PHOTO-FENTON REACTION OF DYES WITH Fe(III)-TREATED HYDROXYAPATITES AS HETEROGENEOUS CATALYSTS IN WATER

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Keywords: Iron-treated hydroxyapatite, Photo-Fenton reaction, Dye

ABSTRACT: Fe(III)-treated hydroxyapatites, HAP-300-Fe and HAP-400-Fe, were prepared from hydroxyapatites, HAP-300 (stoichiometric type) and HAP-400 (Ca-deficient type), respectively, and applied to photo-Fenton reaction of dyes as heterogeneous catalysts in comparison with other Fe catalysts such as Fe_2O_3 , $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$, and FeOOH . Among the Fe catalysts, HAP-400-Fe revealed the highest consumption and mineralization in photo-Fenton reaction of alizarin red S (ARS), and also indicated good catalytic activity to other dyes, alizarin, neutral red, methyl red, methyl orange (MO), Congo red, and indigo carmine. Generation of hydroxyl radical $\cdot\text{OH}$ as an oxidation species from H_2O_2 was observed in the all Fe catalysts by an indirect method using N, N-dimethylamino-4-nitrosoaniline, which is exclusively sensitive to $\cdot\text{OH}$. In order to elucidate catalysis mechanism of Fe catalysts, kinetic investigation based on Michaelis-Menten theory was conducted in the reaction of ARS and MO. As a result, HAP-400-Fe indicated high ability of bearing many dye molecules but low degradative catalysis ability to H_2O_2 , compared with Fe_2O_3 . However, judging from high mineralization ability as well as repeating availability (5 times), HAP-400-Fe is considered as an excellent heterogeneous catalyst for photo-Fenton reaction.

(Received February 24 2011; Accepted March 12 2012)

1. INTRODUCTION

In the previous paper,¹ we reported adsorbability of alizarin red S (ARS) as a born-staining agent² onto Fe(III)- and Pb(II)-treated hydroxyapatites, which are prepared from stoichiometric hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HAP-300 (Ca/P=1.68, slightly containing CO_3), and Ca-deficient hydroxyapatite $\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$, HAP-400 (Ca/P=1.38). Among those metal-treated apatites, Fe(III)-treated hydroxyapatites, HAP-300-Fe and HAP-400-Fe, indicated higher adsorption capacities than the raw apatites, HAP-300 and HAP-400. From IR analysis, an adsorption mechanism between Fe(III)-treated hydroxyapatites and ARS was defined as chelete form constructed by chelation of two functional groups of ARS, 1-C=O⁻ and 9-C=O, to a Fe site of each Fe(III)-treated apatite. The chelete form adsorption is rigid and hardly subjected to desorption even by treatment with an aqueous solution containing phosphate ions.

Judging from those adsorbabilities, we expected that the Fe(III)-treated hydroxyapatites are to be useful as heterogeneous catalysts for photo-Fenton reaction.³⁻¹⁸ Photo-Fenton reaction is one of

advanced oxidation processes,¹⁹ which are essentially used for removal of natural organic matter from drinking water. Although some homogeneous photo-Fenton systems using Fe ions²⁰⁻²⁵ feature a merit of powerful degradation of organic compounds, they have a disadvantage of requirement to remove Fe ions from water after water treatment.⁹ From a viewpoint of smoothing and recycling usage, or running cost in future water purification plants, a heterogeneous system must be superior to homogenous one. As heterogeneous catalysts, iron oxides (Fe_2O_3 , FeOOH),^{3,13} Fe(III)-immobilized zeolites,^{4,11,12} Fe(III)-immobilized silica,^{5,16} Fe(III)-immobilized clay,^{6-9,14,17} Fe(III)-immobilized Al_2O_3 ,¹⁰ Prussian blue colloids,¹⁵ and tungstate salt¹⁸ were employed so far. However, Fe-hybridized hydroxyapatites such as HAP-300-Fe and HAP-400-Fe for heterogeneous photo-Fenton catalysts have never been reported. Since a raw hydroxyapatite as a catalyst base is originally biocompatible²⁶ and has adsorption capacity in water,²⁷ no problem will be raised in water treatment. Therefore, we investigated photo-Fenton reaction of organic dyes with HAP-300-Fe or HAP-400-Fe in water and elucidated its catalysis mechanism by a kinetic study based on Michaelis-Menten theory.

Alizarin Red S (ARS)

Alizarin

Neutral Red

Methyl Red

Methyl Orange (MO)

Congo Red

Indigo Carmine

SCHEME 1 Structures of dyes as substrates in photo-Fenton reaction.

2. MATERIALS AND METHODS

2.1. Materials

Hydroxyapatites, HAP-300 and HAP-400, which were synthesized from CaHPO₄·2H₂O and CaCO₃ and from CaHPO₄·2H₂O and NaOH,^{28, 29} respectively, were supplied from Taihei Chemical Industrial Co., Ltd. Alizarin red S (ARS), alizarin, neutral red, methyl red, methyl orange (MO), Congo red, and indigo carmine as dyes (SCHEME 1), iron (III) chloride hexahydrate FeCl₃·6H₂O (purity 99.9%), distilled water (H₂O), phosphate buffer solution (PBS) at pH 6.86 containing 25 mM KH₂PO₄, 25 mM Na₂HPO₄, and about 0.1% NaN₃, 30% H₂O₂ aqueous solution, and N, N-dimethyl-4-nitrosoaniline (DMNA, SCHEME 2) were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. All chemicals were used without further purification.

2.2. Preparation of Fe(III)-treated hydroxyapatites

According to the previous papers,^{1, 30} Fe(III)-treated hydroxyapatites were synthesized as follows. To 0.50 g of hydroxyapatite (HAP-300 or HAP-400) with a stirrer tip in a 200 mL eggplant-shaped flask was added 100 mL of 25 mM aqueous solution of FeCl₃. The mixture was shaken at 25°C for 4 h in a concussive water incubator, M-100N, (TAITEC Co., Ltd. Saitama, Japan), and centrifuged at 3000 rpm with a centrifuge, Model B (Shimadzu Co., Kyoto, Japan) to recover product material. This material was washed three times with H₂O and dried at 150°C overnight in a drying oven, DZ-54 (Yamato Scientific Co., Ltd., Tokyo, Japan) to obtain Fe(III)-treated hydroxyapatites, HAP-300-Fe (Ca 6.27%, P 15.8%, Fe 28.15%, (Ca+Fe)/P = 1.29) and HAP-400-Fe (Ca 4.39%, P 17.76%, Fe 28.51%, (Ca+Fe)/P = 1.08). These Fe-treated apatites contained 5.0 mmol/g of Fe. Chlorine element was not detected in the Fe(III)-treated apatites. As shown in FIGURE 1 and 2, IR spectra and X-ray diffraction patterns of the Fe-treated apatites were in good accordance with the previous reports.^{1, 30} Specific surface areas and total pore volumes of the Fe-treated apatites were analyzed by BET method with a high speed gas sorption analyzer Nova 1000, type N10-12 (Yuasa Ionics Co., Ltd., Japan, or Malvern Instruments Ltd., UK), and were summarized in TABLE 3. As an alternate, low Fe(III)-loaded

hydroxyapatites containing 1.0 mmol Fe/g and 2.5 mmol Fe/g were prepared by the similar method, in which 5.0 mM and 12.5 mM FeCl_3 aqueous solutions were used, respectively. XRD patterns of those Fe apatites were shown in FIGURE 2, where it was confirmed that Fe(III)-treated hydroxyapatites containing 5.0 mmol Fe/g, HAP-400-Fe, eventually had amorphous FePO_4 phases (FIGURE 2c).³⁰

FIGURE 1 IR spectrum of HAP-400-Fe.^{1, 30} A, surface OH stretching; B, internal OH stretching; C, adsorbed H_2O bending; D, PO_4 stretching.

2.3. Photo-Fenton reaction

A typical reaction method was as follows. To 10 mg of Fe(III)-treated hydroxyapatite, HAP-300-Fe or HAP-400-Fe (2.5 mmol Fe/L), in a transparent glass bottle was added 10 mL of 20 mM H_2O_2 aqueous solution, followed by immediate addition of 10 mL of 46 μM dye (ARS, MO, alizarin, neutral red, methyl red, Congo red, or indigo carmine) aqueous solution, of which pH had been adjusted to 7.0. The actual reaction concentrations were 23 μM for dye and 10 mM for H_2O_2 . After the mixture bottle was tightly closed with a screw cap, it was placed and shaken at 25°C for 6h in the concussive water incubator under black light irradiation (370 mW/cm² at 365 nm). The resulting mixture was filtered via a PTFE membrane filter with 0.45 μm pores, DISMIC-13HP (ADVANTEC Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and the filtrate was analyzed with a UV-VIS spectrophotometer, U-2000, (Hitachi Ltd., Tokyo, JAPAN) equipped with a temperature controller, SDR-30, (KELK Ltd., Kanagawa, JAPAN) for elucidation of consumption of dye, and with a total organic carbon (TOC) analyzer, TOC-V_{CSN} (Shimadzu Co., Kyoto, Japan) for elucidation of mineralization of dye. In the UV-VIS analysis, the following wavelengths were

FIGURE 2 XRD patterns of Fe(III)-treated HAP-400 catalysts: (a) 1.0 mmol Fe/g; (b) 2.5 mmol Fe/g; (c) 5.0 mmol Fe/g (HAP-400-Fe). The peaks with asterisks are assigned to raw hydroxyapatite (HAP-400). X-ray source, Cr.

employed as $\lambda_{\max}$ values: 518 nm for ARS, 463 nm for MO, 518 nm for alizarin, 522 nm for neutral red, 435 nm for methyl red, 498 nm for Congo red, 610 nm for indigo carmine. As control tests, Fe_2O_3 , $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$, and FeOOH were used as catalysts (2.5 mmol Fe/L) in photo-Fenton reaction of dyes under the same condition.

2.4. Observation of Fe elution from Fe catalysts

After the photo-Fenton reaction of ARS using all Fe catalysts described above (Section 2.3), all the filtrates from reaction solutions were analyzed by an inductively coupled plasma atomic emission spectrometer (ICP), SPS3100 (Seiko Instruments Inc., Chiba, Japan) to observe Fe elution concentrations from the Fe catalysts.

2.5. Kinetic method

Kinetic investigation based on Michaelis-Menten theory³¹ for photo-Fenton reaction was carried out as follows. To Fe catalyst, HAP-300-Fe, HAP-400-Fe, or Fe_2O_3 (2.5 mmol Fe/L), in a transparent glass bottle was added 10 mL of 20 mM H_2O_2 aqueous solution, followed by immediate addition of 10 mL of 11.5~96 μM dye (ARS or MO) aqueous solution, of which pH had been adjusted to 7.0. The actual reaction concentrations were 5.6~46 μM for dye and 10 mM for H_2O_2 . After the mixture bottle was tightly closed with a screw cap, it was placed and shaken at 25°C in the concussive water incubator under black light irradiation (370 mW/cm^2 at 365 nm). The reaction times were 15 min for ARS and 4 h for MO. The resulting solution was filtered via the PTFE membrane filter with 0.45 μm pores, and the filtrate was analyzed with the UV-VIS spectrophotometer equipped with the temperature controller for elucidation of consumption of dye. From Lineweaver-Burk plotting³² (referred to FIGURE 9 and 10), the corresponding kinetic constants were evaluated (referred to TABLE 4). As a control test, an investigation using PBS as a solvent was also carried out.

Similar kinetic investigation for observation of hydroxyl radical generation under the photo-Fenton reaction condition was carried out, referred to Bors' method.³³ To an iron catalyst, HAP-400-Fe or Fe_2O_3 (2.5 mmol Fe/L), in a transparent glass bottle was added 10 mL of 20 mM H_2O_2 aqueous solution, followed by immediate addition of 10 mL of 5.0~80 μM DMNA aqueous solution. The actual reaction concentrations were 2.5~40 μM for dye and 10 mM for H_2O_2 . After the mixture bottle was tightly

closed with a screw cap, it was placed and shaken at 25°C for 1 h in the concussive water incubator under black light irradiation (370 mW/cm^2 at 365 nm). Similarly to the above kinetic method using dyes, consumption of DMNA was determined by UV-VIS spectroscopy ($\lambda_{\max}$ 440 nm), and kinetic constants were determined by Lineweaver-Burk plotting (referred to FIGURE 12 and TABLE 4).

FIGURE 3 Consumption, mineralization, and adsorption of ARS (23 μM) under photo-Fenton reaction condition (25°C, 6 h) with Fe(III)-treated HAP-400 catalysts containing 1.0, 2.5, and 5.0 (HAP-400-Fe) mmol Fe/g. The adsorption test was conducted without H_2O_2 and black light irradiation.

3. RESULTS AND DISCUSSION

3.1. Effect of Fe treatment of hydroxyapatite

In order to confirm effect of Fe(III) treatment of hydroxyapatite, photo-Fenton reaction of ARS was investigated at 25°C for 6 h with Fe(III)-treated HAP-400 catalysts, which have different Fe contents, 1.0, 2.5, and 5.0 mmol Fe/g. FIGURE 3 reveals rates of consumption and mineralization of ARS: Consumption means conversion of ARS, and mineralization means ultimate oxidation to CO_2 and H_2O . In addition, ARS adsorption rate under the similar reaction condition without H_2O_2 and black light irradiation was shown in FIGURE 3. In the case of photo-Fenton reaction with a raw apatite, HAP-400 (0 mmol Fe/g), ARS was consumed over 50%. Judging from adsorption result, the consumption rate is mostly attributed to adsorption to HAP-400 and slightly to H_2O_2 oxidation activated by

black light irradiation. The mineralization rate by using HAP-400 is truthfully due to adsorption removal. Photo-Fenton reaction with the Fe(III)-treated HAP-400 catalysts containing 1.0 and 2.5 mmol Fe/g resulted in increase in ARS consumption, which was obviously depended on Fe contents. However, adsorption of ARS using those Fe(III)-treated HAP-400 catalysts resulted in adsorption quantities resembling in the case of HAP-400. We confirmed that the Fe treatment apparently afforded catalytic activity to hydroxyapatite for photo-Fenton reaction. Further increase in Fe content up to 5.0 mmol Fe/g, namely HAP-400-Fe, induced almost complete consumption of ARS and 60% mineralization (referred to TABLE 1 and 2). The mineralization value (60%) was apparently less than the adsorption rate by using HAP-400-Fe, this can be explained by the following reason. The adsorption investigation was conducted in the absence of H_2O_2 , and ARS must be instantly adsorbed on the apatic catalysts. Actually, after the investigation, HAP-400-Fe was stained. On the other hand, photo-Fenton reaction investigation was conducted in the presence of H_2O_2 , and competitive adsorption of both ARS and H_2O_2 molecules onto HAP-400-Fe possibly occurs. Therefore, adsorbability of ARS during photo-Fenton reaction must be depressed. Actually, after the reaction, HAP-400-Fe was hardly stained. In addition, adsorption of H_2O_2 on HAP-400-Fe in the absence of dyes was truly observed, as shown in FIGURE 4 (white circles, “HAP-400-Fe (no light)”). The concentration of H_2O_2 , 10 mM, at the present photo-Fenton reaction was much larger than that of ARS, 23 μM , and adsorbability of ARS must have been further depressed during the reaction. Therefore, the mineralization rate at 60% by using HAP-400-Fe was almost caused by ultimate oxidation to CO_2 and H_2O .

3.2. Difference in hydroxyapatite

As an alternative Fe(III)-treated hydroxyapatite, HAP-300-Fe (5.0 mmol Fe/g) was prepared from hydroxyapatite HAP-300 as a stoichiometric apatite under the same as the preparation condition of HAP-400-Fe. In order to compare catalytic activities between HAP-300-Fe and HAP-400-Fe, photo-Fenton reaction of ARS was investigated at 25°C for 2 h by using those Fe(III)-apatic catalysts. The result was shown by time-dependent consumption curves in FIGURE 5, where filled circles on a solid line are for HAP-400-Fe and filled squares on a solid line are for HAP-300-Fe. As a control test (“no light”), photo-Fenton reaction

FIGURE 4 Degradation of H_2O_2 in the presence of Fe catalysts under black light irradiation.

FIGURE 5 Photo-Fenton reaction of ARS with HAP-300-Fe and HAP-400-Fe.

TABLE 1 Photo-Fenton reaction of dyes for 6 h at 25°C with HAP-400-Fe^(a)

Substrate ^(b)	Photo-Fenton		Self-degradation (%) ^{(c), (e)}	Adsorption (%) ^{(c), (f)}
	Consumption (%) ^(c)	Mineralization (%) ^(d)		
Alizarin red S (ARS)	99	60	<1	83
Alizarin ^(g)	98	59	6	96
Neutral red	55	27	37	<1
Methyl red	42	2	<1	43
Methyl orange (MO)	14	<1	<1	<1
Congo red	89	60	<1	99
Indigo carmine	>99	<1	<1	<1

(a) ARS, 23μM; H₂O₂, 10 mM; HAP-400-Fe, 0.5 g/L. (b) referred to SCHEME 1. (c) determined by UV-VIS. (d) determined by TOC. (e) tested in the absence of H₂O₂. (f) tested in the absence of H₂O₂ without black light irradiation. (g) tested at pH 10.

without black light irradiation was conducted, and similar time-dependent curves were plotted as follows: blank circles on a solid line are for HAP-400-Fe; blank squares on a solid line are for HAP-300-Fe. Time-adsorption curves under the similar reaction condition without H₂O₂ and black light irradiation were also plotted in FIGURE 5, where blank circles on a dashed line are for HAP-400-Fe and blank squares on a dashed line are for HAP-300-Fe. Obviously, HAP-400-Fe indicated much faster ARS consumption rate than HAP-300-Fe, especially within 1 hr. According to the previous paper,¹ adsorption capacity of HAP-400-Fe for ARS at an adsorption equilibrium stage (25°C, 30 hr), 66 μmol/g, was inferior to that of HAP-300-Fe, 91 μmol/g. We recognized that the present ARS consumption rates are subjected to adsorption velocity rather than adsorption capacity, or to kinetic control rather than thermodynamic control. The reactions under no black light irradiation (blank circles and squares) must have involved both adsorption and Fenton reaction; the latter is derived by Fe catalysis of H₂O₂ without light irradiation.^{34, 35} Although both HAP-300-Fe and HAP-400-Fe exhibited a slight possibility of catalysis for Fenton reaction, the ARS consumptions were inferior to the cases of photo-Fenton reactions. Rate difference between photo-Fenton (filled circles and squares) and Fenton (blank circles and squares) reactions in HAP-400-Fe was smaller than that in HAP-300-Fe. However, we evaluated that HAP-400-Fe exhibiting higher ARS consumption rate is more useful than HAP-300-Fe from viewpoint of efficient and fast

decoloration.

3.3. Photo-Fenton reaction of miscellaneous dyes

Photo-Fenton reaction of other dyes, namely alizarin, neutral red, methyl red, methyl orange (MO), Congo red, and indigo carmine, as shown in SCHEME 1, was investigated at 25°C for 6 h by using HAP-400-Fe. Although pH values of the reaction solutions were consistently adjusted to 7.0, the only case of alizarin was carried out at pH 10 because of improving its solubility in water. The reaction result was summarized in TABLE 1, where “self-degradation” means evaluation of direct effect of black light irradiation to a substrate without H₂O₂, and “adsorption” means adsorption rate of a substrate under the similar reaction condition without black light irradiation and H₂O₂. ARS and alizarin, of which structures resemble each other (SCHEME 1), were consumed almost completely and mineralized more than 58%. Similarly, Congo red was consumed at 89% and mineralized at 60%. These three dyes indicating high consumptions exhibited high adsorptions over 80%. Indigo carmine was completely consumed but never mineralized, indicating no adsorption (<1%). Neutral red and methyl red were consumed at approximately half: The former indicated the most self-degradation rate and no adsorption, and the latter indicated a slight mineralization and moderate adsorption. MO indicated the lowest consumption at 14% and no

TABLE 2 Effects of Fe catalyst and reaction time to consumption and mineralization in photo-Fenton reaction of ARS at 25°C.

Catalyst	Amount (mmol Fe/L)	Time (h)	Consumption (%) ^(a)	Mineralization (%) ^(b)
HAP-400-Fe	2.5	6	99	60
	2.5	20	99	72
	5.0	20	99	75
Fe ₂ O ₃	2.5	6	97	25
FePO ₄ •2H ₂ O	2.5	6	82	23
FeOOH	2.5	6	31	1

(c) determined by UV-VIS. (d) determined by TOC.

mineralization. Overviewing relationship between consumption and adsorption rates, it was considered that a substrate having high adsorbability on HAP-400-Fe is apt to indicate high consumption and/or mineralization.

3.4. Comparison with other Fe catalysts

For evaluation of HAP-400-Fe as a catalyst, photo-Fenton reaction of ARS was investigated by using other Fe catalysts, Fe₂O₃, FePO₄•2H₂O, and FeOOH, under a definitive catalyst amount condition as follows: Fe mole number of each catalyst in a reaction solution was unified into 2.5 mmol Fe/L. The resulting time-dependent consumption curves within 2 h were shown in FIGURE 6. The order of apparent consumption rates was regarded as HAP-400-Fe > Fe₂O₃ > FePO₄•2H₂O > FeOOH. This implies that HAP-400-Fe having amorphous FePO₄ on the surface³⁰ has much higher catalytic activity than FePO₄•2H₂O, or FePO₄ itself. The case of no catalyst affected a slight consumption, implying that Fe catalysis should be needed in the present condition. Prolonging reaction time up to 6 h, ARS consumptions were obviously improved in the all Fe catalysts, as shown in TABLE 2. However, mineralization rates in Fe₂O₃, FePO₄•2H₂O, and FeOOH never exceeded the rate in HAP-400-Fe. After the reactions, all solution filtrates were analyzed by ICP to confirm whether Fe ion was eluted or not. All Fe concentrations in the filtrates were found to be under detection limit (< 0.1 ppm), being regarded as no elution. Consequently, we estimated that HAP-400-Fe featured the most

efficient catalysis as a heterogeneous catalyst at least in the reaction of ARS.

FIGURE 6 Time-consumption curves in photo-Fenton reaction of ARS with HAP-400-Fe, Fe₂O₃, FePO₄•2H₂O, FeOOH (2.5 mmol Fe/L).

The difference in effects of the Fe catalysts to ARS consumptions and mineralizations (TABLE 2) seems to be concerned with those surface properties

and/or catalysis mechanisms. FIGURE 7 shows pore distributions of the Fe catalysts, and TABLE 3 summarizes the corresponding specific surface areas and total pore volumes. Obviously, HAP-300-Fe, HAP-400-Fe, and FeOOH bear numerous pores between 10 and 350 Å, or between 1 and 35 nm (FIGURE 7). Simultaneously, they have relatively large total pore volumes and specific surface areas, compared with Fe₂O₃ and FePO₄•2H₂O (TABLE 3). However, those surface areas do not necessarily reflect the corresponding catalytic activities, or degrees of consumption and mineralization. For example, FeOOH, which has the second largest surface area, indicated the lowest ARS consumption rate, and Fe₂O₃, which has the smallest specific surface area and total pore volume, indicated the second fastest consumption rate among the Fe catalysts (FIGURE 6). Adsorption of ARS on Fe₂O₃, FePO₄•2H₂O, and FeOOH was investigated at 25°C for 2 h, but no adsorption on these catalysts was observed. Furthermore, FIGURE 8 indicates kinetic superiority of Fe₂O₃ rather than HAP-400-Fe in photo-Fenton reaction of MO in spite of lower adsorption rate for Fe₂O₃. Consequently, degradation rates of dyes are not always dependent on adsorbabilities of dyes on the present Fe catalysts. We considered that a more detailed kinetics study should be needed in the present photo-Fenton reaction.

FIGURE 7 Pore diameter distribution of HAP-300-Fe, HAP-400-Fe, Fe₂O₃, FePO₄•2H₂O, and FeOOH by a N₂-adsorption method.

FIGURE 8 Time-consumption curves in photo-Fenton reaction of MO with HAP-400-Fe and Fe₂O₃.

TABLE 3 Specific surface areas and total pore volumes of Fe catalysts.

Catalyst	Specific surface area (m ² /g) ^(a)	Total pore volume (cm ³ /g) ^(b)
HAP-300-Fe	170 ^(c)	0.791
HAP-400-Fe	145 ^(c)	0.796
Fe ₂ O ₃	6.38 ^(d)	0.021
FePO ₄ •2H ₂ O	29.7 ^(d)	0.200
FeOOH	169 ^(d)	0.221

(a) BET method. (b) evaluated from FIGURE 7. (c) reference 36. (d) the present data.

3.5. Kinetics based on Michaelis-Menten theory

For a kinetics study, Michaelis-Menten theory³¹ (Eq. 1) is often employed to elucidate enzyme catalysis such as catalytic activity and affinity to a

substrate. In Eq. 1, [S], [E], [ES], and [P] denote concentrations of substrate, enzyme, enzyme-substrate complex, and product, respectively. In the present study, the abbreviations “E” and “ES” can be translated into a Fe catalyst and a catalyst-substrate complex, respectively. The coefficient k_{cat} denotes a kinetic constant of product generation, or turn over number (TON), which means an index of catalytic activity.

Based on the above theory, Eq. 2 is derived and well-known as Michaelis-Menten equation: v , initial velocity; V_{max} , maximum velocity; K_M , Michaelis constant. The initial velocity v increases proportionally with increase in the substrate concentration [S] at an early stage, or relatively low [S] value. However, if [S] becomes considerably large, v turns out to approach to a constant and maximum value, namely V_{max} . The Michaelis constant K_M implies degree of affinity of an enzyme to a substrate; the lower K_M value is regarded as the stronger affinity of the enzyme. Similarly in the present case, K_M can be translated into an affinity constant for a Fe catalyst.

$$v = \frac{V_{\text{max}} [S]}{K_M + [S]} \quad (\text{Eq. 2})$$

If reaction velocity is accomplished to V_{max} , the following equation, Eq. 3, comes into effect: $[E]_0$, initial concentration of enzyme, or that of Fe catalyst, followed by evaluation of k_{cat} .

$$V_{\text{max}} = k_{\text{cat}} [E]_0 \quad (\text{Eq. 3})$$

The Michaelis-Menten equation (Eq. 2) is very useful for kinetic elucidation of almost catalytic reactions, but plotting between [S] (x-axis) and v (y-axis) always features an upward convex curve, which is hardly used for actual evaluation of the constants V_{max} and K_M . Alternatively, as shown in Eq. 4, Lineweaver-Burk equation,³² which is derived by reciprocal transformation from Eq. 2, is useful for instant evaluation of those constants. Plotting a straight line with $1/[S]$ (x-axis) and $1/v$ (y-axis) can give K_M and V_{max} values from a slope and an intercept on the plotting line, respectively.

$$\frac{1}{v} = \frac{K_M}{V_{\text{max}}} \times \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \quad (\text{Eq. 4})$$

FIGURE 9 reveals Lineweaver-Burk plots for the present photo-Fenton reaction of ARS in water by using HAP-300-Fe, HAP-400-Fe, and Fe_2O_3 (solid lines) and in phosphate buffer solution (PBS) by using HAP-400-Fe (a hashed line). In addition, FIGURE 10 indicates similar plots for MO in water by using HAP-400-Fe and Fe_2O_3 . The constants, V_{max} , K_M , and k_{cat} , were evaluated from those FIGURES and summarized in TABLE 4. Subsequently, V_{max}/K_M values were evaluated and sited in TABLE 4. The V_{max}/K_M is from Eq. 5, which is derived from Eq. 2 in the case of $K_M \gg [S]$, and probably regarded as an apparent first-order degradation rate constant, or interpreted as another index of catalytic activity,^{37, 38} similarly to k_{cat} .

$$v \cong \frac{V_{\text{max}}}{K_M} \times [S] \quad (K_M \gg [S]) \quad (\text{Eq. 5})$$

FIGURE 9 Lineweaver-Burk plots in photo-Fenton reaction of ARS with HAP-300-Fe, HAP-400-Fe, and Fe_2O_3 in water (solid line) or PBS (hashed line).

FIGURE 10 Lineweaver-Burk plots in photo-Fenton reaction of MO with HAP-400-Fe and Fe₂O₃ in water.

In photo-Fenton reaction of ARS in water, HAP-400-Fe indicated the largest $V_{\max}$, k_{cat} , and $V_{\max}/K_M$ values (TABLE 4). Among the Fe catalysts employed, HAP-400-Fe apparently exhibited the most efficient catalysis for ARS. However, K_M for HAP-400-Fe indicated the largest value, meaning the poorest affinity to ARS. As reported previously,¹ the order of ARS adsorption capacity of Fe(III)-treated hydroxyapatites was HAP-300-Fe > HAP-400-Fe, but both of them have much larger adsorption capacities than Fe₂O₃, as described above (see in Section 3.4). Consequently, we considered that HAP-400-Fe has relatively large adsorption capacity to bear a substrate, but features weaker affinity to ARS than HAP-300-Fe and Fe₂O₃. Changing the reaction solvent from water to PBS in photo-Fenton reaction of ARS with HAP-400-Fe, $V_{\max}$ and K_M values were lowered to less than half. The decrease in $V_{\max}$ is possibly due to kinetically competitive interaction of ARS and solvent molecules with HAP-400-Fe surface, or inhibition of interaction of ARS molecules with Fe sites by phosphate ions (PO_4^{3-} , HPO_4^{2-} , H_2PO_4^-) from PBS solvent.^{39, 40} The other decrease in K_M or improvement of affinity is possibly due to interaction or adsorption mechanism between ARS and HAP-400-Fe. As described above (see in INTRODUCTION),¹ adsorption mechanism of ARS on HAP-400-Fe is defined as a chelete form, in which neighboring carbonyl (C-9) and phenolate (C-1) groups of ARS make a chelation to a Fe site of HAP-400-Fe. The chelation form intensified ARS adsorption capacity of HAP-400-Fe from 66 μmol/g in water to 101 μmol/g in PBS. Consequently, the decrease in K_M value or increase in affinity is attributed to the increase in adsorption capacity. On

the other hand, photo-Fenton reaction of MO in water resulted in a different feature. Although adsorption rate of Fe₂O₃ was lower than that of HAP-400-Fe (FIGURE 8), Fe₂O₃ indicated a superior catalytic property, or larger $V_{\max}$, k_{cat} , and $V_{\max}/K_M$ values than those of HAP-400-Fe (TABLE 4). In addition, the K_M value of Fe₂O₃ was lower than that of HAP-400-Fe, meaning that Fe₂O₃ has higher affinity to MO than HAP-400-Fe. This result implies that adsorption affinity rather than adsorption amount possibly causes exhibition of efficient catalysis for photo-Fenton reaction of MO.

SCHEME 2 Reaction of DMNA with hydroxyl radical •OH.

As an alternative viewpoint of catalysis, we considered that generation rate of hydroxyl radical •OH as an active oxygen species from H₂O₂ is an important factor for progress of the present reaction. A convenient and indirect method³³ using a nitroso compound, N, N-dimethyl-4-nitrosoaniline (DMNA), was employed for detection of •OH. DMNA is exclusively sensitive to react with •OH, involving oxidation of the original nitroso group into nitro group, as shown in SCHEME 2. Therefore, DMNA was investigated under the similar photo-Fenton reaction condition to evaluate •OH generation ability of the present Fe catalysts. The result of this investigation was shown as time-dependent DMNA consumption plotting in FIGURE 11. Among the Fe catalysts, HAP-400-Fe apparently indicated the fastest rate, but its consumption includes adsorption rate at approximately 10% (white circles on a dashed line). Fe₂O₃ indicated the second fastest consumption rate. Fe₂O₃ possibly makes catalysis in almost exclusive photo-Fenton reaction, since it indicated no adsorbability to DMNA (no plot in FIGURE 11). FePO₄•2H₂O indicated a little more superior rate than the case of no catalyst (“none”). The rate for FeOOH was less than the case of no

TABLE 4 Michaelis-Menten constants^(a) for Fe catalysts in photo-Fenton reaction of dyes and DMNA at 25°C.

Substrate ^(b)	Catalyst ^(c)	Solvent ^(d)	$V_{\max}$ ($\mu\text{M}/\text{min}$)	K_M (μM)	k_{cat} [$\times 10^{-6}$] (s^{-1})	$V_{\max}/K_M$ [$\times 10^{-4}$] (s^{-1})
ARS	HAP-300-Fe	water	0.23	5.26	1.53	7.29
	HAP-400-Fe	water	2.39	40.98	15.9	9.72
	HAP-400-Fe	PBS	0.64	16.64	4.28	6.41
	Fe_2O_3	water	0.67	13.11	4.49	8.52
MO	HAP-400-Fe	water	0.013	20.68	0.0889	0.105
	Fe_2O_3	water	0.019	7.28	0.132	0.435
DMNA	HAP-400-Fe	water	0.15	17.41	1.01	1.44
	Fe_2O_3	water	0.052	4.32	0.345	2.00

(a) equation 2~5. (b) ARS and MO, 5.8~46 μM ; DMNA, 2.5~40 μM . (c) 2.5 mmol Fe/L. (d) adjusted to pH 7.0 in water or to pH 6.8 in PBS.

catalyst, implying the weakest $\bullet\text{OH}$ generation ability of FeOOH . Interestingly, the order of time-dependent DMNA consumption, $\text{HAP-400-Fe} > \text{Fe}_2\text{O}_3 > \text{Fe}_2\text{O}_3 \cdot 2\text{H}_2\text{O} > \text{FeOOH}$, was found to be consistent with that of time-dependent ARS consumption (FIGURE 6). Further, kinetic investigation using DMNA based on Michaelis-Menten theory was conducted to elucidate more detailed catalysis concerned with $\bullet\text{OH}$ generation. Similarly to the case of ARS (FIGURE 9) and MO (FIGURE 10), Lineweaver-Burk plotting was carried out in the reaction of DMNA as shown in FIGURE 12, followed by evaluation of the constants, $V_{\max}$, K_M , k_{cat} , and $V_{\max}/K_M$ as summarized in TABLE 4. The values of $V_{\max}$, K_M , and k_{cat} for HAP-400-Fe were larger than those for Fe_2O_3 , and this tendency is same as the case of ARS. Nevertheless, $V_{\max}/K_M$ value of HAP-400-Fe was lower than that of Fe_2O_3 , implying that apparent first order rate of DMNA consumption rate, or $\bullet\text{OH}$ generation rate for Fe_2O_3 was faster than that for HAP-400-Fe. FIGURE 4 also indicates time-consumption curves in investigation of degradation of H_2O_2 to H_2O and O_2 under black light irradiation by using HAP-400-Fe and Fe_2O_3 , and afforded a definitive proof of superior H_2O_2 degradation rate in Fe_2O_3 , compared with that in HAP-400-Fe. In addition, difference in rates between black light irradiation (filled square) and no irradiation (blank square) by using Fe_2O_3 was much larger than that by using HAP-400-Fe. Since the rate for no irradiation by using Fe_2O_3 was rarely different from that for a blank test (“none”), it is

FIGURE 11 Time-consumption curves in reaction of DMNA under a similar photo-Fenton reaction condition (25°C, 6 h). The expression “none” means the condition without Fe catalyst, and “no light” means without black light irradiation.

translated that Fe_2O_3 can catalyze decomposition of H_2O_2 into $\bullet\text{OH}$ efficiently. On the other hand, H_2O_2 is possibly easy to be adsorbed on HAP-400-Fe rather than Fe_2O_3 . Therefore, catalytic ability of H_2O_2 decomposition into $\bullet\text{OH}$ by Fe_2O_3 is considered to be higher than that by HAP-400-Fe. However, over-viewing whole catalytic behaviors, HAP-400-Fe is regarded as an excellent catalyst since it indicated large pore volume (TABLE 3), relatively high k_{cat} values (TABLE 4), and higher mineralization ability in photo-Fenton reaction of ARS than the other Fe catalysts (TABLE 2).

FIGURE 12 Lineweaver-Burk plots in photo-Fenton reaction of DMNA with HAP-400-Fe and Fe_2O_3 in water.

3.6. Catalysis mechanism

Judging from the kinetic results, the present Fe catalysts simultaneously play two significant roles in photo-Fenton reaction: One is catalysis of hydroxyl radical $\bullet\text{OH}$ generation, and the other is degradation (oxidation) of a dye. Adsorbability of dyes or H_2O_2 on the Fe catalysts also must be deeply concerned with the two roles. Therefore, the following catalysis mechanism, referred to the report of Tao et al.,³ was raised as shown in SCHEME 3. The courses (a) to (d) represent H_2O_2 degradation catalysis or $\bullet\text{OH}$ generation routs, and the other courses (e) and (f) represent degradation catalysis of dyes or substrates. The course (a) indicates that a Fe site interacts with a H_2O_2 molecule to give a catalyst- H_2O_2 complex constructed by coordinate bond, involving equilibrium. The catalyst- H_2O_2 complex is converted to a peroxide surface structure Fe-OOH in the next course (b). Under black light

irradiation, the subsequent photolysis of Fe-OOH instantly occurs as shown in the course (c) to afford a hydroxyl radical $\bullet\text{OH}$ and an unstable super-oxidized structure Fe(IV)=O ,^{3, 41} namely ferryl state iron, on catalyst surface. There are some reports^{6, 11, 12} on an alternative reductive mechanism, which involves reduction of Fe(III) to Fe(II) by treatment of H_2O_2 . However, the present oxidative mechanism from Fe(III) to Fe(IV) state is considered to be reasonable for the present photo-Fenton reaction, judging from other preceding reports,^{42,43} in which Fe(III) -porphyrin complex catalysts can be oxidized to Fe(IV) states by H_2O_2 . We believe that this Fe(IV) producing theory should be applied to the cases using tightly Fe(III) -immobilized catalysts, or similarly to the present heterogeneous Fe catalysts. Especially in HAP-300-Fe and HAP-400-Fe, the Fe sites were immobilized on the amorphous FePO_4 -like surfaces.^{1, 30} The unstable Fe(IV)=O structure is easily subjected to water attack to give the original Fe(III) state and another $\bullet\text{OH}$ as shown in the course (d). Regardless of any catalyst employed, both reaction rates in the course (c) and (d) are probably very fast. Therefore, we considered that total $\bullet\text{OH}$ generation rate is controlled in the course (b) rather than the course (a). HAP-400-Fe can make many H_2O_2 -catalyst complexes in its large pores in the course (a). The catalyst- H_2O_2 complex in HAP-400-Fe are slowly converted to Fe-OOH state in the course (b) for the sake of low affinity to H_2O_2 (the large K_M value for DMNA, TABLE 4), followed by slow generation of $\bullet\text{OH}$ in the course (c) and (d). Fe_2O_3 has small capacity for bearing the complexes in the course (a). Nevertheless, it efficiently and quickly generates Fe-OOH states in the course (b) and $\bullet\text{OH}$ species in the next courses because of its high affinity to H_2O_2 .

TABLE 5 Recycling test of HAP-400-Fe in photo-Fenton reaction of ARS for 6h at 25°C.^(a)

Time(s)	Consumption (%) ^(b)	Mineralization (%) ^(c)
1st	99	60
2nd	99	54
3rd	99	54
4th	99	50
5th	99	50

(a) ARS, 23 μM ; H_2O_2 , 10 mM; HAP-400-Fe, 0.5 g/L. (b) determined by UV-VIS. (c) determined by TOC.

*Complexed (e) or non-complexed substrate

SCHEME 3 A possible catalysis mechanism in the present photo-Fenton reaction.

From a viewpoint of catalyst-substrate complex as shown in the course (e), HAP-400-Fe has a possibility to produce large amount of the catalyst-substrate complexes in equilibrium, due to its large pore volume (TABLE 3) and adsorption capacity,¹ similarly to the case of H₂O₂ mentioned above. On the other hand, Fe₂O₃ affords small amount of the complexes because of its small pore volume and adsorption capacity. Subsequently, a substrate is subjected to attacks of •OH species, followed by degradation and mineralization, regardless of whether the substrate is complexed with a Fe site or not, as shown in the course (f). HAP-400-Fe can bear many complexed substrates to induce effective •OH attacks within its pores. Consequently, its $V_{\max}$ and k_{cat} values for ARS were larger than those of Fe₂O₃ (TABLE 4). In addition, further and repeated attacks of •OH species are essential for effective mineralization of oxidized or degraded substrates into CO₂ and H₂O in Fe catalyst pores. The larger pore volume should enable the higher mineralization in spite of slow •OH generation. Actually, HAP-400-Fe with relatively huge pore volume indicated the most effective mineralization of ARS among the Fe catalysts, and extension of reaction time resulted in increase in ARS mineralization (TABLE 2). On the other hand, Fe₂O₃ can fast generate •OH, and indicate the higher degradation rate (FIGURE 8 and TABLE 4). However, small pore volume of Fe₂O₃ afforded the lower ARS mineralization ability (TABLE 2). Using twice amount of HAP-400-Fe resulted in moderate improvement of ARS mineralization, as shown in TABLE 2. We had supposed that increase

in catalyst amount would improve generation and attack frequency of •OH species to accelerate initial oxidation of ARS. Contrary to the supposition, the increase in catalyst amount might cause lowering concentration of degraded products existing in each pore pocket to decelerate mineralization rate. Furthermore, HAP-400-Fe exhibited good recycling utility as shown in TABLE 5. The catalytic activity was hardly deteriorated: Five times repeating uses never invalidated complete consumption of ARS, and made marginal decrease in mineralization (down to 50%). We found that HAP-400-Fe repeatedly works in the catalysis mechanism mentioned in SCHEME 3. From a comprehensive viewpoint, we considered that HAP-400-Fe is an excellent heterogeneous catalyst for photo-Fenton reaction.

4. CONCLUSION

The Fe(III)-treated hydroxyapatite, HAP-400-Fe, indicated efficient catalytic activity for photo-Fenton reaction. Although rate or generation ability of •OH species of HAP-400-Fe is lower than Fe₂O₃, residence time of substrate and H₂O₂ molecules in HAP-400-Fe pores is longer than that time in Fe₂O₃ pores because of high adsorption capacity of HAP-400-Fe. Organic materials in natural waters such as humic substances⁴⁴ and agrichemicals may be also degraded by using the present photo-Fenton reaction system. In addition, from catalysis of HAP-400-Fe even in PBS, degradation of biomolecules such as proteins and nucleic acids may be accomplished under physiologically suitable conditions existing phosphate ions. In a future, application of Fe(III)-treated hydroxyapatite to water purification system is expected as a high value-added catalyst for photo-Fenton reaction because of its facile repeating availability.

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