 27 to 50°C. This could be the result of the increased temperature accelerating the formation of the free radical products and the coupling reaction leading to polymerization compared with when the temperature was relatively low. However, a descending tendency of the rate of oil removal was observed above 50°C. This could be the result of the higher temperature leading to inactivation of the laccase, and\or the large quantity of polymers produced blocking laccase from attacking the substrate. Laccase inactivation at high temperatures has already been verified (Kim and Nicell 2006a; Kim and Nicell 2006b).

Although the increase of temperature may cause competition between an increase and decrease of the laccase activity, it can be observed that the rate of oil removal by the laccase catalytic oxidation only changed a small extent (between 69 to 71%) in the temperature range of 30 to 70°C. In other words, the rate of oil removal was still high at this temperature range. Therefore, the process of oil removal from wastewater with laccase showed a broad operational temperature range. Generally, thermal inactivation is irreversible and therefore dangerous to laccase treatment of wastewater. On the other hand, low temperature is both economical and convenient for operation; therefore, the temperature should be as low as possible when the rate of oil removal can be kept high. According to these analyses, the suitable temperature in this case was 30°C.

Effect of Reaction Time

In order to evaluate the effect of reaction time on the oil removal rate, experiments were conducted at various concentrations of laccase. The results are shown in Fig.4. It is observed that the majority of transformation occurred in the initial stage. Within the first 6 h of incubation, oil removal rates of approximately 69, 61, and 45% were obtained at laccase concentrations of 3, 2, and 1 U/mL, respectively. After the initial 6 h, the rate of oil removal was almost constant at all laccase concentration levels tested. Therefore, a reaction time of 6 h should be enough for all concentrations of laccase.

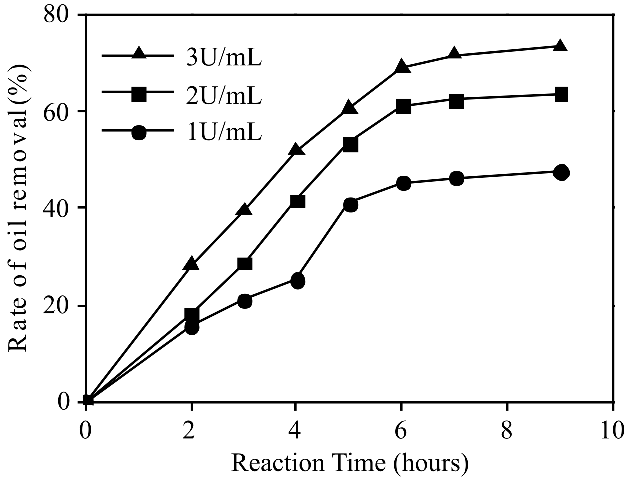

Fig. 4. Effect of reaction time on the rate of oil removal. The reactions were conducted with the initial oil concentration of 120mg/L, laccase concentrations of 1, 2, and 3 U/mL, pH of 6.0, and temperature of 30°C.

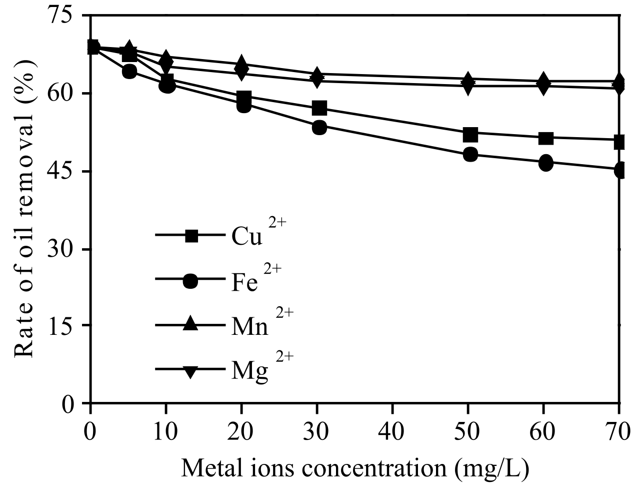

Fig. 5. Effect of metal ions on the rate of oil removal. The reactions were conducted for 6h with the initial oil concentration of 120mg/L, laccase concentration of 3U/mL, pH 6.0, and temperature of 30°C.

According to the deduction above on the mechanism of laccase catalyzing oil removal, the amounts of free radical products converted from the substrates and of polymers produced by the coupling reactions of the products should increase with the passing of time. Therefore, the rate of oil removal increased with the passing of time. However, after a certain time period, the polymerization products would inhibit the generation of free radical products (Durán et al. 2002) such that the subsequent reactions would also be inhibited. Therefore, the rate of oil removal gradually reached a nearly constant value.

Effect of Metal Ions

Generally, there are some types of metal ions in freshwater or circulating water; therefore, it is necessary to investigate their potential effect on the treatment efficiency of synthetic oily wastewater with laccase. Experiments on the effect of the metal ions Mg^{2+}, Mn^{2+}, Cu^{2+}, and Fe^{2+} on the rate of oil removal were designed with an oil concentration of 120 mg/L, a pH of 6.0, a temperature of 30°C, and a laccase concentration of 3.0 U/mL for 6 h. A series of tests were carried out at metal ion concentrations from 0 to 70 mg/L. The results are shown in Fig.5.

The results show that all the metal ions had similar effects on the rate of oil removal. Mg^{2+} and Mn^{2+} ions did not strongly influence the rate of oil removal. At low ion concentrations, it can be observed that the rate of oil removal decreased a little with the increase of the Mg^{2+} or Mn^{2+} ion concentration, and the rate of oil removal almost kept constant when the ion concentration was higher than 30 mg/L. The Fe^{2+} or Cu^{2+} ion strongly decreased the rate of oil removal at all ion concentrations. Even though the concentration of Fe^{2+} was as low as 5mg/L, it caused a 7% decrease of the rate of oil removal. Therefore, Fe^{2+} or Cu^{2+} may restrict the actual application of laccase in the treatment of oily wastewater. The mechanisms of metallic ions inhibiting the removal of oil with laccase are not fully understood. However, in the case of copper and iron ions, it has been reported that these ions could interrupt the electron transport systems of laccase and lead to inhibition of substrate conversion (Torres et al. 2003).

Optimization of Additive Concentrations

Laccase is a major cost component of the water treatment process. Therefore, reductions in the laccase concentration used can promote cost competitiveness of the laccase-catalyzed treatment. Many investigations have reported that some additives can improve catalytic efficiency by forming a protective layer in the vicinity of the active centers of the enzyme and restrict the attack by radicals formed in the catalytic reaction (Vachoud et al. 2001; Delanoy et al. 2005; Modaressi et al. 2005). In recent years, PEG and chitosan were widely studied and applied because they consist of nontoxic organic compounds and are harmless to humans (Harris 1992; Wu et al. 1993).

In order to enhance the effectiveness of laccase performance and determine the suitable additive concentration during the treatment of synthetic oily wastewater, parallel experiments were conducted with laccase in the presence of the additives PEG or chitosan at a laccase concentration of 3 U/mL, reaction time of 6 h, pH of 6.0, oil concentration of 120 mg/L, and additive concentrations in the range of 0 to 160 mg/L.

The results are shown in Fig. 6. Other experiment results showed that almost no oil was removed when the additives were used alone without the addition of laccase. As shown in Fig. 6, PEG slightly decreased the rate of oil removal with laccase. Therefore, PEG has a little inhibition on laccase catalysis of synthetic oily

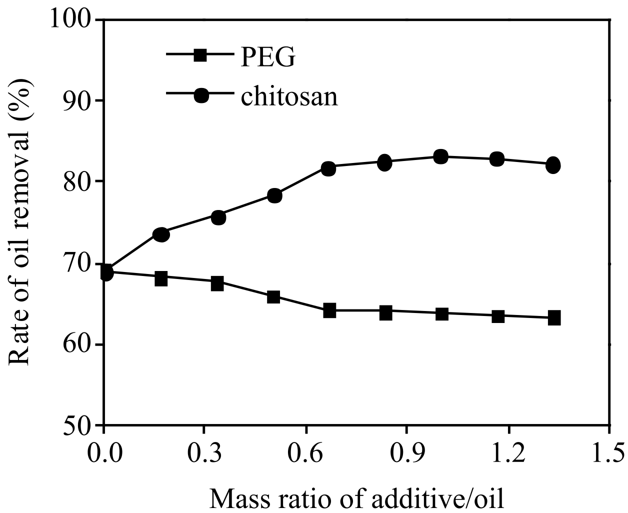

Fig. 6. Additive concentration in the wastewater as a function of the rate of oil removal. The reactions were conducted for 6 h with an initial oil concentration of 120mg/L, laccase concentration of 3 U/mL, pH of 6.0, and temperature of 30°C.

wastewater under our conditions. In contrast to its strong ability to protect peroxidase (Wagner and Nicell 2002), PEG failed to protect laccase here; this could be attributed to the different nature of the oxidized products of these two enzymes.

On the other hand, a significant enhancement in catalytic efficiency was obtained with chitosan as an additive. When the mass ratio of chitosan/oil was in the range from 0 to 0.67, the rate of oil removal was significantly enhanced with the increase of the amount of chitosan. The rate of oil removal gradually reached its maximum value of 84% at the mass ratio of chitosan/oil of 1.0. Further addition of chitosan resulted in a slight reduction of the rate of oil removal, which is in accordance with the result reported by Cheng et al. (2006). When the mass ratio of chitosan/oil was 0.67, the rate of oil removal reached 82%, which was nearly 98% of its maximum value. Therefore, the chitosan addition over the mass ratio of chitosan/oil of 0.67 was unnecessary.

The incremental behaviour of chitosan on the removal of oil with laccase may be associated with the reduction of enzyme inactivity caused by an interaction of chitosan with the inactivity products.

Comparison Between the Synthetic Wastewater and Actual Wastewater

To compare the oil removal effect of laccase in synthetic wastewater versus actual wastewater, parallel batch experiments were conducted under the same conditions of laccase concentration (3 U/mL), reaction time (6 h), and pH (6.0). One of the actual wastewaters studied was generated from the production processes of a petroleum product, and had an oil concentration of 75 mg/L. The other actual wastewater was the circulating water of an oil refinery and had an oil concentration of 89 mg/L. Both kinds of actual wastewater had lower concentrations of oil than that of the synthetic wastewater, so pure oil was added to them so that they had the same total oil concentration as that of the synthetic wastewater of approximately 120 mg/L. The results of the experiments are shown in Fig.7. When only laccase was added, it can be seen that the rate of oil removal from the synthetic wastewater was only 69%, whereas removal from the two actual wastewaters was 80 and 88%, respectively.

In the experiments involving the actual wastewater, increments of the rate of oil removal were obtained. Reduced treatment efficiencies of oil removal were observed for the synthetic wastewater samples. One possible explanation is that some wastewater components could provide a reactive cosubstrate thereby facilitating the removal of the less reactive oil, just like the mechanism mentioned in Roper et al. (1995). Another possible explanation is that protective species in the actual wastewater components protect the enzyme from inactivation by the polymer products. In other words, the enzyme inactivation would be suppressed by protective species in actual wastewater because the inactivating products may interact with the protective species during the reaction process. But it was difficult to analyze the protective species because the wastewater components were too complex.

When both laccase and chitosan were added at the mass ratio of chitosan/oil of 0.67, the rate of oil removal from the synthetic wastewater increased to 82%, whereas removal from both types of the actual wastewater increased to 95%. Therefore, chitosan can enhance the rate of oil removal by laccase in all types of wastewater tested, which may be attributed to the interaction of chitosan and certain components in the actual wastewater.

Different oil removal efficiencies were reported recently for different removal methods of various kinds of oil. After 70 h of treatment, the largest reduction in COD (chemical oxygen demand) was only 57% by electrochemical technology for electrolysis of an oily sample (Santos et al. 2006). The COD removal efficiency reached the range of 62 to 86% on oil-grease by electrocoagulation accompanying with H_2O_2 and a coagulant-aid (Tezcan et al. 2006). The oil removal efficiency reached as high as 99% with the photo-Fenton process for the degradation of the diesel. However, the process needs the combined action of H_2O_2, ferrous ions, and UV radiation (Galvão et al. 2006); the consumptions of the H_2O_2, ferrous ions, and UV radiation power may raise its cost. The method considered in the present study does not require power consumption. The laccase acting as a catalyst can be immobilized on a substrate and be reused in the future industrial process, so the 95% efficiency of oil removal is satisfactory.

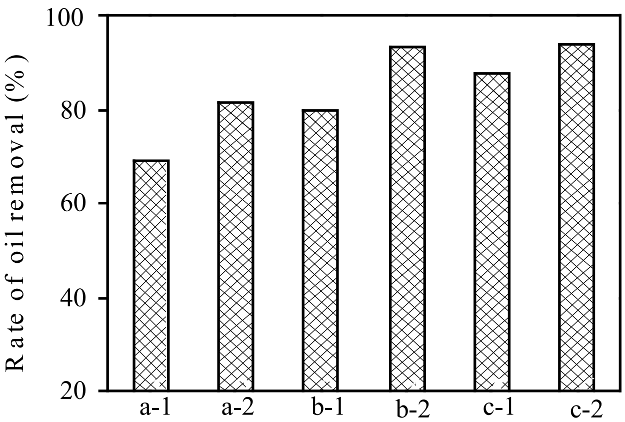

Fig. 7. Effect of water component on the rate of oil removal. The reactions were conducted for a reaction time 6 h with initial oil concentration of 120 mg/L, laccase concentration of 3 U/mL, pH of 6.0, and temperature of 30°C. a-1 = synthetic water; a-2 = synthetic water + 80mg/L chitosan; b-1 = polluted water; b-2 = polluted water + 80 mg/L; c-1 = circulating water; c-2 = circulating water + 80mg/L chitosan.

Conclusions

Treating oily wastewater using laccase was feasible. When only laccase was added to the synthetic oily water, the suitable technological conditions were laccase at 3 U/mL, pH at 6.0, a temperature of 30°C, and a reaction time of 6 h for an initial oil concentration of 120 mg/L. Under these condition, the rate of oil removal was as high as 69%.

At all ranges of concentration tested, the Mg^{2+} or Mn^{2+} ion in the wastewater slightly inhibited the laccase catalytic process, but Cu^{2+} or Fe^{2+} ion obviously inhibited the process. Significant concentrations of Cu^{2+} or Fe^{2+} ion may restrict the actual application of laccase in treating certain oily wastewater.

Of the additives studied, PEG slightly inhibited the oil conversion with laccase, but chitosan enhanced the conversion. When the mass ratio of chitosan/oil was 0.67, the rate of oil removal reached as high as 82%. Because chitosan is biodegradable and nontoxic, it can be a good additive for the treatment of oily wastewater with laccase.

The rate of oil removal of 95% could be obtained when the actual wastewater was treated with both laccase and the chitosan additive under the optimal conditions with an initial oil concentration of 120 mg/L.

Acknowledgment

The authors wish to thank the Research Institute of Yanshan Petrochemical Co., Ltd., SINOPEC (Beijing, China) for financial support.

References

Andreozzi R, Caprio V, Insola A, Marotta R and Sanchirico R. 2000. Advanced oxidation processes for the treatment of mineral oil-contaminated wastewaters. Water Res. **34:**620-628.

Badawy MI and Ali MEM. 2006. Fenton's peroxidation and coagulation processes for the treatment of combined industrial and domestic wastewater. J. Hazard. Mater. B. **136:**961-966.

Barreca AM, Fabbrini MF, Galli CP And Ljunggren SJ. 2003. Laccase/mediated oxidation of a lignin model for improved delignification procedures. J. Mol. Catal. B:Enzym. **26:**105-110.

Braun AM and Oliveros E. 1997. How to evaluate photochemical methods for water treatment. Water Sci. Technol. **35:**17-23.

Chang IS, Chung CM and Han SH. 2001. Treatment of oily wastewater by ultrafiltration and ozone. Desalination. **133:**225-232.

Cheng J, Yu SM and Zuo P. 2006. Horseradish peroxidase immobilized on aluminum-pillared interlayered clay for the catalytic oxidation of phenolic wastewater. Water Res. **40:**283-290.

Claus H. 2004. Laccases: structure, reactions, distribution. Micron. **35:**93-96.

Delanoy G, Li QX and Yu J. 2005. Activity and stability of laccase in conjugation with chitosan. Int. J. Biol. Macro. **35:** 89-95.

Durán N, Rosa MA, Annibale AD and Gianfreda L. 2002. Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review. Enzym. Microb. Technol. **31:**907-931.

Eggert C, Temp U and Eriksson KL. 1996. The ligninolytic system of the white-rot fungus Pycnoporus cinnabarinus: purification and characterization of the laccase. Appl. Environ. Micorbiol. **62:**1151–1158.

Galvão SAO, Mota ALN, Silva DN, Moraes JEF, Nascimento CAO and Chiavone-Filho O. 2006. Application of the photo-Fenton process to the treatment of wastewaters contaminated with diesel. Sci. Total Environ. **367:**42-49.

Ganjidoust H, Tatsumi K, Wada S and Kawase M. 1996. Role of peroxidase and chitosan in removing chlorophenols from aqueous solution. Water Sci. Technol. **34:**151-159.

Gianfreda L, Sannino F, Filazzola MT and Leonowicz A. 1998. Catalytic behavior and detoxifying ability of a laccase from the fungal strain Cerrena unicolor. J. Mol. Catal B: Enzym. **4:**13-23.

Harris JM. 1992. Polyethylene Glycol Chemistry: Biotechnical and Biomedical Applications. p. 1-12. Plenum Press, New York.

Ishihara T. 1980. Lignin Biodegradation Microbiology. Chem. Potential Appl. **2:**17-23.

Kim YJ and Nicell JA. 2006a. Impact of reaction conditions on the laccase-catalyzed conversion of bisphenol A . Biores. Technol. **97:**1431-1442.

Kim YJ and Nicell JA. 2006b. Laccase-catalyzed oxidation of bisphenol A with the aid of additives. Process Biochem. **41:** 1029-1037.

Kurian R, Nakhla G and Bassi A. 2006. Biodegradation kinetics of high strength oily pet food wastewater in a membrane-coupled bioreactor (MBR). Chemosphere. **65:**1204–1211.

Li K, Xu F and Eriksson KL. 1999. Comparsion of fungal laccases and redox mediators in oxidation of a non-phenolic lignin model compound. Appl. Environ. Microb. **65:**2654-2660.

Mayer AM and Staples RC. 2002a. Laccase: new functions for an old enzyme. Phytochemistry. **60:**551-565.

Mayer AM and Staples RC. 2002b. Mechanisms of survival of necrotrophic fungal plant pathogens in hosts expressing the hypersensitive response. Phytochemistry. **58:**33-41.

Modaressi K, Taylor KE, Bewtra JK and Biswas N. 2005. Laccase-catalyzed removal of bisphenol-A from water: Protective effect of PEG on enzyme activity .Water Res. **39:**4309-4316.

Rajakovic V, Aleksic G, Radetic M and Rajakovic L. 2007. Efficiency of oil removal from real wastewater with different sorbent materials. J. Hazard. Mater. **143:**494–499.

Roper JC, Sarkar JM, Dec J and Bollag JM. 1995. Enhanced enzymatic removal of chlorophenols in the presence of co-substrates. Water Res. **29:**2720-2724.

Santos MRG, Goulart MOF, Tonholo J and Zanta CLPS. 2006. The application of electrochemical technology to the remediation of oily wastewater, Chemosphere, **64:** 393-9.

Setti L, Giuliani S, Spinozzi G and Pifferi PG. 1999. Laccase catalyzed-oxidative coupling of 3-methyl 2-benzothiazolinone hydrazone and methoxyphenols. Enzyme Microb. Technol. **25:**285–289.

Srebotnik E and Hammel KE. 2000. Degradation of nonphenolic by laccase/1-hydroxybenzotriazole system. J. Biotechnol. **81:**179-188.

Tezcan Ün Ü, Ugur S, Koparal AS and Bakır Ögütveren Ü. 2006. Electrocoagulation of olive mill wastewaters. Sep. Purif. Technol. **52:**136-141.

Torres E, Bustos JI and Borgne SL. 2003. Potential use of oxidative enzymes for the detoxification of organic pollutants. Appl. Catal. B:Environ. **46:**1-15.

Tsioulpas A, Dimou D and Iconomou D. 2002. Phenolic removal in olive oil mill wastewater by strains of Pleurotus spp. in respect to their phenol oxidase(laccase) activity. Biores. Technol. **84:**251-257.

Vachoud L, Chen TH, Payne GF and Vazquez-Duhalt R. 2001. Peroxidase catalyzed grafting of gallate esters onto the polysaccharide chitosan. Enzyme Microb. Tech. **29:**380-385.

Verner L, Ludmila L, Jan A and Franch B. 2000. Identification of compounds and specific functional groups in the wavelength region 168–330 nm using gas chromatography with UV detection. J. Chromatogr. A. **867:**187-206.

Wagner M and Nicell JA. 2002. Detoxification of phenolic solutions with horseradish peroxidase and hydrogen peroxide. Water Res. **36:** 4041-4052.

Water and Wastewater Monitoring Analysis Committee of Chinese National Environment Bureau. 1989. Water and Wastewater Monitoring Analysis Method. 1st edn, Water and Wastewater Monitoring Analysis Committee of Chinese National Environment Bureau, Beijing, China.

Wolfenden BS and Wilson RL. 1982. Radical cations as reference chromogens in studies of one-electron transfer reactions. J. Chem. Soc. Perkin. Trans. **2:**805–812.

Wong YX and Yu J. 1999. Laccase-catalyzed decolorization of synthetic dyes. Water Res. **33:**3512-3520.

Wu J, Taylor KE, Bewtra JK and Biswas N. 1993. Optimization of the reaction conditions for enzymatic removal of phenol from wastewater in the presence of polyethylene glycol. Water Res. **27:**1701-1706.

Yaropalov AI, Skorobogatko OV, Vartanov SS and Varforlomeyev SD. 1994. Laccase properties, catalytic mechanism and applicability. Appl. Biochem. Biotechnol. **49:** 257-279.

Zhao X, Wang Y, Ye Z, Borthwick AGL and Ni J. 2006. Oil field wastewater treatment in Biological Aerated Filter by immobilized microorganisms. Process Biochem. **41:**1475-1483.

Zouboulis AI and Avranas A. 2000. Treatment of oil-in-water emulsions by coagulation and dissolved-air flotation. Colloids Surf. A. **172:**153–161.

Received: 8 January 2009; accepted: 9 July 2009.