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Evaluation of kinetic competition among formation and degradation processes of dissolved humic-like substances based on hydrothermal reactions measured by Ultraviolet–visible spectroscopy

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Abstract

Hydrothermal decolorization (color loss) of Nordic fulvic and humic acid solutions at 80–180 °C for 0–600 hours was traced by ultraviolet–visible (UV–Vis) spectroscopy. These changes were considered to be due to degradation of humic-like chemical structure and represented by decreases in absorbance at 254 nm (UV_{254}). The temperature dependence of their apparent rate constants obtained by the first order reaction model was well described by the Arrhenius equation giving activation energies of 87.6 and 101 kJ mol^{-1} for degradation of fulvic and humic acids, respectively. The degradation rates of humic substances were slower than the formation rates of humic-like substances by the Maillard-type browning reaction, suggesting that humic-like substances can be preserved in the aquatic environments, where their formation (browning) and degradation (decolorization) processes are occurring together. By extrapolation to 15 °C of the Arrhenius equation, 99–1980 years of time scales for these processes were estimated. The time scale of changes in UV_{254} (18 years) in natural aquatic environments estimated by kinetic analyses on Ago Bay bottom sediments were closer to the formation time scales of humic-like substances. (183 words < 350)

Introduction

Dissolved organic matter (DOM), defined as organic compounds passing through a

0.45μm filter, is common in natural waters such as rivers, lakes, oceans and ground waters. Only 20 % of DOM in the natural aquatic environments is identifiable and the 80 % of DOM is dissolved humic substances (Thurman, 1985). The term "humic substances" is often used as a general term of polymerized high molecular weight organic matter in natural environments, whose structure is not well defined. It should be noted that International Humic Substances Society (IHSS) proposes extraction methods of humic substances by using alkali solutions and resins (Aiken, 1985; Kuwatsuka *et al.*, 1992; Swift, 1996). In this paper, "humic substances" is used for samples which are obtained from natural environments based on the IHSS extraction methods and "humic-like substances" is used as a general term for those with similar properties to "humic substances" regardless of using the IHSS methods.

Humic-like substances are known to have broad and featureless UV–Vis absorption spectra (Wang *et al.*, 1990), which are derived from presence of aromatic rings or conjugated C=C or C=O double bonds, in the ultraviolet (UV) (200–400 nm) and visible (Vis) (400–800 nm) regions (Stevenson, 1994). In spite of the featureless absorption spectra, absorption intensities and their ratios at particular wavelengths were used as surrogate parameters of quantity and property of humic substances. For example, absorbance at 254 nm (UV_{254}) has been often used as a measure of DOM including humic-like substances (Edzwald *et al.*, 1985; Korshin *et al.*, 1997).

Humic-like substances as well as DOM have a key role in biogeochemical cycling (Hedges, 2002), agriculture (Stevenson, 1994), marine chemistry and forest ecology (Coble, 1996; Berg and McClaugherty, 2003). They have abundant hydrophilic functional groups such as –COOH and –OH which are responsible to their aqueous solubility, binding sites for metals, buffer capacities and other reactive characteristics (Stevenson, 1994). Because of their high abundances and reactivities, humic-like substances also play an important role in environmental pollution studies (Kördel *et al.*, 1997). In spite of these importance in natural environments, the rates of formation and degradation of humic-like substances in aqueous conditions are poorly understood, because of complex multiple pathways including chemical and biological processes. Although 250–1900 years of mean residence time, which are ages of humic-like substances measured by the ^{14}C dating method, are reported by Stevenson (1994), few studies examine kinetic competition among the formation and degradation processes.

Based on these problems, our previous researches (Nakaya and Nakashima, 2016; Nakaya et al., 2018a) preliminarily examined kinetic analyses on the Maillard-type browning reactions simulating formation of humic-like substances. In the one of our paper (Nakaya et al., 2018a), we conducted in situ UV–Vis spectroscopy of 0.1 mol l^{-1}

glycine + ribose solution at 60–80 °C for 0–144 hours by an in situ hydrothermal cell. The UV–Vis spectra indicated that absorbance at shorter wavelengths increased more for longer heating durations in association with increases in a band around 280 nm. By correlation analyses among the UV–Vis spectra, fluorescence spectra and size exclusion liquid chromatograms for the product solutions in batch experiments, we suggested that increases in UV_{254} correspond to increases in products with humic substances-like fluorescent patterns (Nagao et al., 2003). We also suggested that increases in band area around 280 nm (base-line: 245–315 nm) correspond to increases in furfural-like intermediates. These increases with time were fitted by an exponential equation assuming the first order reaction. Increase rates at 280 nm were twice larger than those at 254 nm. Kinetic analyses of the obtained rates by the Arrhenius equation gave activation energies of 91.4–96.6 kJ mol^{-1}. In the present paper, for kinetic comparison between simulated formation and degradation of humic(-like) substances, we kinetically reanalyzed the changes in UV_{254} in the heating experiments of glycine + ribose mixture solutions during the Maillard-type browning processes.

On the other hand, some previous researches reported thermal degradation of humic substances in solid state (Ioselis *et al.*, 1980; Lu *et al.*, 1997; Martyniuk *et al.*, 2001; Giovanela *et al.*, 2004). They focused on decarboxylation at much higher temperature than typical aquatic environments. However, few studies examined thermal degradation of humic substances in solution state, in spite of presence of aqueous humic substances. For quantitative evaluation of competition between the simulated formation and degradation of humic(-like) substances in water, this paper aims at kinetic analyses on thermal degradation of dissolved humic substances by monitoring hydrothermal decolorization of fulvic and humic acid solutions. Fulvic and humic acids are humic substances extracted from soils and sediments by alkaline solutions and from natural water by resin adsorption. The fulvic acid is hydrophilic dissolved humic substance at most of pH conditions. The humic acid is the fraction of humic substances soluble in alkaline solutions, but is precipitated under acidic conditions (pH<2). As a representative index of their color loss, UV_{254} were used. Kinetic parameters were compared with those for the Maillard-type simulated formation of humic-like substances by Nakaya *et al.* (2018a).

This study also examined time scales for changes of humic-like substances in natural aquatic environment, by taking an example of changes in UV_{254} of bottom sediments. The samples were collected at the center of Ago Bay (Takonobori), Mie Prefecture. Chemical oxygen demand (COD: an indicator of organic pollution in a closed water area such as a lake) at bottom of the sea in Ago Bay was reported to be increasing possibly because of overcrowded pearl culture (Momoshima *et al.*, 2008). The values of UV_{254} and total organic carbon (TOC) for solutions of humic acids extracted from bottom sediments at three depth fractions (0–3, 3–6, 6–9 cm) were measured and their changing rates were evaluated by using sedimentation rates (0.25 g cm^{-2} y^{-1}, Momoshima *et al.*, 2008). Finally, these rates were compared with experimentally evaluated time scales for simulated formation (browning) and degradation (decolorization) of humic(-like) substances.

Samples and Methods

Hydrothermal experiments

In order to trace thermal decolorization of humic substances, batch hydrothermal experiments and UV–Vis spectroscopy were conducted. Nordic fulvic acid and Nordic humic acid reference samples were obtained from IHSS. Nordic fulvic and humic acid sample solutions (100 mg l^{-1}) were prepared by dissolving them in pure water (MilliQ > 18.2MΩ cm). Initial pHs were about 3.6 for both solutions. 8 ml of humic substance solutions were introduced into PTFE (polytetrafluoroethylene) inner bottles (10 ml inner volume) in stainless steel containers. These reaction vessels were sealed and heated in ovens at 80–180 °C for 0–600 hours. Although pressures in the sealed bottles were not controlled and monitored, inside of the bottles are considered to be a closed system. Assuming that the bottles were sealed at room temperature under 1 atm, pressures in the bottles during heating experiments at 80–180 °C are calculated to be 0.17–1.2 MPa. Temperatures during hydrothermal experiments were recorded on a data logger through thermocouples placed at the bottom of the PTFE inner bottles. The temperature precision of these hydrothermal vessels was maintained within ±2 °C. After the heating experiments, the sample solutions were diluted 10 times by pure water. UV–Vis spectra were measured in a quartz cell (optical pass length: 10 mm) by a UV–Vis spectrometer (V-570, Jasco) at a scanning speed of 100 nm min^{-1} with a resolution of 0.5 nm in the 190–800 nm spectral range.

Changes with time in UV_{254} were fitted by an exponential equation assuming the first order reaction. The obtained apparent first order reaction rate constants at 80–180 °C were plotted in an Arrhenius diagram. Activation energy [E_a (kJ mol^{-1})] values were obtained by fitting the experimental data by the Arrhenius equation:

$$\ln k = \ln A - E_a/RT$$

where k is the reaction rate constant, A is the frequency factor (s^{-1}), R is the gas constant (8.31×10^{-3} kJ mol^{-1} K^{-1}) and T is the absolute temperature (K). It should be noted that the fitting by the Arrhenius equation for the Maillard-type browning reactions (Nakaya *et al.*, 2018a) was re-conducted by the same fitting program as for other data in this study. The apparent first order rate constants can be extrapolated to lower temperatures by the fitted lines. As a representative temperature of Earth's aquatic environment, 15 °C was selected. In order to better represent time scales of these processes at Earth's surface environments, their half-time $t_{1/2}$ values can be calculated by $(\ln 2)/k$.

Analyses on natural samples

Bottom sediments of the center of Ago Bay (Takonobori), Mie Prefecture were collected in July 2004 by a core sampler (6 cm diameter; 30 cm height). The core sample was divided into three parts: 0–3, 3–6 and 6–9 cm. Humic acid fractions were extracted from these fractions in reference to the IHSS method (Ishiwatari *et al.*, 1977; Swift, 1996) at Mie University. The humic acid fractions were obtained as powders precipitated at low pH. They were then dissolved in pure water at the same concentration (mg l^{-1}). UV–Vis spectra for the humic acid solutions were measured by the UV–Vis spectrometer (V-570, Jasco) with the quartz cell (optical pass length: 10 mm). Concentrations of total organic carbon (*TOC*) in the extracted solutions were measured by a total organic carbon (TOC) analyzer (TOC-V, Shimadzu). It should be noted that these chemical analyses were conducted in 2004, soon after the sample collection.

Since concentrations of the extracted humic acid solutions were different from each other, absorbance at 254 nm per unit mass concentration of TOC (UV_{254}/TOC, unit: ml mgC^{-1}) can be used as appropriate indicators for quantitative comparison. Their changes with the average time (year) from the deposition (average ages) were fitted by an exponential equation assuming the first order reaction.

Results

UV–Vis spectral changes in the hydrothermal experiments

Representative UV–Vis spectra (200–600 nm) for (a) Nordic fulvic acid and (b) Nordic humic acid solutions (180 °C, 0–18 hours) in the batch hydrothermal experiments are shown in Fig. 1. Absorption in the UV–Vis range decreased with time. In fact, concentrations of Nordic fulvic acid (10–100 mg l^{-1}) have a good linear correlation with UV_{254} (Supplemental Materials: Fig. s1). Decreases in UV_{254} of (a, b) Nordic fulvic acid and (c, d) Nordic humic acid solutions in the batch hydrothermal experiments for different heating temperatures (80–180 °C) are plotted in Fig. 2. They show exponential decreases in UV_{254} with heating duration (t). The higher heating temperature, the faster UV_{254} decreases. Moreover, quasi-linear trends were recognized in semi-logarithmic plots of UV_{254} against heating duration (Supplemental Materials: Fig. s2). This suggests the apparent first order reactions for the decreases in UV_{254}.

A fitting of the data by

$UV_{254} = C\ exp(-kt)$

gave the apparent first order reaction rate constant k (fitting parameters: k and C) for each heating temperature. The fitting curves are shown in Fig. 2 on UV_{254} changes. The obtained k and C values are listed in Table 1. Larger rate constants k are obtained for higher temperatures.

On the other hand, Fig. 3 shows representative UV–Vis spectra (200–600 nm) for 0.1 mol l^{-1} glycine and ribose mixture solution (80 °C, 0–144 hours) measured by the in situ hydrothermal cell in our previous research (Nakaya *et al.*, 2018a). It shows increases in ultraviolet tails and in band areas around 280 nm possibly due to furfural-like intermediates. Since our previous research suggested that changes in UV_{254} represent increases in humic-like products rather than the furfural-like intermediates (absorbing at 280 nm) in the 0.1 mol l^{-1} glycine + ribose aqueous system, changes with heating duration in UV_{254} were extracted from Fig. 3 for each heating temperature (60–80 °C). The absorbance at 254 nm was determined by subtracting the absorbance at 600 nm from the raw absorbance at 254 nm for correcting the baseline effects, since the absorption tails from ultraviolet to visible region disappears around 600 nm. Fig. 4 shows increases in UV_{254} of the sample solutions heated at 60–80 °C in the in situ hydrothermal spectroscopic measurements.

The later stages ($UV_{254} > 0.095$) were fitted by the first order reaction model:

$UV_{254} = 3.23(1-\exp(-k(t-\tau))) + 0.095$

where τ is a starting time ($UV_{254} = 0.095$) of the later stage (Nakaya *et al.*, 2018a). The fitting curves are shown in Fig. 4 on UV_{254} changes. The obtained rate constants k are listed in Table 1.

The apparent first order reaction rate constants were plotted in an Arrhenius diagram (Fig. 5) and fitted by the Arrhenius equation. The values of E_a and their errors are listed in Table 2. The fitting lines in the experimental temperature range (60–180 °C) are shown in Fig. 5.

The apparent first order rate constants at 60–180 °C obtained in the hydrothermal experiments on Nordic fulvic and humic acids and the Maillard-type browning (Nakaya *et al.*, 2018a) were extrapolated to 15 °C by the fitted lines. The extrapolated rate constants are shown in the Arrhenius diagram (Fig. 5). The estimated time scales (half-time $t_{1/2}$ values) and their errors at 15 °C are listed in Table 2.

Analyses on natural samples

The solutions of the humic acids extracted from Takonobori (3 depth fractions) showed brown colors (Supplemental Materials: Fig. s3) and their molecular weights were from 14500 to 22800 Dalton by size exclusion liquid chromatography (SEC). Since Momoshima *et al.* (2008) reported 0.25 g cm^{-2} y^{-1} of sedimentation rate at Takonobori, average times from the deposition (average ages) can be estimated for each fraction. Since the average times were calculated by the reported sedimentation rates (Momoshima *et al.*, 2008) from weights of every 3 cm fraction, they do not have the same interval. Table 3 lists the average times and values of UV_{254}, TOC and UV_{254}/TOC for the sample solutions. Fig. 6 shows (a) UV_{254}, (b) TOC and (c) UV_{254}/TOC for each depth fraction and (d–f) those against the average time (year) from the deposition.

In order to evaluate rate constant values in a natural environment, the increases with time of UV_{254}/TOC values of humic acid solutions at Takonobori in about 15 years [Fig. 6 (f)] were fitted by the first order reaction model:

$UV_{254}/TOC = C(1\text{-}\exp(\text{-}kt))$

Although these fitting curves include large errors due to very small data points, they gave an apparent first order rate constant $k = 1.23 \pm 1.48$ ($\times 10^{-9}$ s^{-1}) and $C = 0.074$ (ml mgC^{-1}). The obtained rate constants k are plotted in the Arrhenius diagram assuming an average temperature of 15 °C for the bottom sediments at Takonobori with an error of ±15 °C (Fig. 5). The half-time ($t_{1/2}$) values at 15 °C are in the range of 8 to 28 years.

Discussion

The first order reaction model well reproduced changes in UV_{254} (Figs. 2 and 4) for the hydrothermal experiments and the temperature dependence of the apparent first order rate constants was well described by the Arrhenius equation (Fig. 5). The activation energy values for these processes are in the similar value ranges (87.6–101 kJ mol^{-1}). The hydrothermal decolorization and browning processes of humic-like substances might be rate-limited by the first order-like chemical reactions. These apparent first order reaction models suggest that increases and decreases of brownish colors of humic-like substances have apparent saturation values without linear increases/decreases and can be proportional to the concentrations of the initial components.

In this study, decreases and increases in UV_{254} respectively represented decolorization and browning processes. Absorption in UV region of natural organic matter is considered to reflect substituted aromatic structures (Korshin *et al.*, 1997). Therefore, it is suggested that the changes in UV_{254} by the hydrothermal experiments corresponds to degradation and formation of aromatic structure and/or substituted group of aromatic rings such as carboxyl groups. Moreover, the time scales of 490 and 1980 years for decolorization of humic substance solutions (Table 2) are similar to a reported mean residence time of 250–1900 years (Stevenson, 1994), which are ages of humic substances measured by the ^{14}C dating method. These may also suggest that decolorization of humic substances represented by decreases in UV_{254} can be corresponding to degradation of their structures.

The degradation (decolorization) rates of humic substances are slower than the formation (browning) rates of humic-like substances by the Maillard-type browning reaction (Fig. 5). This may suggest that humic-like substances can be preserved in the aquatic environments, where their formation and degradation processes are occurring together.

On the other hand, this study examined changes in UV_{254} together with TOC of humic acid solutions extracted from the 3 depth fractions of Ago Bay bottom sediments as a function of time from their deposition (Table 3, Fig.6). Despite the same weights of humic acid fractions at three depths (mg l^{-1}), the TOC values increased with depth [Fig. 6 (b), (e)]. This means that percentages of organic carbon in the humic acid molecules increased with depth. Increases with time in UV_{254} suggest increases in aromatic rings and/or substituted functional groups including double bonds [Fig. 6 (a), (d)]. Since UV_{254} normalized for amount of DOM ($SUVA_{254}$) is suggested to be an indicator of aromaticity (Weishaar et al., 2003), UV_{254}/TOC can be taken in our study as an indicator of high molecular weight structural components of the humic acid fractions.

The increases in UV_{254}/TOC (Fig. 6; Table 3) possibly reflect increases in ratio of carbon atoms relating to UV active structures, which have π electron conjugated systems by aromatic rings and unsaturated bonds. This process can be interpreted as a formation process of humic-like substances.

The increases in UV_{254}/TOC with time from the deposition were fitted by the first order reaction model. This may be due to existence of a rate-limiting first order reaction. There is a possibility that competition between formation of the π electron conjugated systems and back reactions (their degradation) caused the "apparent" first order trends. Unfortunately, few data points (UV–Vis spectroscopy for 3 depth fractions) limit further detailed analyses. However, the half-time value in the fitting of the Ago Bay data (18 years) had similar orders of magnitude to those of simulated formation and degradation processes of humic-like substances at 15 °C estimated by extrapolation of the experimental data at 60–180 °C (Fig. 5). This may suggest validity of kinetic extrapolation of the experimental data.

These evaluations are only the first trials with limited data by UV–Vis spectroscopy and further detailed studies are required. We had expected an intermediate time scales on the natural samples between the experimental formation and degradation of humic-like substances because of balance of their rates: the formation is faster than the degradation (Fig. 5). However, the half-time for changes of the Ago Bay sediments (18 years) are closer to the values at 15 °C extrapolated from the experimental formation (browning) rates (99 years), rather than those (470–1980 years) from the experimental degradation (decolorization) rates of humic-like substances (Fig. 5). Therefore, in the future studies, precisions of data and effects of environmental conditions should be evaluated.

For the hydrothermal experiments on browning and decolorization processes, effects of pH, ionic strength, ions, minerals and organic molecular sizes should be considered. For the analyses of natural samples, sedimentation rates, coexisting minerals, bioactivities, etc. should be taken into consideration. These environmental factors can increase/decrease apparent reaction rate constants k_{app} and so that apparent frequency factors A_{app}, leading to vertical upward/downward shifts of the linear trends (Fig. 5). For example, stabilization of organic matter by association with minerals have been suggested (Mikutta *et al.*, 2006), possibly leading to smaller degradation rates.

On the other hand, enhancement of transformation of humic(-like) substances by minerals have also been suggested (e.g. Taguchi and Sampei, 1985; Otsuka and Nakashima, 2007; Nakaya *et al.*, 2018b). These catalytic effects are expected to lower activation energy values E_a leading to smaller gradients of the linear trends in Fig. 5.

Additionally, chemical structures such as substituted aromatic structures generated/decomposed in the experimental and natural systems should be identified by combining UV–Vis, infrared, fluorescence and nuclear magnetic resonance spectroscopy. For example, increase and decrease in functional groups during formation and degradation of humic(-like) substances should be evaluated, because of their large abilities for complexation of heavy metal cations. Martyniuk et al. (2001) and Nakada et al. (2016) reported that thermal decomposition of humic substances including decarboxylation was related to the release of metal ions. These future studies will provide quantitative bases for evaluation of time scales of formation/degradation and fates of humic-like substances interacting with inorganic materials and metal ions in the natural environment.

Conclusion

For quantitative evaluation of kinetic competition between the simulated formation and degradation processes of humic(-like) substances, this paper conducted kinetic analyses on thermal degradation of humic substances by hydrothermal decolorization of fulvic and humic acids at 80–180 °C for 0–600 hours. They were traced by UV–Vis spectroscopy, and these changes were considered to be due to degradation of humic-like chemical structure and represented by decreases in absorbance at 254 nm (UV_{254}). The temperature dependence of their apparent rate constants obtained by the first order reaction model was well described by the Arrhenius equation giving activation energies of 87.6 and 101 kJ mol^{-1} for degradation of fulvic and humic acids, respectively. These degradation rates of humic substances are slower than the formation rates of humic-like substances by the Maillard-type browning reaction, suggesting that humic-like substances can be preserved in the aquatic environments where their formation (browning) and degradation (decolorization) processes are occurring together. In fact, kinetic analyses of increases with time in UV_{254}/TOC for humic acids extracted from Ago Bay bottom sediments indicated time scales close to those for the formation processes of humic-like substances extrapolated to 15 °C.

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References

Aiken, G. R. (1985) Isolation and concentration techniques for aquatic humic substances. *Humic substances in soil, sediment and water: geochemistry and isolation*. (Aiken, G. R., McKnight, D. M., Wershaw, R. L. and MacCarthy, P., eds.), 363–385, Wiley-Interscience.

Berg, B. and McClaugherty, C. (2003) Plant litter decomposition, humus formation, carbon sequestation, Springer, 1–10 pp.

Coble P. G. (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine chemistr*. 51, 325–346.

Edzwald, J. K., Becker, W. C. and Wattier, K. L. (1985) Surrogate parameters for monitoring organic matter and THM precursors. *J. Am. Wat. Works Assoc.* 77, 122–132.

Giovanela, E, Parlanti, E., Soriano-Sierra, E. J., Soldi, M. S. and Sierra, M. M. D. (2004) Elemental compositions, FT-IR spectra and thermal behavior of sedimentary fulvic and humic acids from aquatic and terrestrial environments. *Geochem. J.* 38, 255–264.

Hedges, J. I. (2002) Why dissolved organic matter?, *Biogeochemistry of Marine Dissolved Organic Matter* (Hansell, D. A. and Carson, C. A., eds.), 1–33, Academic Press.

Ishiwatari, R., Ishiwatari, M., Rohrback, B. G. and Kaplan, I. R. (1977) Thermal alteration experiments on organic matter from recent marine sediments in relation to petroleum genesis. *Geochim. Cosmochim. Acta* 41, 815–828.

Ioselis, P., Ikan, R. and Frenkel, M. (1980) Thermal degradation of metal-complexed humic substances. *Phys. Chem. Earth* 12, 567–577.

Kuwatsuka, S., Watanabe, A., Itoh, K. and Arai, S. (1992) Comparison of two methods of preparation of humic and fulvic acids, IHSS method and NAGOYA method. *Soil Sci. Plant Nutr.* 38, 23–30.

Kördel, W., Dassenakis,M., Lintelmann, J. and Padberg, S. (1997) The importance of natural organic material for environmental processes in waters and soils (Technical Report). *Pure Appl. Chem.* 69, 1571–1600.

Korshin, G. V., Li, C. W. and Benjamin, M. M. (1997) Monitoring the properties of natural organic matter through UV spectroscopy: a consistent theory. *Wat. Res.* 31, 1187–1795.

Lu, X. Q., Vassallo, A. M. and Johnson, W. D. (1997) Thermal stability of humic substances and their metal forms: an investigation using FTIR emission spectroscopy. *J. Anal. Appl. Pyrolysis.* 43, 103–113.

Martyniuk, H., Więkowska, J. and Lipman, J. (2001) The study of influence of metal ions on thermal decomposition of humic acids. *J. Therm. Anal. Calorim.* 65, 711–721.

Mikutta, R., Kleber, M., Torn, M. S. and Jahn, R. (2006) Stabilization of soil organic matter: association with minerals or chemical recalcitrance?. *Biogeochemistry* 77, 25–56.

Momoshima, N., Ueda, Y., Sugihara, S., Yamagata, Y. and Kokubu, H. (2008) Investigation of sedimentation rate in Ago Bay, Japan by lead-210 dating. *Chikyukagaku* (*Geochemistry*) 42, 99–111. (in Japanese with English abstract)

Nagao, S., Matsunaga, T., Suzuki, Y., Ueno, T. and Amano., H. (2003) Characteristics of humic substances in the Kuji River waters as determined by high-performance size exclusion chromatography with fluorescence detection. *Wat. Res.* 37, 4159–4170.

Nakada, R., Waseda, A., Okumira, F. and Takahashi, Y. (2016) Impact of the decarboxylation reaction on rare earth elements binding to organic matter: From humic substances to crude oil. *Chem. Geol.* 420, 231–239.

Nakaya, Y and Nakashima, S. (2016) In situ IR transmission spectroscopic observation and kinetic analyses of initial stage of the Maillard reaction as a simulated formation process of humic substances. *Chem. Lett.* 45, 1204–1206.

Nakaya, Y., Nakashima, S. and Moriizumi, M. (2018a) Non-destructive spectroscopic tracing of simulated formation processes of humic-like substances based on the Maillard reaction. *Appl. Spectrosc.* 72, 1189–1198.

Nakaya, Y., Okada, K., Yudai I. and Nakashima, S. (2018b) Spectroscopic study of effects of goethite surfaces on the simulated maillard reaction forming humic-like substances. e-J. Surf. Sci. Nanotechnol. 16, 411–418.

Otsuka, T. and Nakashima., S. (2007) The formation of CO_2 by fulvic acid on the surface of goethite studied using ultraviolet and infrared spectroscopy. *J. Mineral. Petrol. Sci.* 102, 302–305.

Stevenson, F. J. (1994) *Humus Chemistry: Genesis, Composition, Reactions*, Wiley, 1–23, 212–235, 304 pp.

Swift, R. S. (1996) Organic matter characterization. *Methods of soil analysis. Part 3. Chemical methods* (Sparks, D. L., Page, A. L., Helmke, P. A., Loeppert, R. H., Soltanpour, P. N., Tabatabai, M. A., Johnson, C. T. and Summer, M. E., eds.), 1018–1020, Soil Science Society of America.

Taguchi, K. and Sampei, Y. (1986) The formation, and clay mineral and $CaCO_3$ association reactions of melanoidins. *Org. Geochem.* 10, 1081–1089.

Thurman. E. M. (1985) *Organic geochemistry of Natural waters*, Martinus Nijhoff/Dr. W. Junl Publishers, 1–4 pp.

Wang, Z., Pant, B. C. and Langford, C. H. (1990) Spectroscopic and structural characterization of a Laurentian fulvic acid: Notes on the origin of the color. *Anal. Chim. Acta* 232, 43–49.

Weishaar, J. L., Aiken, G. L., Bergamaschi, B. A., Fram, M. S., Fujii, R. and Mopper, K. (2003) Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon. *Environ. Sci. Technol.* 37, 4702–4708.

Tables

Table 1. The values of k and C in the first order reaction fittings in the batch heating experiments on Nordic fulvic and humic acid solutions at 80–180 °C. The values of k in the first order reaction fittings in the in situ heating measurements on glycine and ribose mixture solutions (0.1 mol l^{-1}) at 60–80 °C (Nakaya et al.., 2018a) are also listed.

	Temp. (°C)	180	160	140	120	100	80
Fulvic acid	k (10^{-7} s^{-1})	268	116	34.9	6.81	1.50	0.497
	C	0.195	0.222	0.224	0.229	0.218	0.231
Humic acid	k (10^{-7} s^{-1})	459	177	44.3	8.85	1.70	0.254
	C	0.232	0.259	0.243	0.250	0.236	0.255

	Temp. (°C)	80	75	70	65	60
Browning	k (10^{-7} s^{-1})	6.52	5.34	3.02	2.62	1.60

Table 2. The activation energy E_a values obtained from the fitting of the apparent first order reaction rate constants by the Arrhenius equation and half-time ($t_{1/2}$) values at 15 °C estimated by extrapolation of the fitted lines.

Sample	E_a (kJ mol^{-1})	$t_{1/2}$ at 15 °C (s^{-1})
Fulvic acid	87.6 ± 6.7	470 ± 2
Humic acid	102 ± 3	1980 ± 2
Browning	96.8 ± 17.8	99 ± 3

Table 3. Average times and values of UV_{254}, TOC, and UV_{254}/TOC for the sample solutions extracted from three depth fractions of bottom sediments at Takonobori, Ago Bay, Mie Prefecture, Japan.

Depth (cm)	Average time (y)	UV_{254}	*TOC* (mgC ml^{-1})	UV_{254}/*TOC* (ml mgO^{-1})
0–3	6.5	0.677	30.5	0.0222
3–6	13.5	0.853	36.1	0.0236
6–9	21.5	2.872	65.6	0.0438

Figures

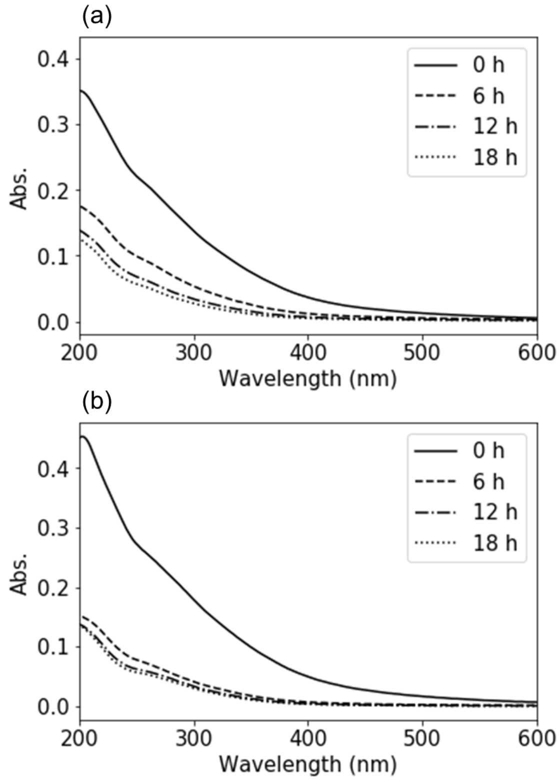

Fig. 1. Representative UV–Vis spectra for (a) Nordic fulvic acid and (b) Nordic humic acid solutions (180 °C, 0–18 hours) in the batch hydrothermal experiments.

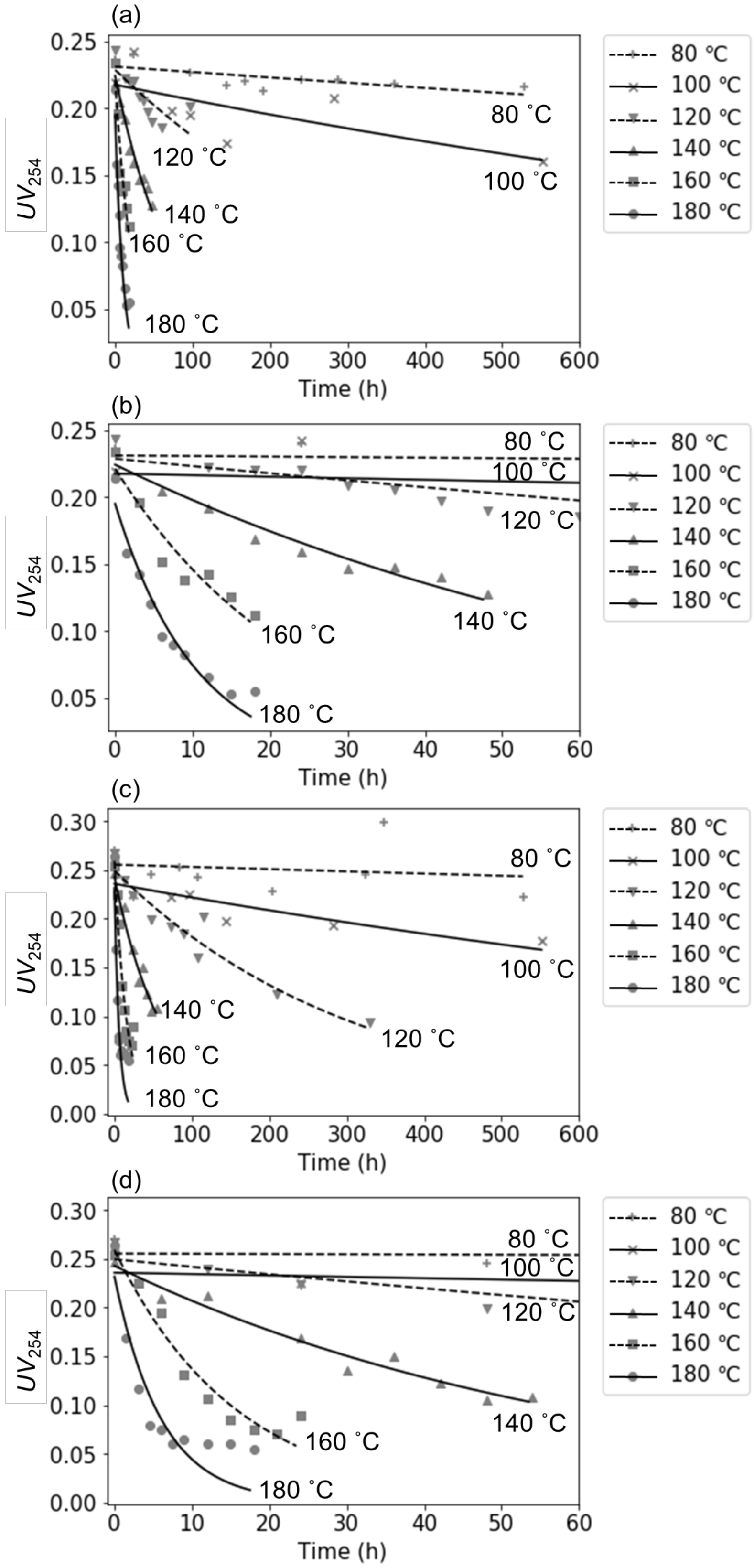

Fig. 2. Decreases in UV_{254} of (a, b) Nordic fulvic acid and (c, d) Nordic humic acid

solutions in the batch hydrothermal experiments for different heating temperatures (80–180 °C, 600 hours) with fitting curves by the first order reaction. Figures (b) and (d) are enlarged drawings of figures (a) and (c), respectively, in the range of 0–60 hours.

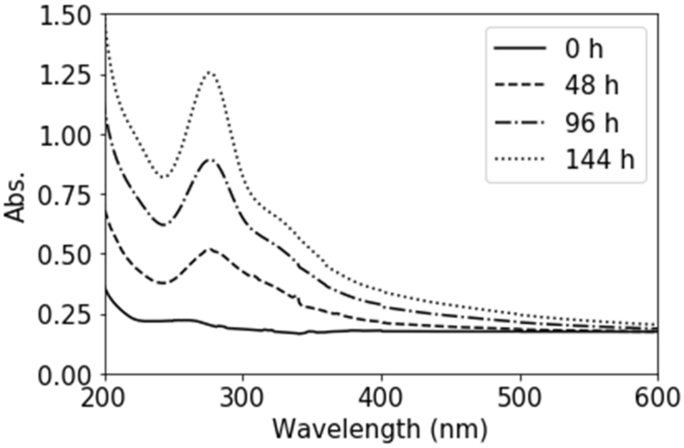

Fig. 3. Representative in situ UV–Vis spectra for 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 80 °C for about 0, 48, 96 and 144 hours (modified after Nakaya et al., 2018a).

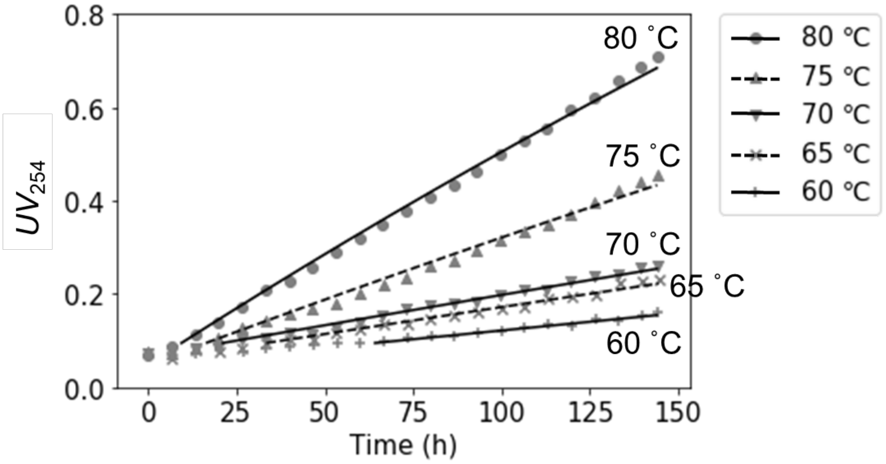

Fig. 4. Changes with time in UV_{254} during the Maillard-type browning reactions of 0.1 mol l^{-1} glycine + ribose mixture solutions heated at 60, 65, 70, 75 and 80 °C with fitting curves by the first order reaction model (modified after Nakaya et al.., 2018a). Only representative data from numerous UV_{254} values are shown as dots for clarity.

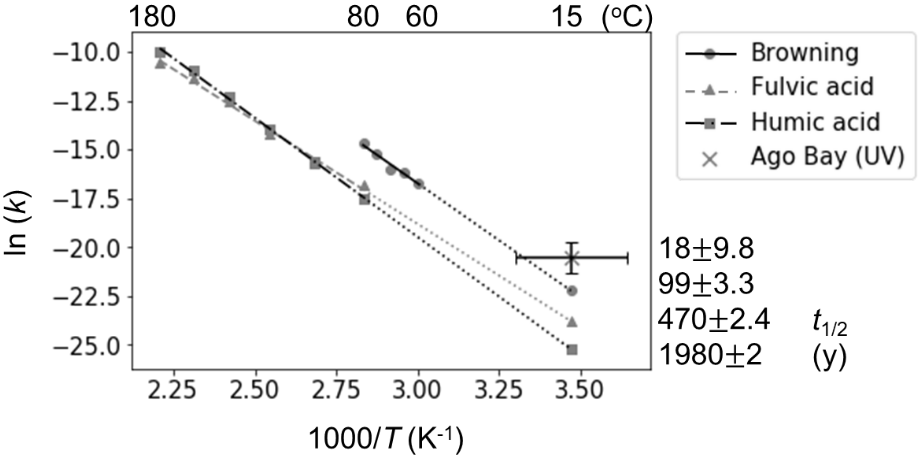

Fig. 5. Arrhenius plots of the first order reaction rate constants k values of hydrothermal changes in UV_{254} for fulvic and humic acids (decolorization) and for browning reactions with fitting lines. The extrapolation of these fitted lines (dashed lines) to 15 °C are also shown. The k value estimated by UV_{254} on Ago Bay bottom sediments is plotted with error bars. The half-time ($t_{1/2}$) values calculated by (ln 2)/k are indicated on the right side of the frame.

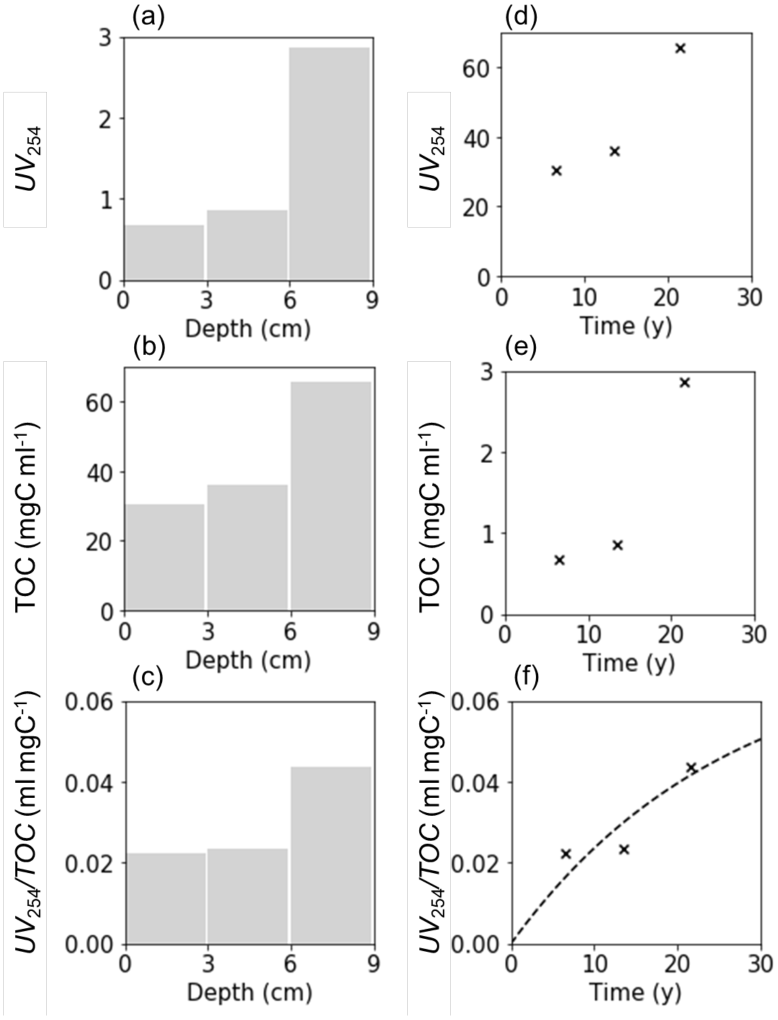

Fig. 6. The values of (a) UV_{254}, (b) TOC and (c) UV_{254}/TOC for the humic acid solutions extracted from 3 depth fractions of Takonobori and (d) UV_{254}, (e) TOC and (f) UV_{254}/TOC against the average time from the deposition with a fitting curve by the first order reaction model.