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Treatment of bleaching wastewater from pulp-paper plants in China using enzymes and coagulants

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Abstract: The treatment of wastewater from pulp-paper plants in China by horseradish peroxidase was investigated in this study. The effects of horseradish peroxidase and coagulants were discussed in detail. The results indicated that enzymes might improve the removal of AOX, TOC and colour for pulp-paper wastewater and modified chitosan is far more effective than $\text{Al}_2(\text{SO}_4)_3$ to remove AOX, TOC and colour.

Key words: pulp-paper wastewater; coagulant; horseradish peroxidase

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Introduction

Bleaching wastewater from pulp-paper plants contains many kinds of chlorinated organic compounds which are proved to be carcinogenic, mutagenic and refractory (Gellman, 1988; Paasivirta, 1988; Garden, 1990; Suntio, 1988; Vuorinen, 1987; Seppala, 1988). Several methods, including microbial degradation, adsorption on activated carbon and so on (Milstein, 1988; Tokeay, 1994; Jackson, 1992; Luonsi, 1988; Bryant, 1992; Jokela, 1993; Bumpus, 1985; Amy, 1988; Mileski, 1988), are currently used to treat the pulp-paper wastewater containing those chemicals. Some researchers suggested to remove potentially carcinogenic chemicals such as chlorinated phenols and aromatic amines in pulp-paper wastewater by horseradish peroxidase (HRP) and other enzymes (Klibanov, 1980; 1981; 1983; Pacice, 1984; Atlow, 1984; Hoff, 1985; Chapsal, 1986; Maloney, 1986; Hakulinen, 1988; Schreiner, 1988), an enzymatic approach to treat chlorinated organic compounds in pulp-paper wastewater has been attracting interest (Simmons, 1989; Davis, 1990; Dec, 1990; Ferrer, 1991; Michel, 1991; Royacand, 1991; Galliano, 1991; Bollag, 1992; Nakamoto, 1992; Tatsumi, 1994; Morimoto, 1995).

Horseradish peroxidase was demonstrated to remove chlorinated phenols and aromatic amines from wastewater. This enzyme oxidizes numerous chlorinated organic compounds in the presence of hydrogen peroxide to generate corresponding radicals which diffuse from the active center into solution and form insoluble polymers (Klibanov, 1980; 1981; 1983). These insoluble polymers then precipitate from wastewater and can be separated by simple filtration or flocculation. Most researches have been conducted using lab prepared wastewater. About the use of HRP in real wastewater, few papers were available.

The treatment of wastewater from pulp-paper plants in China by horseradish peroxidase was investigated in this study. The effects of horseradish peroxidase and coagulants were discussed in detail.

1 Material and methods

1.1 Horseradish peroxidase and its activity measurement

Horseradish peroxidase (EC 1.11.1.7) was obtained commercially from Wako Chemicals (Tokyo, Japan) and had a specific activity of 100 units/mg. Peroxidase activity was determined by

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using a spectrophotometer (Jasco Ubest-55, Japan Spectroscopic, Tokyo, Japan). One unit of peroxidase activity was defined from an increase in the optical density ($A_{405\text{nm}}$) in 2.4 ml reaction mixture containing 2, 2'-azino-bis[3-ethyl-benzothiazoline]-(6)-sulfonic acid] diammonium salt (Wako Chemicals) per minute at pH 6.0 at 25°C.

1.2 Coagulants

$\text{Al}_2(\text{SO}_4)_3$ was commercially available and of reagent grade. Modified chitosan (V2/P39) was produced in National Institute of Resources and Environment, Japan.

The wastewater sample was from E-stage of bleaching process in one pulp-paper plant in Shanghai, China. Straw was used as the raw material for pulping in this factory. The bleaching sequence was CED. The sample was stored at 4°C after collection. The sample was centrifuged at 3000 r/min for 10 min before treated using enzyme or coagulants. The supernatant was adjusted to pH 7.0 with NaOH solution and used in the following tests. Characteristics of the sample after centrifuge are as follows: pH = 10.8, AOX (absorbable organic halogen) = 65.3 mg/L, TOC (total organic carbon) = 669 mg/L and AOX/TOC = 0.104.

1.3 Treatment methods

1.3.1 Using enzyme alone

All reactions were carried in 3 ml of wastewater. An enzyme solution was first added into wastewater, followed by addition of hydrogen peroxide. The concentrations of HRP and H_2O_2 were 500 unit/L and 1 mmol/L. Test solution was incubated under aerobic conditions using a stirrer (Tokyo Rika Kikai MDC-RT, Tokyo, Japan) at 25°C. After a prescribed time (60 min) the sample was centrifuged at 3000 r/min for 10 min and measured for AOX, TOC and colour ($A_{400\text{nm}}$).

1.3.2 Using coagulants alone

All reactions were carried in 3ml of wastewater after different additions of coagulants [$\text{Al}_2(\text{SO}_4)_3$ or V2/P39]. Test solution was shaken using the same stirrer as the above at 25°C. After a prescribed time (60 min) the sample was centrifuged at 3000 r/min for 10 min and measured for AOX, TOC and colour ($A_{400\text{nm}}$).

1.3.3 Combination treatment with enzyme and coagulants

After the same enzymatic treatment as the above, different additions of coagulants were added. Then test sample was shaken using the same stirrer as the above at 25°C. After 60 min the sample was centrifuged at 3000 r/min for 10 min and measured for AOX, TOC and colour ($A_{400\text{nm}}$).

1.4 Analysis

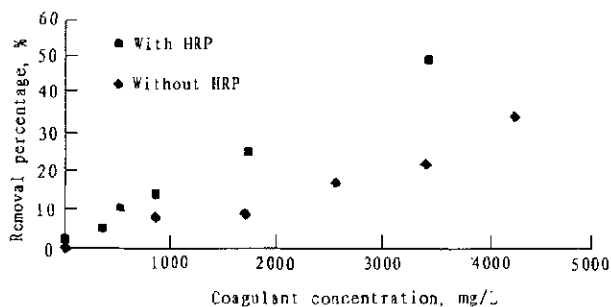
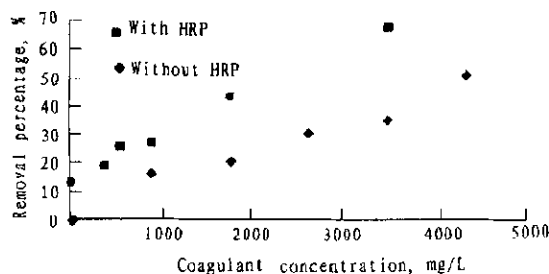
The determination of TOC was made using a Shimadzu TOC analyzer, Model 500 (Kyoto, Japan), and AOX was determined with a Mitsubishi AOX analyzer, TOX-10Σ (Tokyo, Japan). The absorbency at 400 nm of reaction wastewater sample was measured by using the same spectrometer as above.

2 Results and discussion

2.1 Using $\text{Al}_2(\text{SO}_4)_3$ and HRP

The removal of AOX, TOC and colour in wastewater samples using $\text{Al}_2(\text{SO}_4)_3$ is indicated in Figs. 1—3.

If using $\text{Al}_2(\text{SO}_4)_3$ alone, while the concentration of $\text{Al}_2(\text{SO}_4)_3$ increased from 857 mg/L to 4285 mg/L, the removal percentages for TOC of the wastewater were from 8% to 34%. The removal percentages for AOX in the wastewater were from 16% to 50% and the removal percentages for colour of the wastewater were from 20% to 49%. When using enzyme (HRP) before coagulation, the removal efficiency almost increased two times at the same concentration of coagulants. For the same removal percentage of TOC, AOX, or colour, the concentrations of

Fig. 1 TOC removal using $\text{Al}_2(\text{SO}_4)_3$ as the coagulantFig. 2 AOX removal using $\text{Al}_2(\text{SO}_4)_3$ as the coagulant

from water and the settlement of soluble polymers would be improved by coagulants.

As to the molecular weight of polymers in precipitate, Dec and Bollag reported that the average weight of precipitate produced during removal of 2, 4-dichlorophenol using HRP was about 800 dilutions, i. e., it contained tetramers and parameters (Dec, 1990). As to the soluble polymers, analysis results of GC-MS by the author of this article indicated that there is a di-polymer after the reaction (data in detail will be published later).

Its concentration increased along with reaction time while the concentration of 4-chlorophenol decreased. The molecular formula of this polymer is $\text{C}_{12}\text{Cl}_2\text{OH}_8$ and its molecule weight is 254. According to there search results of GC-MS library, this chemical is 2, 4'-dichloro-5-hydroxy-diphenyl ester, a di-polymer of 4-CP. Stephen reported one product with similar structure from removal of 2, 4-dichlorophenol catalyzed by HRP (Maloney, 1986). Using coagulants will increase the removal of such by-products from the enzyme reaction.

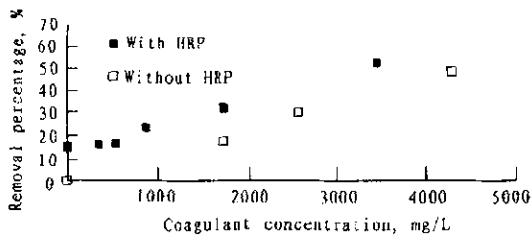
From these results, it might also be clear that, at the same concentration of $\text{Al}_2(\text{SO}_4)_3$, the removal percentage of AOX is higher than that of TOC. This means that organic chlorine in the wastewater is easier to be removed by coagulants than other kinds of chemicals in this wastewater. The reason for such result is not very clear now. We suggested that it perhaps is due to that there are more chlorine components in the part of high-molecule-weight compounds. This needs more tests to prove.

2.2 Using V2/P39 and HRP

Chitosan is one common coagulant used in wastewater treatment. V2/P39 is one kind of

coagulants might reduce by two times.

These results supported the conclusion that enzymes might improve the removal of AOX, TOC and colour for pulp-paper wastewater. The reason for such results was that some organic chlorine might be catalyzed by horseradish peroxidase to form radicals, radicals would polymerize with other radicals or organic chlorine compounds to form polymers. These polymers with high molecular weight might precipitate

Fig. 3 Colour removal using $\text{Al}_2(\text{SO}_4)_3$ as the coagulant

modified chitosan. V2/P39 has more groups containing N atoms than natural chitosan and is more suitable as one coagulant used to remove precipitate catalyzed by enzymes (Morimoto, 1995).

The test results are shown in Fig. 4—Fig. 6.

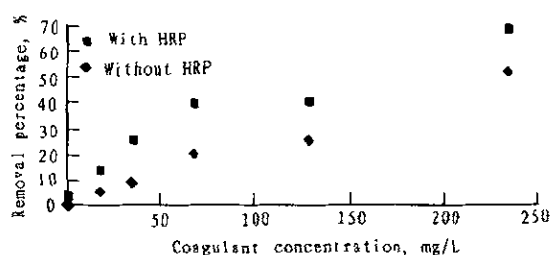


Fig. 4 AOX removal using coagulant V2/P39

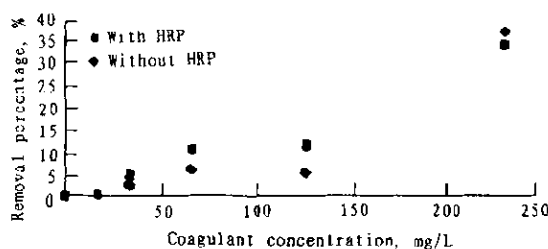


Fig. 5 TOC removal using coagulant V2/P39

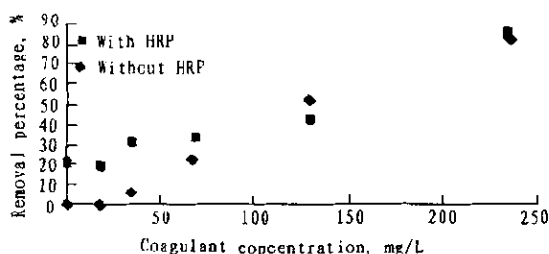


Fig. 6 Colour removal using V2/P39 as the coagulant

According to the results, V2/P39 was far more effective than $\text{Al}_2(\text{SO}_4)_3$ to remove AOX and colour in the wastewater. For example, using coagulants alone, 4285 mg/L $\text{Al}_2(\text{SO}_4)_3$ should be used while only 233 mg/L V2/P39 was required to get the AOX removal of 50%. The efficiency of V2/P39 was almost 20 times higher than $\text{Al}_2(\text{SO}_4)_3$. TOC determination results also supported the same conclusion.

One same result both in tests using $\text{Al}_2(\text{SO}_4)_3$ and tests using V2/P39 was that AOX/TOC ratio in wastewater decrease after treatment. It was an interesting phenomena although the mechanism is not known now.

The results also demonstrated that V2/P39 was especially effective for colour removal. At the concentration of 233 mg/L, the colour was almost decreased 90%. For $\text{Al}_2(\text{SO}_4)_3$, the colour removal is only 50% even the concentration of $\text{Al}_2(\text{SO}_4)_3$ is as high as 4285 mg/L.

However, the increasing of removal percentage due to HRP addition was not so high as that in the test using $\text{Al}_2(\text{SO}_4)_3$. The reason perhaps was that there is difference between the mechanisms

of $\text{Al}_2(\text{SO}_4)_3$ and V2/P39 as the coagulant. V2/P39 was more effective than $\text{Al}_2(\text{SO}_4)_3$ for the low-molecule-weight compounds in the wastewater. Thus without polymerization catalyzed by enzyme, some low-molecule-weight chemicals still might be removed from wastewater by V2/P39.

The process using the enzyme and coagulants is a promising method for paper-pulp wastewater containing organic chlorides, although this method is still under development and has the following problems shown in this study, such as high dose of coagulants especially for $\text{Al}_2(\text{SO}_4)_3$ more complex than usual methods and high cost.

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