Evaluation of the Effect of Humic Acids on the Reductive Elution of Fe from Fe$_2$O$_3$ in a Saline, Seawater-like Medium

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A fertilizer composed of steelmaking slag and compost, which would be expected to supply dissolved Fe to the sea, was evaluated for use in a seaweed-bed restoration technique. The Fe species in steelmaking slag is present in the form of insoluble Fe(III)-oxides, but could be eluted by seawater via reducing reactions. Humic acid (HA) would contribute to such a reductive Fe elution because it can act as an electron donor/acceptor. In this study, the effect of HAs with added ascorbic acid (ASC) on the reductive elution of Fe from Fe$_2$O$_3$ into a saline medium (pH 8, I = 0.7 as NaCl) was evaluated by a laboratory-based method. After a 3-day incubation, approximately 3 μM of Fe(II) was eluted in the presence of 5 mM of ASC. The effect of reductive Fe elution was clearly enhanced by the co-presence of low concentrations of HA, due to the electron shuttling function of HA. When 5 mM ASC was used in conjunction with 5 mg of L$^{-1}$ HA, which was derived from hard-wood bark compost, about 5 μM of Fe(II) was eluted. The function was attributed to the structural features of HAs, polyaromatic compounds, N-content and highly substituted aromatic compounds, all of which could enhance the reductive Fe elution process. The present study also provided a simple method to evaluate the efficiency of HA on the reductive Fe elution.

KEY WORDS: steelmaking slag; restoration of seaweed beds; Fe elution; hematite; humic acid.

1. Introduction

It is well known that Fe is an essential nutrient for organisms. Although Fe is the fourth most abundant element in the earth, seawater contains only limited amounts of Fe because Fe is one of the least soluble metals in oxygenated and alkaline water. As brown macroalgal reproductive growth is regulated by dissolved Fe, their life cycle and biomass hence could be easily limited by a lack of dissolved Fe. Seaweed bed depletion is regarded as one of the serious coastal problems, in particular, along the coast of Japan. To clarify the role of Fe on brown macroalgal growth, the restoration of seaweed beds has been examined by supplying Fe using a fertilizer composed of a mixture of steelmaking slag and compost. An increase in dissolved Fe concentration and the restoration of a seaweed bed were observed after applying such a fertilizer to a barren coastal area. Previous studies reported that compost could supply dissolved organic matter, which can bind dissolved Fe and improve the bioavailability of Fe to brown alga. While the dissolution of Fe and other minerals from steelmaking slag in seawater were investigated, the mechanism responsible for the elution of Fe has not been well-clarified.

Fe(III)-oxides, such as hematite (Fe$_2$O$_3$) and magnetite, are found on the surface of steelmaking slag. Fe(III)-oxides have to be reduced to Fe(II) species in order to be soluble form in seawater. It is known that the microbial reduction of Fe under anaerobic conditions is a relevant mechanism that explains why soluble Fe(II) can be produced from insoluble Fe(III)-oxides. The previous study observed reductive Fe elution from Fe$_2$O$_3$ by an anaerobic bacterium, E. oxidotolerans, isolated from the fertilizer, and the reductive Fe elution was enhanced by co-existed antraquinone. Quinone moieties in humic substances would also play a role as an electron acceptor/donor in the natural sediment under aquatic condition. Attention has focused on the electron shuttle effect of humic substances which can accelerate the metal reductive reaction in sediments or aquatic environments. Labile organic substances, such as saccharides, could also contribute to the production of electrons by becoming a microbial energy source. A structural analysis of humic acid (HA) indicated that anaerobic bacteria are activated in the presence of a fertilizer composed of steelmaking slag and compost during a 6-month period of incubation in coastal seawater. Based on these findings, it is possible that humic substances can contribute to the elution of Fe, which will then serve as an activator of bacterial growth or promote electron shuttle from bacteria to steelmaking slag in the fertilizer. Although the efficiency of humic substances on Fe elution is an essential factor for improving the extent of restoration, the role of humic substances has not been elucidated. To better understand and development of the Fe supplying technique, the capability of HA in reductive Fe elution should be evalu-
ated as key function of HA.

The present study reports on the contribution of HA on the reductive elution of Fe into seawater-like conditions (pH 8, I = 0.7) carried out in laboratory-based experiments. The performance of eluting Fe from insoluble Fe source is a key mechanism in the Fe supplying technique. Fe₂O₃, an insoluble Fe(III)-oxide found on the surface of steelmaking slag, was employed as a model of an insoluble Fe source in the fertilizer.²⁻² Anaerobic bacteria should be employed as a reducing agents, however, specialized isolation and cultivation techniques are required when the bacteria used in the evaluation. Ascorbic acid can also perform a well-reducing agent in seawater condition.³ In this study, to provide the simple evaluation process, we used ascorbic acid as a model of reducing agent. The capability of HA to participate in this process would largely depend on the chemical structures. To investigate what the structural feature can contribute to the capability, HAs from three different origins were used. The relationship between the structural characteristics of HAs and the Fe elution efficiency was also investigated.

2. Experimental Methods

2.1. Materials and Chemicals

To avoid side reactions with HAs and reagents, Mg²⁺, Ca²⁺ and K⁺ were not used in the experiments. A saline medium, which contained 0.7 M NaCl, 0.05 M Tris-HCl and 100 μM 1,10-phenanthroline (o-Phen) in ultrapure water, was used as media for the Fe elution experiment. The concentration of NaCl was based on the known concentration and ionic strength (I = 0.7) of typical seawater. An 80% final volume of medium was prepared. The pH was adjusted to 8.00 ± 0.02, and the medium was then autoclaved (121°C, 20 min). To avoid any influence of bacteria, several drops of the preservative (1-chlorobutane:o-fluorotoluene:1, 2-dichloroethane:100) were added in a tightly closed glass bottle until used.

HAs were extracted from hardwood bark compost (Sanyo-Bark, Sanyo-Chip Industry, Japan), softwood bark compost (Daishizien, Green-Sogyo Co., Ltd., Japan) and peat powder (River-Import, Japan), and then purified according to IHSS procedures.¹⁸,¹⁹ Yields of HAs were evaluated by dividing the HA (g, dw) obtained in a slag, was employed as a model of an insoluble Fe source in the fertilizer.²⁻² Anaerobic bacteria should be employed as a reducing agents, however, specialized isolation and cultivation techniques are required when the bacteria used in the evaluation. Ascorbic acid can also perform a well-reducing agent in seawater condition.³ In this study, to provide the simple evaluation process, we used ascorbic acid as a model of reducing agent. The capability of HA to participate in this process would largely depend on the chemical structures. To investigate what the structural feature can contribute to the capability, HAs from three different origins were used. The relationship between the structural characteristics of HAs and the Fe elution efficiency was also investigated.

2.2. Analyses of Humic Acid Characteristics

The C, H, N and ash compositions of humic acids were determined by means of a CHN CORDER MT-5 (Yanako, Japan). The percent of O was estimated by subtracting the sum of the percent C, H, N and ash from 100%. The total acidity and carboxylic group content were determined by the Ba(OH)₂ and Ca(CH₃COO)₂ methods, respectively.²¹ The phenolic hydroxyl group content was calculated by subtracting the carboxylic group content from the total acidity. The absorbances of HAs prepared to 0.025 g L⁻¹ in 0.1 M phosphate buffer (pH 8.0) were measured by means of a V-630 spectrophotometer (JASCO, Japan). The absorbivities at 280 (ε₂₈₀) and 600 nm (ε₆₀₀) were evaluated by the following equation:²³

\[
\frac{\text{Absorbivity at 280 or 600 nm (cm}^{-1} \text{) \times HA (g L}^{-1} \text{)}}{\text{ε}} = \frac{\text{Absorbivity at 280 or 600 nm (cm}^{-1} \text{) \times HA (g L}^{-1} \text{)}}{\text{ε}}.
\]

E₂₈₀/E₆₀₀ ratio was calculated as the ratio of the absorbances at 254 and 220 nm, corresponding to the electron transfer band and the benzenoid band, respectively.²³ FT-IR spectra for the HAs were recorded by a FTIR/4100 spectrometer (JASCO, Japan), and the spectral bands were assigned according to previous reports.²⁰,²⁴,²₅

2.3. Fe Elution Assay

A 12.0–13.0 mg sample of Fe₂O₃ powder (Nacalai, Japan) was added to a 15-mL poly-centrifuge tube. 9.6 mL of the saline medium and the HA solution then added, following which, the volume was adjusted to 12 mL with ultrapure water. After adding 0–60 μL of the ASC solution, the sample was then shaken by means of a rotary incubator (AG, FINEPCR, Korea) for arbitrary incubation period in the dark at room temperature (20 ± 3°C). The sample was centrifuged (10 000 rpm, 10 min and 5°C) after the incubation, a 10 mL portion of the supernatant was then transferred to a fresh 15-mL centrifuge tube. Fe(II)-(o-Phen)₃, a divalent cation, accompanied by counterions such as ClO₄⁻, can be dissolved in an organic solvent, and has a large permittivity.²⁶,²⁷ A 1 mL aliquot of 1 M aqueous NaClO₄ was added to the transferred samples, following which the Fe(II)-(o-Phen)₃ complex was obtained by 3 extractions with 1 mL of chloroform each time.²⁶,²⁷ The resulting chloroform phase surface that was activated by the action of HAs was isolated by centrifugation (10 000 rpm, 3–5 min and 5°C).²⁰ To concentrate and adjust the volume of the sample, the solvent was removed under a stream of N₂ gas at 70–75°C, the dried sample was then dissolved in 1 mL of 50% EtOH. After centrifugation (3 000 rpm, 3 min), the Fe(II)-(o-Phen) concentration in the supernatant was determined based on the absorbance at 510 nm by means of a V-630 spectrophotometer (JASCO, Japan) using 1-cm pass length quartz microcuvette.

3. Results

3.1. Reductive Fe Elution with Ascorbic Acid

The Fe(III) in Fe₂O₃ is not soluble seawater, however, if the Fe(III) is reduced to Fe(II), the resulting ferrous iron species are soluble. ASC is a suitable reducing agent under seawater conditions.³ In our experimental condition, soluble Fe(II) species can be detected in the following equilibria:

\[
\text{ASC} \rightleftharpoons \text{AS Crab} \quad (1)
\]
ASCred + Fe(III) ⇌ ASC + Fe(II) \[\text{(2)}\]

Fe(II) + 3(o-Phen) ⇌ Fe(II) -(o-Phen) \[\text{(3)}\]

where ASCred represents the reduced form of ASC. Fe(III) can be reduced by the oxidation of ASCred, Fe(II) is then produced as a result (Eq. (2)). The eluted Fe(II) is immediately complexed with o-Phen, resulting in the Fe(II) being dissolved as colored complexes in the medium (Eq. (3)).

**Figure 1** shows the Fe elution behavior during a 3-day period. The eluted Fe was not detected in the absence of ASC. In the presence of 5 mM of ASC, the amount of Fe eluted was increased for the first 1-day incubation period. The amount of Fe eluted increased gently during 1–3 day of incubation. When the concentrations of ASC were 1 and 2 mM, only negligible amounts of Fe were eluted during a 3-day incubation. **Figure 2** shows the effect of reductive Fe elution with 0–5 mM of ASC after a 3-day incubation. When the concentration of ASC was under 3 mM, the amount of Fe eluted increased linearly with increasing the concentration of ASC. The proportion in the amount of Fe elution against the concentration of ASC was drastically enlarged when the concentration of ASC increased to more than 3 mM ASC. These results confirm that a sufficient amount of ASC can function as a reducing agent of Fe, and dissolved Fe can be supplied to seawater-like conditions from Fe₂O₃ via a reducing reaction. For this reason, an incubation period of 3 days and a concentration of ASC of 5 mM were used in the Fe elution assay.

### 3.2. Effect of Humic Acid on Fe Elution

**Figure 3** shows the effect of HA on the elution of Fe. All plots represent the values with the corresponding values in the absence of Fe₂O₃ being subtracted (Fig. 3). No reductive elution of Fe was observed in the absence of ASC (open plots in Fig. 3), suggesting that a reducing agent is needed to reduce the Fe in Fe₂O₃ even in the presence of HAs. The reductive Fe elution was observed when the presence of ASC, and the amount of Fe eluted in the presence of HAs was larger than that of the absence of HAs. The maximum
eluted Fe concentration was found to be under the condition of 5 mg L\(^{-1}\) HHA with ASC (ca. 5 \(\mu\)M-Fe(II)), however, the amount of Fe that was eluted drastically decreased with increasing HHA concentration (Fig. 3). In SHA and PHA, an increase in the concentration of eluted Fe was also observed in the low concentration of SHA (ca. 4.5 \(\mu\)M-Fe(II)) and PHA with ASC (ca. 4 \(\mu\)M-Fe(II)), and the Fe concentration decreased with increasing of HAs concentrations as well as the case of HHA.

### 3.3. Structural Features of HAs

The yields, elemental compositions, atomic ratios and acidic functional group contents of the HAs used in this study are summarized in **Table 1**. The yield of HHA was significantly higher than those of SHA and PHA, indicating that the hard-wood compost was a highly matured material. The N content and C/N ratio in the HHA were significantly larger and smaller, respectively, than those values in SHA and PHA. Source materials and humidity conditions influence the structural features of HAs. A large value of the C/N ratio for PHA may be associated with a decreased level of N-containing compounds in a peat source, which is mainly derived from grasses and algae. H/C and O/C ratios can be used to indicate the degree of saturation of C–C bonds and the degree of O-containing groups, respectively. There were no significant differences in the ratios in these HAs. SHA and PHA contained a high amount of acidic functional groups compared with that for the HHA.

UV-vis absorptivities are summarized in **Table 2**. Absorption at 600 nm is indicative of an increase in the extent of condensation and increasing molecular size, \(\varepsilon_{600}\) increases with proceeding the humification reaction. This parameter indicates that HHA was a highly condensed HAs compared with the SHA and PHA. Because the peat soil under anaerobic conditions, such as a bog, humification reactions in the environmental condition would be more limited than that for an aerobic composting process. The result of \(\varepsilon_{280}\) was consistent with the yields. The \(\varepsilon_{280}\) value is commonly used as an index of the degree of aromaticity of humic substances. The higher value of \(\varepsilon_{280}\) for the HHA indicates that it contains a higher degree of aromatic structures than SHA and PHA. Aromatization of carbohydrates could also occur during the enzymatic humification process. The higher value of \(\varepsilon_{280}\) for the HHA indicates that the hardwood bark compost was a highly humified material, consistent with the results of higher values for the yield and the \(\varepsilon_{600}\) values. An absorption at around 250 nm is designated an electron transfer band, and this absorption \(\varepsilon_{ET}\) increases with increasing substitution of O on the aromatic rings. A high \(\varepsilon_{ET}/\varepsilon_{280}\) ratio thus indicates the presence of O-containing functional groups on aromatic rings. This value for the HHA was smaller than those for the SHA and PHA, suggesting that the SHA and PHA contained many aromatic compounds that were substituted by O-containing functional groups. The result of \(\varepsilon_{ET}/\varepsilon_{280}\) can not only be attributed to acidic functional group content, but also attributed to aromaticity. The HHA recorded a higher value of \(\varepsilon_{280}\) and a lower value of \(\varepsilon_{ET}/\varepsilon_{280}\) while the H/C ratios for HAs were not significantly different. It could thus be concluded that the HHA sample contained large amounts of polyaromatic compounds.

**Figure 4** shows the FT-IR spectra of the HAs. The spectral bands were assigned as follows,\(^{20,24,25}\) broad bands at 3 400–300 cm\(^{-1}\) for O–H stretching of phenolic hydroxyl groups, and N–H stretching; the peak at 2 980 cm\(^{-1}\) for C–H stretching in the CH\(_3\) and CH\(_2\); the peaks at 2 920 and 2 850 cm\(^{-1}\) for aliphatic C–H stretching; the weak peak at 1 260 cm\(^{-1}\) for aromatic or ester C–O stretching; peaks at 1 720 cm\(^{-1}\) for COOH or ketone; peak at 1 640–1 620 cm\(^{-1}\) for aromatic or C=C stretching and C=O stretching of quinone or conjugated ketones; the peak or shoulder at 1 450 cm\(^{-1}\) for aliphatic C–H deformation; the peak at 1 400 cm\(^{-1}\) for O–H deformation or phenolic C–O stretching; the weak peak at 1 260 cm\(^{-1}\) for aromatic or ester C–O stretching; peaks at 1 080–1 025 cm\(^{-1}\) for C-O stretching of saccharides. Spectra for SHA and PHA are similar shape (Fig. 4). The strong peaks at 2 915 cm\(^{-1}\) and 2 850 cm\(^{-1}\) for PHA indicated that PHA contained more aromatic structural features than other HAs, consistent with a previous report.\(^{28}\) The intensity of the peaks at 1 080 cm\(^{-1}\) and 1 050 cm\(^{-1}\) are indicative of the presence of saccharides in the HHA. The large peak at 3 400 cm\(^{-1}\) for HHA could be attributed to large nitrogen content. The stronger peak at 1 630 cm\(^{-1}\) indicated that the HHA contained a higher

### Table 1. Yields, elemental compositions, atomic ratios and acidic functional group contents of the humic acids used in this study.

|                  | HHA | SHA | PHA |
|------------------|-----|-----|-----|
| Yields (g kg\(^{-1}\)) | 42.3| 12.7| 10.1|
| Elemental compositions |     |     |     |
| C%               | 50.0| 51.3| 53.1|
| H%               | 4.49| 4.65| 4.59|
| N%               | 5.29| 2.91| 2.21|
| O%               | 37.7| 37.2| 40.1|
| Ash%             | 2.48| 3.97| n.d.\(^\alpha\)|
| Atomic ratio     |     |     |     |
| H/C              | 1.07| 1.08| 1.03|
| O/C              | 0.566| 0.545| 0.566|
| C/N              | 11.0| 20.5| 28.0|
| Acidic functional group content (mmol g\(^{-1}\)) |     |     |     |
| Total\(^\beta\)  | 5.54 ± 0.72| 6.28 ± 0.46| 6.16 ± 0.45|
| Carboxylic acid\(^\gamma\) | 3.29 ± 0.13| 3.43 ± 0.05| 3.76 ± 0.24|
| Phenolic hydroxyl group\(^\gamma\) | 2.25 ± 0.84| 2.85 ± 0.51| 2.40 ± 0.69|

\(^\alpha\) Not detected.

\(^\beta\) The average values ± S.D. of the triplicate experiment.

\(^\gamma\) The values were calculated by subtracting the carboxylic acid content from the total acidity.

### Table 2. UV-vis absorptivities on humic acids.

| UV-vis parameters\(^\alpha\) | HHA | SHA | PHA |
|-----------------------------|-----|-----|-----|
| \(\varepsilon_{600}\)      | 1.21| 0.808| 0.682|
| \(\varepsilon_{280}\)      | 30.9| 26.5| 25.8|
| \(\varepsilon_{ET}/\varepsilon_{280}\) | 0.687| 0.726| 0.739|

\(^\alpha\) These parameters were measured by 0.025 g L\(^{-1}\) of HA in 0.1 M phosphate buffer (pH 8.00).
amount of aromatic compounds and quinones than the other HAs, consistent with the UV-vis analysis data.

4. Discussion

Reductive Fe elution from Fe$_2$O$_3$ was stimulated by ASC in the presence of a low concentration of HAs (Fig. 3), indicating that humic substances are capable of functioning as electron donors/acceptors. In this study, HA appears to contribute to the reduction of Fe, as described by the following equilibria:

$$\text{ASC}^{\text{red}} + \text{HA} \rightleftharpoons \text{ASC} + \text{HA}^{\text{red}} \quad \ldots \ldots \ldots (4)$$

$$\text{HA}^{\text{red}} + \text{Fe}(\text{III}) \rightleftharpoons \text{HA} + \text{Fe}(\text{II}) \quad \ldots \ldots \ldots (5)$$

where HA$^{\text{red}}$ represents the reduced form HA. Previous studies demonstrated that the electron shuttling effect of dissolved humic substances can occur, even at low concentrations of 1 to 10 mg L$^{-1}$, and the result shown in Fig. 3 are consonant with those reports.$^{28,31}$ This electron shuttling function is largely induced by quinone moieties.$^{12,16,30,31}$ The electron shuttling function depends on the structural features of HA. A significantly large amount of aromatic compounds was detected as fragments of the HHA by TMAH-py-GC/MS analysis in a previous study.$^{28}$ The fragment structure of aromatic compounds is mainly derived from lignin-derivatives,$^{32}$ hence the chemical structure would be expected to significant depending on the original source of the HA. The coumaryl type is a typical type of lignin found in grass or bamboo, the coniferyl type is major lignin found in soft-wood plants, and the sinapyl types are characterized as lignin derived from hard-wood plants.$^{32}$ A di-phenolic structure is necessary to generate a quinonic structure, an aromatic compound multi-substituted by O-containing functional groups, such as the sinapyl type, is a favorable structure. Electron-accepting capacity would be associated with aromaticity.$^{4,15}$ It is known that an aromatic network increases with the degree of humification, the above structural analyses indicate that the HHA contains a highly poly-aromatic structure compared to the SHA and PHA. N-containing compounds, which were detected as pyroles and indoles,$^{28}$ may contribute to electron transfer due to their aromatic properties resulting from covalent N–H bonds.$^{15}$ The structural analyses indicated that the HHA contains a highly substituted aromatic structure, polyaromatic compounds, and a significant amount of N, therefore, the HHA had more favorable structural features for assisting in the Fe reduction process than the SHA and PHA. The maximum values of Fe elution were found for 5 mg L$^{-1}$ of HHA and SHA, while the corresponding value for PHA was at 10 mg L$^{-1}$. This could be attributed to a lower capacity or stability of free electron in the PHA.

The concentration of o-Phen in the medium (100 μM) was enough amount to the target Fe(II) concentration, eluted Fe(II) could assuredly complex with o-Phen as a result. However, the high concentration of HA would inhibit the complexation of Fe(II) with o-Phen because the complexation of Fe(II) with HA could occur as the competing reaction.$^{61}$ Accordingly, the percentage of detectable Fe(II) species with o-Phen in the presence of 5–50 mg L$^{-1}$ HAs was investigated based on absorbance at 510 nm after Fe(II) added (Fig. 5). In the presence of HAs, the detectable Fe(II) with o-Phen decreased in 2–10% lower than the total Fe(II) (5 μM). The percentage of detectable Fe(II) decreased with increasing the concentration of HAs. Considering the result in the Fig. 5, about 5 μM Fe(II) could be reductive eluted in the presence of 10 mg L$^{-1}$ of HA (Fig. 3). The complexation Fe(II) with HAs would be a reason of why the reductive Fe elution decreased with increasing of the concentration of HA.

A variety of side reactions could also occur by electron accepted HA with a neighboring HA, as described by the equations below:$^{9}$

$$\text{HA}^{\text{red}} + \text{HA} \rightarrow \text{HA}^{\prime} \quad \ldots \ldots \ldots (6)$$

$$\text{HA}^{\text{red}} \rightarrow \text{HA}^{\prime} \quad \ldots \ldots \ldots \ldots \ldots (7)$$

where HA$^{\prime}$ represent a structurally altered HA by self-oxidation. These side-reactions could inhibit the reductive Fe elution when the concentration of HA was enlarged. To investigate the influence of the concentration of quinone

\[\text{Detectable Fe(II) (mg/L)}\]

\[5 \text{ mg L}^{-1}, 10 \text{ mg L}^{-1}, 50 \text{ mg L}^{-1}\]

\[\text{HHA, SHA, PHA}\]

Fig. 5. Percentage of detectable Fe(II) in the saline medium in the presence of HAs. Total Fe(II) concentration was 5 μM, and the incubation period was 30 min. Open, meshed and filled bars represent the average values (S.D., n = 3) in the presence of 5, 10 and 50 mg L$^{-1}$ of HAs, respectively. The corresponding values of the absence of Fe(II) were subtracted from the original values.
moieties, the reductive Fe elution was evaluated in the presence of a variety concentration of AQDS (Fig. 6). When the presence of 10 μ AQDS with 5 mM ASC, the amount of Fe eluted increased competing with that value in the absence of AQDS (Fig. 6). However, the amount of Fe eluted slightly decreased in the condition of high concentration AQDS (100 μM). When the presence of high concentration of AQDS, the reduction could also be occurred between reduced form and oxidized form of AQDSs resulting in the decrease of the amount of reductive Fe eluted. This result suggests that the reduction-oxidation reaction between quinicin structures in HA could be attributed to decreasing the effect of reductive Fe elution (Fig. 3). The nucleophilic addition reaction, which causes the self-decomposition or condensation of HA, might also proceed along with Fe(III) reduction in the presence of high concentrations of HA. With increasing the concentration HHA, the Fe elution drastically decreased compared to those case of SHA and PHA (Fig. 3). A peak at around 1 050 cm⁻¹ of FT-IR spectrum for HHA stronger than those for SHA and PHA, indicating that the greater the amount of saccharide components was contained in HHA. The self-computation of accepted-electron by labile components, such as saccharides, could cause of the drastically decrease of reductive Fe elution in the presence of high concentration HHA. The method in this study evaluates only the electron-shutting capability of HA. Saccharides can play a role as an energy source of microorganism, thereby, it could be expected that these labile structural part in HA could indirectly contribute to Fe elution. To avoid the influence of the side reactions, the evaluation has to be carried out in the presence of low concentration of HA (5 mg L⁻¹).

5. Conclusions

This study reports on the effect of humic acid on reductive Fe elution from Fe₂O₃ into a seawater-like saline medium (pH 8, I = 0.7) using a laboratory-based experiment. Reductive Fe elution with ascorbic acid as a reducing agent was evidenced by coloration caused by the presence of Fe(II) when it immediately complexed with 1,10-phenanthroline.

At low HA concentration condition, the capability of enhancing the reductive Fe elution by HA was evaluated. This function was found to be largely dependent on the structural features of the co-existing humic acid. Based on the performance of reductive Fe elution, we conducted that hard-wood bark compost was a suitable humic acid source for use in the fertilizing technique to seawater containing limited amounts of dissolved Fe species.

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