

# Effects of peat humic acids on the catalytic oxidation of pentachlorophenol using metalloporphyrins and metallophthalocyanines

Sinta Rismayani<sup>1</sup>, Masami Fukushima\*, Akira Sawada, Hiroyasu Ichikawa, Kenji Tatsumi

*National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba-West, Tsukuba 305-8569, Japan*

Received 8 October 2003; received in revised form 30 January 2004; accepted 3 March 2004

## Abstract

Iron(III)-tetra(*p*-sulfophenyl)porphine (Fe(III)-TPPS), manganese(III)-5,10,15,20-tetra(4-pyridyl)-21H,23H-porphine (Mn(III)-TPyP), iron(III)-phthalocyanine-tetrasulfonic acid (Fe(III)-PcTS) and copper(II)-phthalocyanine-tetrasulfonic acid (Cu(II)-PcTS) were evaluated in terms of the catalytic activity for the oxidation of pentachlorophenol (PCP). Although Cu(II)-PcTS had no activity, 40–70% of the PCP was oxidized in the Fe(III)-TPPS, Fe(III)-PcTS and Mn(III)-TPyP systems. In addition, the effects of the addition of peat humic acids (HAs) on PCP oxidation were also investigated. In the presence of HAs, 65–85% of the PCP was oxidized in the Mn(III)-TPyP system, and approximately 100% of the PCP was oxidized in the Fe(III)-TPPS and Fe(III)-PcTS systems. These results show that the addition of peat HAs is effective in enhancing the PCP oxidation. The effects of peat HAs were investigated using hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) as a model for the hydrophobic portions in HAs, because peat HA contains a hydrophobic core similar to that of cyclodextrin. The conversion of PCP increased with increasing concentrations of HP- $\beta$ -CD. This suggests that structural moieties analogous to cyclodextrins in peat HAs contribute to the enhanced PCP oxidation via the catalytic system with Fe(III)- and Mn(III)-complexes.

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**Keywords:** Metalloporphyrins; Metallophthalocyanines; Pentachlorophenol; Oxidation; Humic acids

## 1. Introduction

In soil environments, some biotransformations via aerobic microorganisms such as white-rot fungi have been attended to the oxidative degradation of persistent organic pollutants (POPs), such as chlorinated phenols [1]. The enzymes produced from aerobic microorganisms can play an important role in the degradation of POPs in soil environments. Although humic acids (HAs) are widely distributed in soils, their effects on the efficiencies of biotransformation are not fully understood. We previously examined the effects of HAs on the oxidation of pentachlorophenol (PCP) via a biomimetic catalytic system using iron(III)-porphyrin complex (Fe(III)-Por), which can be regarded as a catalytic center of an enzyme [2,3]. It is generally known that Fe(III)-Por serves as a cofactor for certain oxidative enzymes such as

peroxidase and lignase. However, other oxidative enzymes such as manganese-peroxidase and laccase utilize Mn and Cu as cofactors [4,5]. To understand the remedial processes of organic pollutants via microorganisms, other catalysts in addition to Fe(III)-Por should be examined.

In practical use in soil remediation, microorganisms require the maintenance of conditions conducive to their viability and growth. However, the use of a chemical catalyst for converting POPs such as PCP could circumvent many of the problems associated with the introduction of non-indigenous microorganisms into contaminated soils. Therefore, metalloporphyrins might simplify and accelerate the overall reaction. However, because the costs of metalloporphyrins are high, other biomimetic systems should be examined.

It is known that metallophthalocyanines have many of the characteristics of metalloporphyrins [6]. They are used as electronic photoconductors, solar batteries, solar energy storage systems and catalysts for organic synthesis. Because metallophthalocyanines are commercially available, the cost is lower than that of metalloporphyrins. Thus, to apply a biomimetic catalytic system to soil remediation, it is worth examining the degradation of organic

\* Corresponding author. Tel.: +81-29-861-8786; fax: +81-29-861-8328.

E-mail address: [m-fukushima@aist.go.jp](mailto:m-fukushima@aist.go.jp) (M. Fukushima).

<sup>1</sup> Present address: Institute for R&D of Textile Industry (Balai Besar Tekstil), Jl. Ahmad Yani 390, Bandung 40172, Indonesia.

pollutants by metallophthalocyanines. In the present study, we report on an investigation of the catalytic oxidation of PCP using metalloporphyrins and metallophthalocyanines. Moreover, the effects of HA on the efficiency of PCP oxidation were also investigated using peat HAs and hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) as a representative model compound.

## 2. Material and methods

### 2.1. Materials

The chemical structures of catalysts used in the present study are shown in Fig. 1: iron(III)-tetrakis(sulfonatophenyl) porphyrin (Fe(III)-TPPS); iron(III)-phthalocyanine-tetrasulfonic acid (Fe(III)-PcTS); manganese(III)-tetra(4-pyridyl) porphyrin (Mn(III)-TPyP); copper(II) phthalocyanine tetrasulfonic acid (Cu(II)-PcTS). The catalysts, except for Fe(III)-TPPS, were purchased from Aldrich. Fe(III)-TPPS was synthesized according to a previous method [3]. The HAs were extracted with aqueous 0.1 M NaOH from Bibai (BHA) and Shinshinotsu (SHA) peat soils, and powdered samples of HAs were obtained by purification according to a method of the International Humic Substances Society [7]. The elemental compositions of these materials are summarized in Table 1. KHSO<sub>5</sub> was purchased from Merck. PCP (99.0% purity) was purchased from Nacalai Tesque, and a stock solution (0.01 M) was prepared by dissolving it in acetonitrile. HP- $\beta$ -CD was purchased from Aldrich. 2-Hydroxyl (2H-NCDE) and 4-hydroxyl (4H-NCDE) nonachlorodiphenyl ethers were prepared according to a previous report [2]. A <sup>14</sup>C-labeled PCP (22 mCi mmol<sup>-1</sup>,

Table 1

Results of elemental analyses for the HAs

HAs	%C	%H	%N	%O	%S	%ash	H/C	O/C
BHA	57.24	5.70	2.44	31.30	0.58	2.74	1.19	0.41
SHA	57.93	5.46	1.78	33.90	0.93	n.d.	1.12	0.44

n.d., not detected.

0.5 mCi ml<sup>-1</sup> in toluene; American Radiolabeled Chemicals Inc.) was used for the measurement of the amounts of <sup>14</sup>CO<sub>2</sub> generated as a result of PCP degradation.

### 2.2. Oxidation test of PCP

A 2 ml aliquot of 0.02 M NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>/citrate buffer at pH 6 was placed in a 20 ml L-shaped test tube. The 10–40  $\mu$ l aliquot of 3.8 mM PCP in acetonitrile, the 10–100  $\mu$ l aliquot of aqueous catalyst (200  $\mu$ M) and 100  $\mu$ l aliquot of aqueous HA (1 g l<sup>-1</sup>) were added to the buffer solution. Subsequently, 25  $\mu$ l of aqueous 0.01 M KHSO<sub>5</sub> was added, and the tube was then shaken in a Monosin IIA-type thermostatic shaking water bath (Taitec) at 25  $\pm$  0.1  $^{\circ}$ C. After a 60 min reaction period, an 800  $\mu$ l aliquot of the test solution was mixed with 400  $\mu$ l of 2-propanol. To analyze the level of PCP in the test solution, a 20  $\mu$ l aliquot of the mixture was injected into an HPLC system.

### 2.3. HPLC analysis

The HPLC system used in the present study was a JASCO PU-980-type HPLC pumping system. The mobile phase consisted of a mixture of 0.08% aqueous H<sub>3</sub>PO<sub>4</sub> and methanol (20/80 = v/v), and the flow rate was set at 1 ml min<sup>-1</sup>. A 5C18-MS Cosmosil packed column (4.6 mm i.d.  $\times$  250 mm, Nacalai Tesque) was used as a solid phase, and the column temperature was maintained at 50  $^{\circ}$ C. PCP was measured by UV absorption at a wavelength of 220 nm. The detection limit of PCP in the HPLC analysis was estimated at 0.3  $\mu$ M.

The elution behavior of Fe(III)-TPPS was investigated using the same HPLC system. However, the mobile phase was a mixture of 0.08% aqueous H<sub>3</sub>PO<sub>4</sub> and methanol (70/30 = v/v), and the detection wavelength of Fe(III)-TPPS was set at 419 nm.

### 2.4. Analyses of byproducts

A 25 ml aliquot of 0.02 M NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>/citrate buffer at pH 6, which contained 50 mg l<sup>-1</sup> of HA, was placed in a 100 ml Erlenmeyer flask. A 125  $\mu$ l aliquot of 0.01 M PCP in acetonitrile and a 625  $\mu$ l aliquot of aqueous Fe(III)-TPPS (200  $\mu$ M) was added to the buffer solution. Subsequently, 313  $\mu$ l of aqueous 0.01 M KHSO<sub>5</sub> was added, and the flask was then shaken in a thermostatic shaking water bath at 25  $^{\circ}$ C. After a 60 min reaction period, 2.5 ml of aqueous 1 M ascorbic acid was added, and the pH of the solution was adjusted to 11–11.5 by adding aqueous K<sub>2</sub>CO<sub>3</sub>. Subsequently, 5 ml of acetic anhydride was added dropwise,

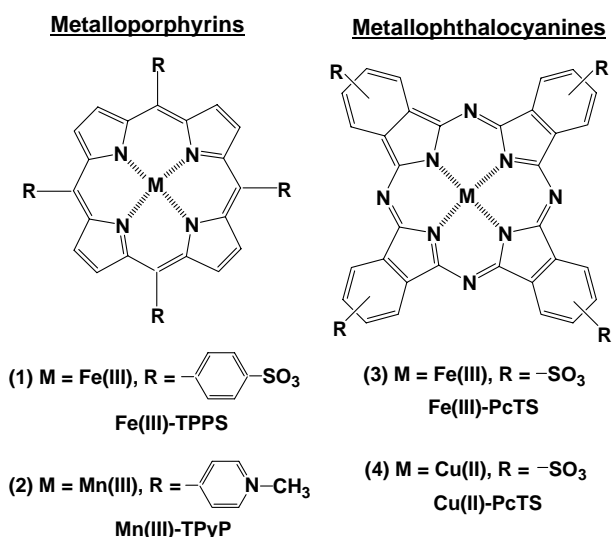


Fig. 1. Chemical structures of metalloporphyrins and metallophthalocyanines: (1) iron(III)-tetrakis(sulfonatophenyl)porphyrin (Fe(III)-TPPS); (2) manganese(III)-tetra(4-pyridyl)porphyrin (Mn(III)-TPyP); (3) iron(III)-phthalocyanine-tetrasulfonic acid (Fe(III)-PcTS); (4) copper(II) phthalocyanine-tetrasulfonic acid (Cu(II)-PcTS).

and 0.6 ml of a 1 mM anthracene in hexane was added as an internal standard for the GC/MS analyses. This mixture was extracted three times with 30 ml of *n*-hexane, and the combined extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration, the extract was concentrated to 500 μl under a stream of dry N<sub>2</sub>. A 1 μl aliquot of the extract was introduced into an HP5971/HP5890 series II (Hewlett Packard) GC/MS system. A Quadrex methyl silicon capillary column (0.25 mm i.d. × 25 m) was employed in the separation. The temperature protocol was as follows: 65 °C for 1.5 min, 65–120 °C at 35 °C min<sup>-1</sup>, 120–300 °C at 7 °C min<sup>-1</sup> and a 300 °C hold for 5 min. The concentration of PCP in the test solution before and after the reaction was also determined by HPLC. All runs were conducted in duplicate.

### 2.5. Analysis of CO<sub>2</sub> using <sup>14</sup>C-labeled PCP

After removing toluene from the <sup>14</sup>C-labeled PCP stock by a stream of dry N<sub>2</sub>, the labeled PCP was dissolved in acetonitrile. A 33 μl aliquot of the <sup>14</sup>C-labeled PCP in acetonitrile (4.55 mM) was placed in a 50 ml Erlenmeyer flask, which included 5 ml of NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>/citrate buffer (pH 6) containing 50 mg l<sup>-1</sup> HA and 5 μM catalyst. After adding 62.5 μl of 0.01 M KHSO<sub>5</sub>, the flask was fitted with a stopper, which contained a polyethylene center-well including a plug of glass wool impregnated with 400 μl of 2 M NaOH aqueous as a <sup>14</sup>CO<sub>2</sub> trap. After 60 min of reaction period, 2 ml of 2-propanol and 0.5 ml of 1 M H<sub>2</sub>SO<sub>4</sub> was injected via a syringe needle from the top of stopper to drive off <sup>14</sup>CO<sub>2</sub> from the test solution. After stirring for 2 h, 1 ml of the test solution was pipetted into a glass vial that contained the mixture of methanol (1 ml) and scintillation cocktail (9 ml). The glass wool and aqueous washings (600 μl) from the center-well were transferred to a vial that contained the mixture of methanol and scintillation cocktail. The radioactivities (dpm) in the vials were measured by an LS 6000 Series liquid scintillation counter (Beckman Instruments Inc.). To evaluate the amounts of PCP oxidized, a test solution containing 30 μM of non-labeled PCP was reacted and the resulting [PCP]<sub>60</sub> measured by HPLC.

### 2.6. UV-Vis absorption spectra of Fe(III)-TPPS

The visible absorption spectra of the aqueous solution at pH 6, which contained catalysts (5 μM) and HAs (or HP-β-CD), were measured by using a Jasco V-550-type spectrophotometer (Japan Spectroscopic Co.) with a quartz cell (1 cm × 1 cm) at 25 °C.

## 3. Results and discussion

### 3.1. Oxidation characteristics of PCP

Fig. 2 shows the conversion of PCP in the presence of the Fe(III)-, Mn(III)- and Cu(II)-complexes at pH 6 for a

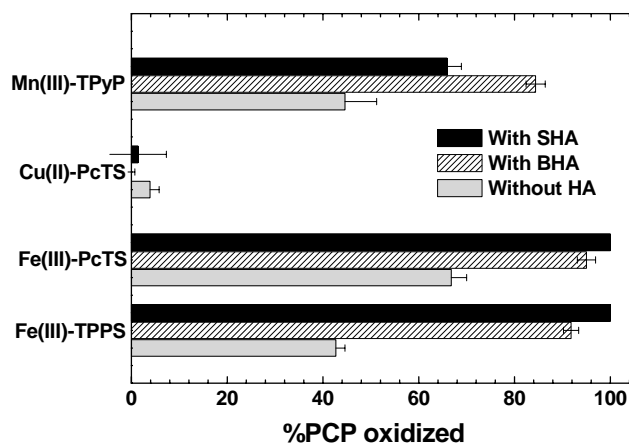
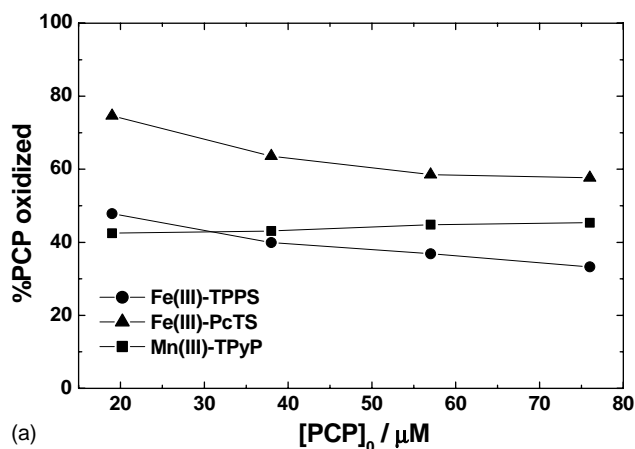


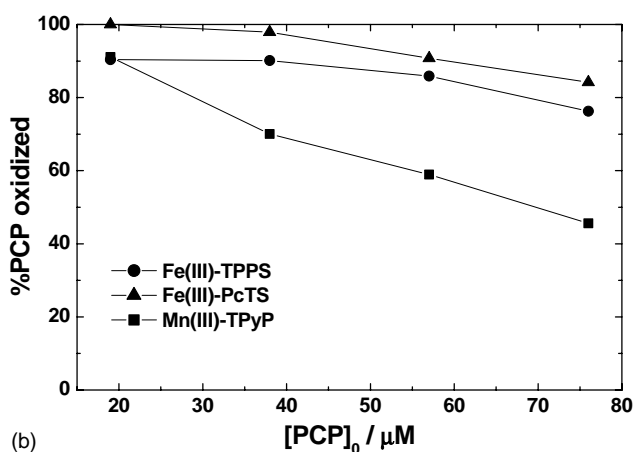
Fig. 2. Conversions of PCP in the absence and presence of HAs—[PCP]<sub>0</sub>: 38 μM; [catalysts]: 5 μM; [KHSO<sub>5</sub>]: 125 μM; pH 6; reaction time: 60 min.

reaction period of 60 min. For the Cu(II)-PcTS system, no PCP oxidation was observed irrespective of the absence or presence of HAs. In the absence of HAs, 40–70% of the PCP was oxidized for the Fe(III)-TPPS, Mn(III)-TPyP and Fe(III)-PcTS systems. It is noteworthy that the highest level of PCP oxidation was observed in the Fe(III)-PcTS system. For the control experiments, no PCP oxidation was observed in the presence of the Mn(II)- or Fe(III)-complexes. In addition, no PCP oxidation was observed in the presence of only KHSO<sub>5</sub>. These results show that Mn(III)- and Fe(III)-complexes are effective in oxidizing PCP via a catalytic oxidation with KHSO<sub>5</sub>.

In a previous report [3], the addition of HAs, which were of a lower degree of humification, was found to enhance PCP oxidation in a Fe(III)-TPPS/KHSO<sub>5</sub> system. The HAs with a lower degree of humification was originated from peat soil. To confirm whether the addition of HA is effective in enhancing PCP oxidation in the presence of Fe(III)-PcTS and Mn(III)-TPyP or not, we examined the effect of the addition of peat HAs to each catalytic system. Fig. 2 also shows the effects of peat HAs (BHA and SHA) on the conversion of PCP. As shown in Table 1, the H/C and O/C atomic ratios for BHA and SHA, which were the indices of the degree of humification [8], were in the ranges of 1.12–1.19 and 0.41–0.44, respectively. Thus, the degrees of humification for BHA and SHA are similar. For the Mn(III)-TPyP system, the conversion of PCP increased from 45 to 67–85% as a result of adding BHA and SHA. However, in the cases of Fe(III) complexes (the Fe(III)-TPPS and Fe(III)-PcTS systems), 90–100% of PCP was oxidized by adding BHA and SHA. In the control experiments (HA only, catalysts + HA and KHSO<sub>5</sub> + HA), no conversion of PCP was observed. These results demonstrate that PCP oxidation is enhanced by adding peat HA in the Fe(III)-PcTS and Mn(III)-TPyP systems as well as the Fe(III)-TPPS system.



(a)



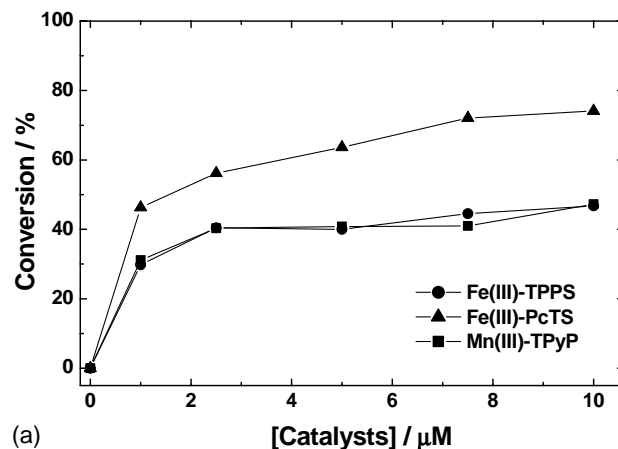
(b)

Fig. 3. Influence of initial concentration of PCP on the conversion of PCP in the absence (a) and presence (b) of HA—[catalysts]: 5 μM; [KHSO<sub>5</sub>]: 125 μM; pH 6; reaction: time 60 min; [SHA]: 50 mg l<sup>-1</sup> for (b).

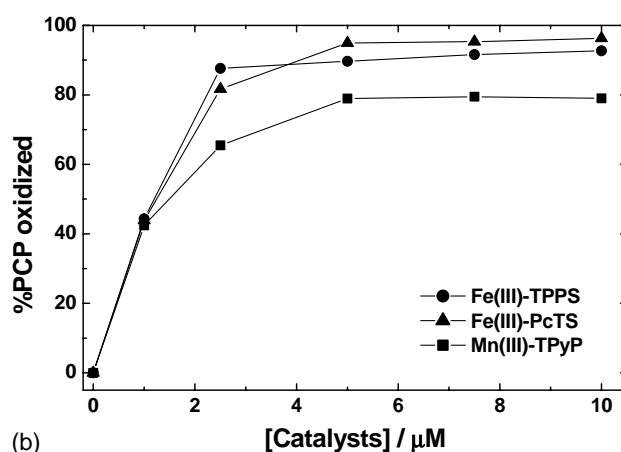
### 3.2. Effects of initial concentrations of PCP and catalysts

Fig. 3a and b shows the influences of the initial concentration of PCP on the conversion. In the absence of SHA, the conversion of PCP slightly decreased with increasing initial concentration of PCP, but remained constant in the Mn(III)-TPyP system. In the presence of SHA, the conversion of PCP also decreased with increasing initial substrate concentration. However, the conversion of PCP for the Mn(III)-TPyP system decreased with increasing [PCP]<sub>0</sub> in the presence of SHA.

Fig. 4a and b shows the influence of catalyst concentration on the conversion of PCP. In the absence of SHA, the conversion of PCP for the Fe(III)-PcTS system steeply increased up to 1 μM and then increased more gradually. For the Fe(III)-TPPS and Mn(III)-TPyP systems, the conversion of PCP increased up to 2.5 μM and then reached a plateau. In the presence of SHA, the conversion for the Fe(III)-TPPS and Fe(III)-PcTS systems increased up to 5 μM and then reached a plateau. For the Mn(III)-TPyP system, the conversion reached a plateau for concentrations above 2.5 μM. As shown in Figs. 3 and 4, the initial concentrations of PCP



(a)



(b)

Fig. 4. Influence of initial concentration of catalysts on the conversion of PCP in the absence (a) and presence (b) of HA—[PCP]<sub>0</sub>: 38 μM; [KHSO<sub>5</sub>]: 125 μM; pH 6; reaction time: 60 min; [SHA]: 50 mg l<sup>-1</sup> for (b).

and catalysts influenced the conversions of PCP. However, the levels of PCP oxidation in the presence of SHA were significantly higher than those in its absence. These results indicate that the addition of peat HA is effective in enhancing the oxidation of PCP.

### 3.3. Byproducts

For the Fe(III)-TPPS, Fe(III)-PcTS and Mn(III)-TPyP systems, PCP was effectively oxidized whether HAs were present or not. Thus, we attempted to identify and determine the byproducts by GC/MS. Fig. 5 shows the distribution of byproducts for the Fe(III)-TPPS, Fe(III)-PcTS and Mn(III)-TPyP systems. The amounts of CO<sub>2</sub> were determined using <sup>14</sup>C-labeled PCP. In the absence of HAs, *o*-TeCQ, 2H-NCDE, 4H-NCDE and OCDD were identified as byproducts of the Fe(III)-TPPS and Mn(III)-TPyP reactions. However, in the presence of peat HAs, only *o*-TeCQ was found as a byproduct. These results are consistent with our previous study of Fe(III)-TPPS [2]. In all catalysts, the addition of peat HAs was effective in preventing the formation of more toxic dimers such as 2H-NCDE, 4H-NCDE and OCDD. As

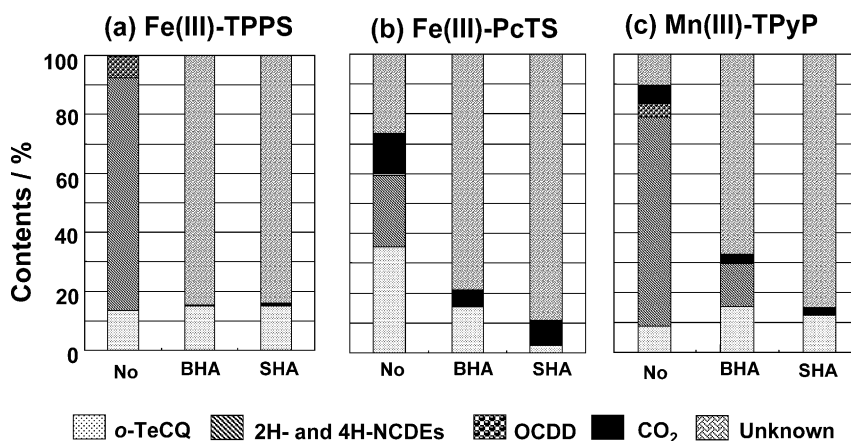


Fig. 5. Distribution of byproducts for catalytic reactions using Fe(III)-TPPS, Fe(III)-PcTS and Mn(III)-TPyP in the absence and presence of HAs.

described in a previous paper [2], the “unknown” species in the presence of peat HAs can be attributed to the formation of polymeric compounds by reactions of HAs and PCP-derived intermediates. In this study, the fact that forming the polymeric compounds largely reduced the toxicities of PCP-derived intermediates was demonstrated by a Microtox test. Although CO<sub>2</sub> generation was not observed in the Fe(III)-TPPS system, 3–13% of PCP was mineralized to CO<sub>2</sub> in the Fe(III)-TPPS and Mn(III)-TPyP systems. For the Fe(III)-PcTS and Mn(III)-TPyP systems, the CO<sub>2</sub> generation decreased in the presence of HAs. A possible explanation for this is that, in the presence of HAs, the mineralization of PCP competes with the formation of polymeric compounds. In addition, even in the absence of HAs, no toxic dimers such as OCDD were observed in the Fe(III)-PcTS system. These results indicate that Fe(III)-PcTS is the most effective catalyst of the three complexes.

#### 3.4. Possible effects of HAs

The interesting issue is why the addition of peat HAs led to an enhancement in PCP oxidation. In the peroxidase oxidation of PCP, the conversion was enhanced by the addition of humic precursors such as phenolic acids [9]. An HA with a lower degree of humification may contain larger amounts of humic precursors such as phenolic acids. If the enhancement in the oxidation of PCP is related to humic precursors, structural fragments containing phenolic acids might explain this enhancement. In general, humic precursors are known to be lignin-like compounds, which consist of a variety of phenolic acids [10]. Thus, we first investigated the effects of humic precursors on PCP disappearance. Gallic, caffeic, ferulic, syringic, vanillic and protocatechuic acids, catechol and *p*-hydroquinone were examined as models of humic precursors. When an aqueous solution containing 50 μM of PCP, 125 μM of phenolic acids and 5 μM of Fe(III)-TPPS was allowed to react with 125 μM of KHSO<sub>5</sub> for 60 min, no significant enhancement in the conversion of PCP was detected. Secondly, the addition of axial ligands for Por-Fe(III)

such as cysteine and histidine has been reported to be useful in enhancing the oxidation of substrates [11–13]. Therefore, the addition of cysteine and histidine (125 μM), which are contained in HAs as amino acid residues [10], was examined. However, no enhancement was detected.

On the other hand, computational chemistry [14] indicates a preferred helical structure for aliphatic chains in HAs. That is, to accommodate the aliphatic carbohydrate structure inside, HA contains a hydrophobic cavity similar to that of a cyclodextrin. Peat HAs with a lower degree of humification include such moieties, and this may contribute to the interaction with catalysts. To show the effects of such moieties in HAs, we examined the addition of HP-β-CD as a model compound of HAs. Fig. 6 shows the effect of HP-β-CD concentration on the conversion of PCP. The conversion of PCP increased with increasing concentrations of HP-β-CD. Moreover, the byproducts for the oxidation via the Fe(III)-TPPS system with HP-β-CD were measured by GC/MS (contents of the byproducts: *o*-TeCQ, 16.7%; NCDEs, 9.6%; OCDD, 0.3%; unknown, 63.6%). Although the toxic dimers such as NCDEs and OCDD did not completely disappear,

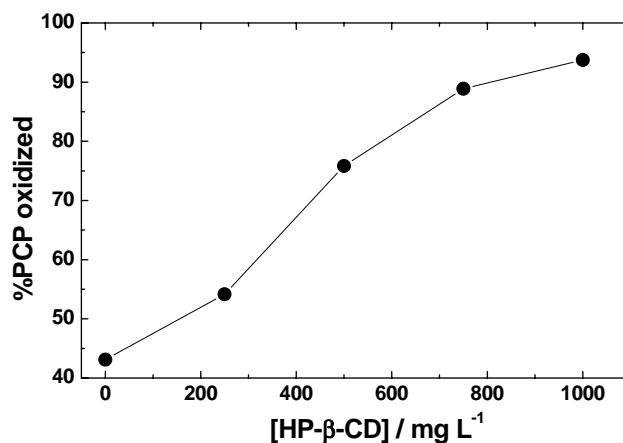


Fig. 6. Effect of HP-β-CD concentration on the conversion of PCP—[PCP]<sub>0</sub>: 50 μM; [Fe(III)-TPPS]: 5 μM; [KHSO<sub>5</sub>]: 125 μM; pH 6.0; reaction time: 60 min.

the contents of such dimers were much lower than those in the absence of additives (NCDEs, 80%; OCDD, 7%). Such a tendency is similar to that in the HAs. These results suggest that the structural part of peat HAs that are similar to cyclodextrin contributes to the enhancement of catalytic oxidation of PCP with Fe(III)-TPPS.

### 3.5. Interactions between Fe(III)-TPPS and HP- $\beta$ -CD or HAs

It has been reported that polymer-supported metalloporphyrin catalysts are effective in enhancing substrate conversion [4]. This suggests that the binding of catalysts to HA can be an important process for enhancing the oxidation of PCP. Thus, we investigated the interactions between Fe(III)-TPPS and HP- $\beta$ -CD or HAs. It is known that the complexation of molecules with metalloporphyrins brings about the red-shift in the UV-Vis absorption spectra [15]. Therefore, the changes of UV-Vis absorption spectra of Fe(III)-TPPS were observed whether HP- $\beta$ -CD or HAs was present or not.

Fig. 7 shows the UV-Vis absorption spectra of Fe(III)-TPPS in the absence and presence of HP- $\beta$ -CD. In the absence of HP- $\beta$ -CD, the spectrum of Fe(III)-TPPS had three peaks at 327, 394 and 529 nm. When HP- $\beta$ -CD was added to an aqueous solution of Fe(III)-TPPS, these peaks shifted to higher wavelengths. In particular, the largest peak at 394 nm, corresponding to the Soret band, was significantly shifted to 419 nm, and the absorbance at 419 nm increased with increasing concentration of HP- $\beta$ -CD. It has been reported that the aromatic substituents in meso-porphyrin can be incorporated into a cyclodextrin [16]. These results suggest the existence of interactions between Fe(III)-TPPS and HP- $\beta$ -CD.

Fig. 8a shows the UV-Vis absorption spectra of Fe(III)-TPPS in the absence and presence of BHA. The absorbance increased with increasing concentration of BHA. If the spectra would not be varied by adding BHA, subtraction of the spectrum for the mixture of Fe(III)-TPPS and BHA from

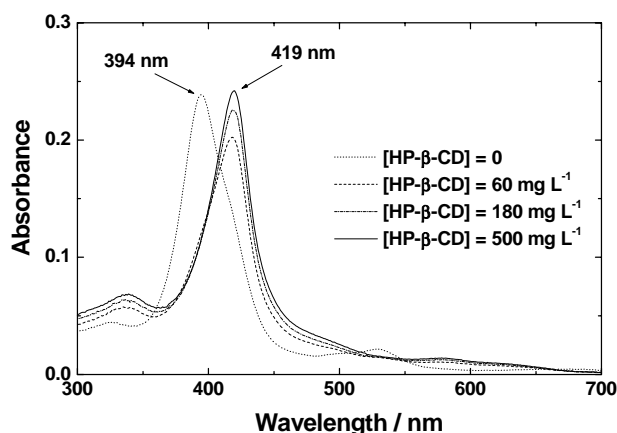


Fig. 7. UV-Vis absorption spectra of Fe(III)-TPPS in the absence and presence of HP- $\beta$ -CD.

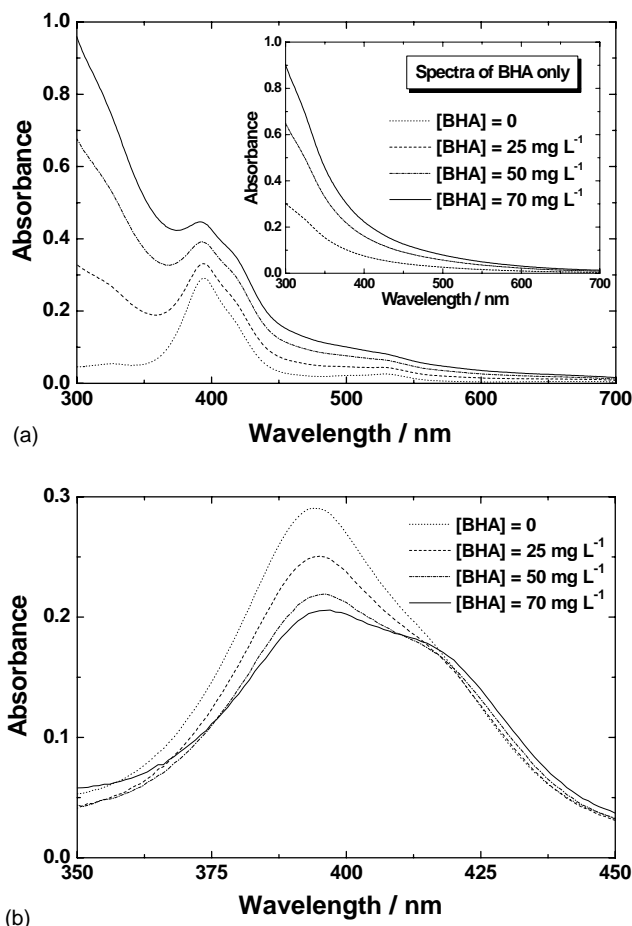


Fig. 8. UV-Vis absorption spectra of Fe(III)-TPPS in the absence and presence of BHA: pH 6; [Fe(III)-TPPS], 5  $\mu$ M. (a) The spectra of the mixture of Fe(III)-TPPS and BHA (insert: spectra of BHA only). (b) Subtraction of the spectra of the mixtures from the spectra of BHA only.

that for BHA only (insert in Fig. 8a) could be similar to the spectrum in the absence of BHA. Fig. 8b shows the subtraction of the spectra for the mixture from those for BHA only. The subtracted spectra were clearly different from the spectrum for Fe(III)-TPPS only. With increasing concentration of BHA, the absorbance at 394 nm decreased, and a shoulder at around 420 nm appeared. Similar results were observed in the case of SHA. These results suggest the existence of interactions between Fe(III)-TPPS and HAs.

If benzenesulfonate, which is a more hydrophilic part in Fe(III)-TPPS, would bind to HA or HP- $\beta$ -CD, the hydrophobicity of Fe(III)-TPPS would be enhanced. In the case of a C<sub>18</sub>-type column, the elution of Fe(III)-TPPS may be retarded by binding with HA or HP- $\beta$ -CD. Thus, the elution behavior of Fe(III)-TPPS in the HPLC system using C<sub>18</sub>-type column was investigated in the absence and presence of HAs or HP- $\beta$ -CD. In the absence of HAs and HP- $\beta$ -CD, the retention time of Fe(III)-TPPS was  $5.05 \pm 0.01$  min. However, new peaks at  $5.15 \pm 0.03$ ,  $5.19 \pm 0.05$  and  $5.29 \pm 0.08$  min appeared in the presence of BHA, SHA and HP- $\beta$ -CD, respectively. These retention times were

significantly longer than for Fe(III)-TPPS only. These results support the incorporation of Fe(III)-TPPS into HAs or HP- $\beta$ -CD. Therefore, we conclude that the interaction between HA and Fe(III)-TPPS contributed to an enhanced PCP oxidation.

#### 4. Conclusion

We confirmed that the addition of HAs with a lower degree of humification was effective in PCP oxidation using Fe(III)-PcTS and Mn(III)-TPyP as well as in a Fe(III)-TPPS system. Because the cost of Fe(III)-PcTS is relatively low, the results presented here provide useful information concerning the use of biomimetic catalysts in the in situ remediation of PCP-contaminated soils. Moreover, the effect of peat HAs can be attributed to interactions between the catalyst and hydrophobic regions, analogous to a cyclodextrin resulting in an enhanced PCP oxidation. These results indicate that the addition of peat HAs can be effective in the remediation of PCP-contaminated soil.

#### Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research in Japan Society for the Promotion of Science (14380283).

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