Removal of organic contaminants from iron sulfides as a pretreatment for mineral-mediated chemical synthesis under prebiotic hydrothermal conditions

Manabu Nishizawa,[1]* Masafumi Saitoh,[2,3] Yohei Matsui,[2,3] Yoichi Usui[3,4] and Takazo Shibuya[1,2,3]

[1]Department of Subsurface Geobiological Analysis and Research (D-SUGAR), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka 237-0061, Japan
[2]Research and Development (R&D) Center for Submarine Resources, JAMSTEC, Yokosuka 237-0061, Japan
[3]Project Team for Development of New-generation Research Protocol for Submarine Resources, JAMSTEC, Yokosuka 237-0061, Japan
[4]Department of Deep Earth Structure and Dynamics Research, JAMSTEC, Yokosuka 237-0061, Japan

Iron sulfides are thought to play critical roles in prebiotic chemistry as a synthesis reactant or a mineral catalyst to form key organic compounds and as an active center for proto-enzymes. Laboratory experiments with iron sulfides and other reactants at geologically reasonable low concentrations are essential to test this hypothesis. Therefore, organic contaminants potentially attached to iron sulfides should be removed as much as possible prior to experiments. This study measured amounts of organic contaminants in troilite and pyrite that are/were likely present in extraterrestrial and Hadean Earth hydrothermal environments, respectively. In addition, we evaluated the effectiveness of the anoxic heating (450–1000°C and 0.1 MPa), hydrothermal processing (300°C and 50 MPa), and wet chemical treatment (acid and/or organic solvents) of these reagents as pretreatments. Commercially available iron sulfide reagents contain considerable amounts of organic matter with up to 1560 ppm carbon and 30 ppm nitrogen. With the best cleaning procedure, anoxic heating removed 66% and 53% of the carbon and nitrogen from troilite, respectively, whereas it removed 25% and <10% of the carbon and nitrogen from pyrite, respectively. Anoxic heating and hydrothermal processing of the troilite reagents reduced the metallic iron impurity contents but produced small amounts of iron oxides. Clearly, future work is needed to establish more efficient protocols to substantially remove persistent organic components. However, appropriate sample selection combined with anoxic heating and mineral separation would allow the preparation of troilite or pyrite fractions with a relatively small amount of refractory organic contaminants (≤40 ppm C, ≤2 ppm N) that could not contribute to the synthesis of ammonia and polymeric biomolecules in simulated extraterrestrial and Hadean Earth hydrothermal conditions, respectively.

Keywords: iron sulfides, chemical evolution, hadean, extraterrestrial objects, hydrothermal activity

INTRODUCTION

Seafloor hydrothermal environments are candidate locations for the emergence of life (e.g., Russell *et al.*, 2014). A key foundation of this hypothesis is the predominance of iron sulfides in such environments. Experiments have shown that pyrrhotite ($Fe_{1-x}S$) and pyrite (FeS_2) promote the reduction of carbon monoxide (CO) to form methanethiol (CH_3SH), a key reactant for methyl thioacetate (a potential prototype of Acetyl CoA), in the presence of hydrogen sulfide (H_2S) at 100°C (Heinen and Lauwers, 1996). Further, the crystal structure of mackinawite (FeS) is affine with the active centers of hydrogenases (McGlynn *et al.*, 2009). This implies that hydrothermal mackinawite associated with peptide nests might have operated as the precursor to metalloenzymes (Russell and Hall, 1997; Russell *et al.*, 2003, 2014). Iron sulfides may, therefore, have played critical roles in prebiotic metabolisms (e.g., Wächtershäuser, 1988; Cody, 2004).

An unresolved issue in chemical evolution is whether these reactions occurred in the early earth environments, because previous experiments used reactants at high concentrations that are geologically unreasonable. In particular, ammonia or cyanide at the sub-molar level has been used as a nitrogen source in amino acid synthesis in prebiotic conditions (e.g., Marshall, 1994; Huber and Wächtershäuser, 2006). However, it is highly uncertain whether these compounds or their potential precursors (i.e., nitrogen oxides) were sufficiently abundant in hydrothermal environments to allow these reactions before the potential emergence of a nitrogen fixation metabo-

*Corresponding author (e-mail: m_nishizawa@jamstec.go.jp)

Table 1. Mineral compositions and carbon and nitrogen concentrations of solid samples

Sample	Mineral composition	Carbon conc. [μgC/g]	Nitrogen conc. [μgN/g]	Notes
Reagent 1: FeS (99%-Fe in total metal, Strem chemicals)				
Initial reagent	Major: Troilite, Minor: Monoclinic pyrrhotite, Metallic iron	25	5.6	No chemical treatment
		27	2.2	No chemical treatment
		79	3.4	Rinse with hexane and dry at 60°C in air
Solid product after anoxic heating at 1000°C[a]	Major: Troilite, Minor: Metallic iron, W stite	9	1.4	No chemical treatment after anoxic heating
		36	3.6	Rinse with hexane and dry at 60°C in air after anoxic heating
Solid product after hydrothermal processing at 300°C[b]	Major: Troilite, Minor: Metallic iron, Magnetite	58	1.7	Rinse with hexane and dry at 60°C in air after hydrothermal processing
		59	1.8	Rinse with acetone and then pure water after hydrothermal processing. Freeze-dry in vacuum.
		60	1.4	Rinse with acetone and then pure water after hydrothermal processing. Freeze-dry in vacuum.
		64	1.9	Rinse with acetone and then pure water after hydrothermal processing. Freeze-dry in vacuum.
		64	1.9	Rinse with acetone and then pure water after hydrothermal processing. Freeze-dry in vacuum.
Reagent 2: FeS (99.9%-Fe in total metal, Alfa aesar)				
Initial reagent	Major: Troilite, Minor: Monoclinic pyrrhotite, Metallic iron	37	3.5	No chemical treatment
		45	2.9	No chemical treatment
		73	3.1	Rinse with hexane and dry at 60°C in air
		95	3.8	Rinse with hexane and dry at 60°C in air
Outer part of solid product in a Pt-Rh crucible ("rim") after anoxic heating at 1000°C[a]	Major: 1C-pyrrhotite, Minor: Magnetite	33	0.6	No chemical treatment after anoxic heating
		23	1.2	Rinse with hexane and dry at 60°C in air after anoxic heating
Inner part of solid product in a Pt-Rh crucible ("core") after anoxic heating at 1000°C[a]	Major: 1C-pyrrhotite, Minor: Magnetite	19	0.9	No chemical treatment after anoxic heating
		22	0.6	No chemical treatment after anoxic heating
		38	1.2	Rinse with hexane and dry at 60°C in air after anoxic heating

Sample	Mineral composition	Carbon conc. [µgC/g]	Nitrogen conc. [µgN/g]	Notes
Reagent 3: FeS$_2$ (Alfa aesar 12842)				
Initial reagent	pyrite	1443	25	No chemical treatment
Solid after sequential rinse	pyrite	1560	30	Rinse with pure water and freeze-dry in vacuum
	pyrite	1560	25	Rinse with 6N HCl, pure water, methanol, and hexane sequentially. Freeze-dry in vacuum.
Solid product after anoxic heating at 450°C[c]	pyrite	1090	25	No chemical treatment after anoxic heating
Solid product after anoxic heating at 600°C[d]	pyrite	1081	22	No chemical treatment after anoxic heating
	pyrrhotite	1147	13	No chemical treatment after anoxic heating
Reagent 4: Powder of large euhedral pyrite crystals from Peruvian mine (2 cm in length)				
Initial reagent	pyrite	35	1.6	No chemical treatment
Solid after sequential rinse	pyrite	82	2.3	Rinse with 6N HCl, pure water, methanol, and hexane sequentially. Freeze-dry in vacuum.
Synthetic komatiite[e]		209	0.6	No chemical treatment
		208	0.5	Rinse with acetone and dry at 60°C in air

a. The conditions for the anoxic heating were 1000°C, 180 min, and oxygen fugacity at the quartz–fayalite–magnetite (QFM) buffer; b. The conditions for the hydrothermal processing were 300°C, 50 MPa, and 60 12 min; c. The conditions for the anoxic heating were 450°C and 90 min under a CO$_2$ gas atmosphere; d. The conditions for the anoxic heating were 650°C and 180 min under an H$_2$–CO$_2$ mixed gas atmosphere (H$_2$:CO$_2$ = 1:40); e. The protocol for the komatiite synthesis is given in Ueda et al. (2016).

lism as early as 3.5 Ga (Nihsizawa *et al.*, 2014). Even though N$_2$ would have been the most abundant nitrogen compound in ancient hydrothermal fluids (250–1500 µmol/kg; Nishizawa *et al.*, 2007; Marty *et al.*, 2013), it remains to be revealed if N$_2$ was quantitatively reduced appreciably to ammonia in prebiotic hydrothermal environments.

Further, the chemical evolution associated with the hydrothermal activity may have occurred not only on the early Earth but also on extraterrestrial objects. Ongoing hydrothermal reactions of aqueously unaltered chondritic cores above 50°C are thought to occur within the Saturnian icy satellite Enceladus (Hsu *et al.*, 2015; Sekine *et al.*, 2015). In addition, aqueous alterations of the parent bodies of carbonaceous chondrites (up to 150°C) are thought to have occurred in the early stages of solar system formation (e.g., Clayton and Mayeda, 1999). Because troilite (FeS) is the primary sulfide mineral in chondrites and bio-related organics (e.g., amino acids) exist in carbonaceous chondrites (Lauretta *et al.*, 1996; Botta and Bada, 2002; Bullock *et al.*, 2005), these findings suggest the possibility of the extraterrestrial hydrothermal synthesis of polymeric biomolecules (e.g., peptides) in the presence of troilite, which has not yet been studied but is testable via a comparison of the organic composition of the experimental products and those of carbonaceous chondrites or returned samples from C-type asteroids and Enceladus in future space missions (e.g., HAYABUSA2).

To test the role of iron sulfides in the synthesis of ammonia and polymeric biomolecules, laboratory experiments under controlled chemical conditions relevant to Hadean or extraterrestrial hydrothermal environments are necessary. The removal of organic contaminants attached to iron sulfides is a key pretreatment in such experiments to detect low-yield products; this minimizes their blanks originating from organic contaminants and prevents interference with polymerization due to undesired reactions of starting organic compounds (e.g., AA) with organic contaminants. However, criteria for sample selection and cleaning protocols have not been reported for iron sulfides in previous studies. This paper reports the results of the anoxic heating, hydrothermal processing, and wet chemical treatment of troilite and pyrite to evaluate the effectiveness of the decomposition of organic contaminants and its influences on the mineralogy.

MATERIALS AND METHODS

Sample selection and experiments

Two types of synthetic FeS reagents with different metal purities (R1: Strem chemical 93-2640, 99%-Fe in total metal and R2: Alfa aesar 14024, 99.9%-Fe in total metal) were used as starting materials for troilite, because troilite rarely occurs in terrestrial environments. For the

anoxic heating of the reagents, 12 g of R1 and 30 g of R2 were heated separately in a Pt-Rh crucible at 1000°C for 180 min in an electric furnace, regulating the oxygen fugacity at the quartz-fayalite-magnetite (QFM) buffer under an H_2-CO_2 mixed gas atmosphere. The solid products were subsequently quenched in air to room temperature and were crushed in an agate mortar. For the anoxic heating of R2, the outer part of the solid product (the "rim") in a crucible was blackish in color, whereas the inner part (the "core") was yellowish in color. Therefore, they were separated via visual inspection. The hydrothermal experiment was performed by reacting 12 g of R1 and 48.6 g of pure water in a gold reaction cell at 300°C and 50 MPa for 6012 min. Before the experiment, headspace gas (i.e., air) in the reaction cell was extruded via hand compression. Approximately 3 g of reacted fluid was collected from the reaction cell to measure the ammonium concentration just before the completion of the experiment. Some of the solid products after anoxic heating or hydrothermal processing were rinsed with hexane (HPLC analysis grade, Wako), acetone (Reagent grade, Wako), and/or pure water (18.4 MΩ·cm at 25°C) to remove lipophilic and/or hydrophilic organic components that can potentially be produced from thermal or hydrothermal decomposition of organic contaminants that initially existed in the FeS reagents. Approximately 3–4 g of the solid products and 50 mL of the rinse solution were loaded in centrifuge glass tubes (pre-combusted at 450°C for 4 h) and were placed in an ultrasound bath at room temperature for 20 min. After rinsing and decantation, the resultant deposits were freeze-dried in a vacuum or dried at 60°C in air until the analyses (Table 1). For FeS, we did not use hydrochloric acid (HCl) to hydrolyze the organic components (e.g., proteins) due to the high solubility of FeS in HCl solutions.

Two pyrite samples were selected in this study. One was a fine-grained FeS_2 reagent (R3: Alfa aesar 12842) and the other was a powder sample made from large euhedral pyrite crystals (approximately 2 cm in length) from a Peruvian Mine (R4). Rinsing and anoxic heating were applied separately to the samples. We used 6N HCl (Nakarai), pure water (18.4 MΩ·cm at 25°C), and organic solvents (methanol, Wako reagent grade; hexane, Wako HPLC analysis grade) as rinse solutions to hydrolyze the proteins and remove the organic components with hydrophilic (e.g., amino acids) and lipophilic (e.g., lipids) properties, respectively. Anoxic heating was applied to 2 g of R3 in a Pt-Rh crucible at 450°C for 90 min or 650°C for 180 min in an electric furnace under a CO_2 or an H_2-CO_2 mixed gas atmosphere, respectively, and then the sample was cooled to room temperature in O_2-free conditions.

Mineralogical and chemical analyses
The mineral composition was analyzed using X-ray diffraction (XRD; Rigaku MiniFlex II) and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDS; Hitachi TM3000). The XRD peak assignment was made with the aid of the software PDXL using the Inorganic Crystal Structure Database. The temperature dependence of the low-field magnetic susceptibility of the solid samples was also measured to obtain qualitative and quantitative data of the iron speciation at a bulk scale. Magnetic measurements were conducted using an AGICO Kappabridge KLY-4/CS-3 during heating from –172°C to room temperature and when cycling from room temperature to 700°C in argon.

The carbon and nitrogen concentrations in the solid samples were measured via flush combustion in a FLASH EA1112 elemental analyzer online with a Finnigan DELTAplus Advantage mass spectrometer. The precision achieved by repeated analyses of an in-house standard was better than 10% for the carbon and nitrogen concentrations.

The ammonium concentration in the reacted fluid from the hydrothermal experiment was determined with fluorescence photometry after reacting at 50°C with a 20 mM orthophthaldialdehyde solution and a 200 mM sodium borate solution containing 2 mM of sodium sulfite.

Calculation of the maximum concentration of ammonia in the N_2-NH_3-H_2-H_2O system
The maximum concentration of ammonia in the N_2-NH_3-H_2-H_2O system was calculated assuming chemical equilibrium, $N_2 + 3H_2 \rightleftarrows 2NH_3$. Considering the mass balance of nitrogen and hydrogen in the closed system, the concentrations of N_2, H_2, and NH_3 in equilibrium can be expressed as $C_{N2,initial} - 0.5C_{NH3,eq}$ μmol/kg, $C_{H2,initial} - 1.5C_{NH3,eq}$ μmol/kg, and $C_{NH3,eq}$ μmol/kg, respectively. $C_{N2,initial}$ and $C_{H2,initial}$ denote the initial concentrations of N_2 and H_2, respectively, in the fluid prior to N_2 reduction. $C_{NH3,eq}$ denotes the ammonia concentration in equilibrium. Therefore, $C_{NH3,eq}$ was estimated from equation (1): $C_{NH3,eq} = K^{0.5} (C_{N2,initial} - 0.5C_{NH3,eq})^{0.5}(C_{H2,initial} - 1.5C_{NH3,eq})^{1.5}$. K denotes the equilibrium constant K for $N_2 + 3H_2 \rightleftarrows 2NH_3$ and was estimated using the software SUPCRT92 (Johnson *et al.*, 1992).

RESULTS

Mineral composition
Prior to the experiments, synthetic FeS reagents R1 and R2 had similar XRD patterns with main peaks at 29.8°, 33.5°, 43.1°, 47.1°, and 53.1° (2θ) corresponding to the main peaks of hexagonal troilite with a = b = 5.965 Å and c = 11.759 Å (space group: P-6 2c, No. 190) (Fig. 1a). Troilite with this space group has been found in chondrites and a lunar sample (Evans, 1970; Skala *et al.*, 2006). In addition, the XRD peak at 43.8° suggests the existence of pyrrhotite in R1 and R2. Further informa-
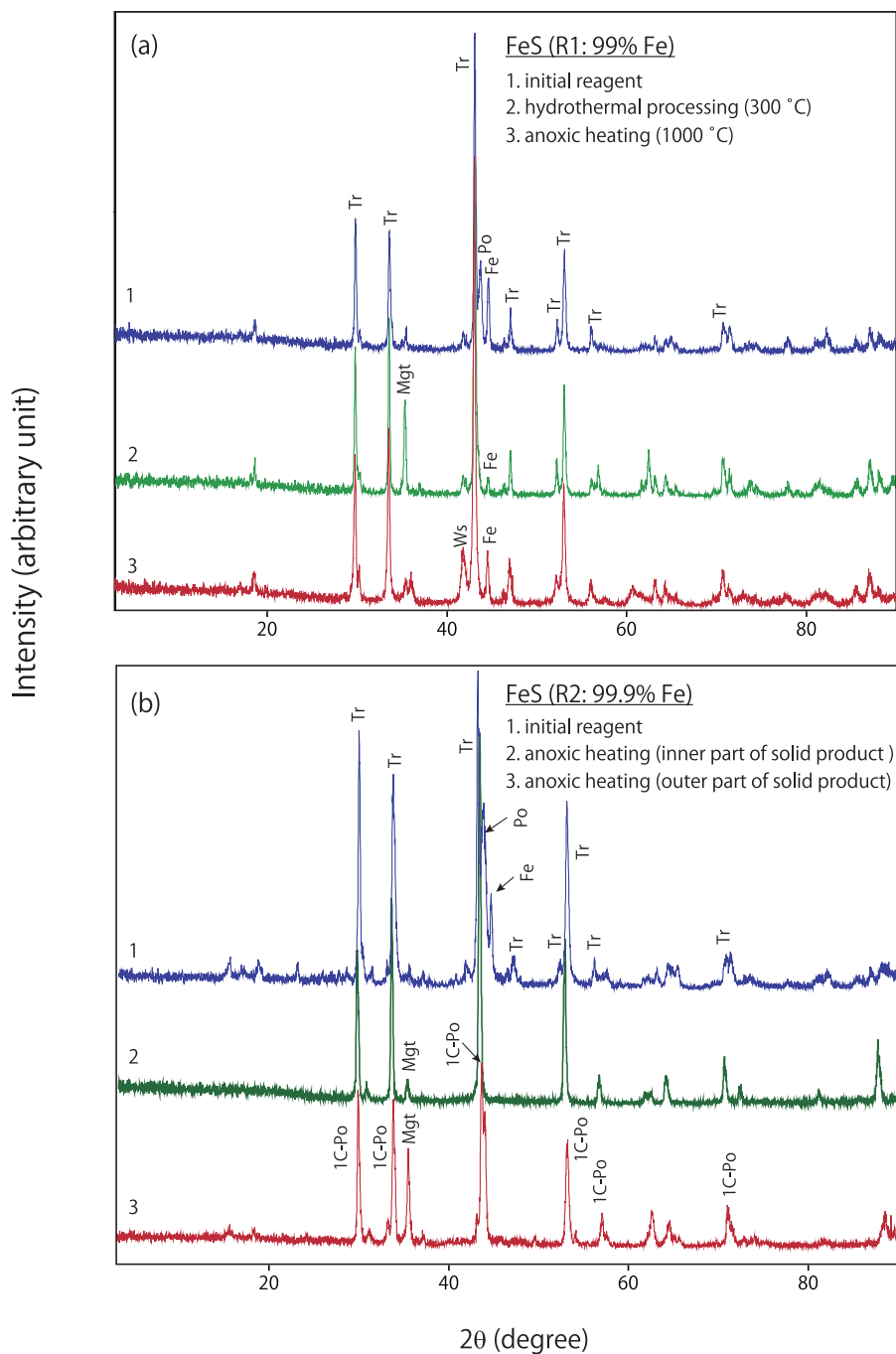
Fig. 1. X-ray diffraction spectra of the FeS reagents before and after thermal or hydrothermal processing. Tr: troilite (space group P-6 2c, No. 190); 1C-Po: Pyrrhotite (space group P 63 mc, No. 186); Po: pyrrhotite; Fe: metallic iron; Mgt: magnetite; Ws: wüstite.

tion can be obtained from the magnetic susceptibility data. The magnetic susceptibility curves of R1 exhibit little change and remain high even at 700°C (Fig. 2a). This is not expected for ferrimagnetic iron sulfides (Curie temperature < 700°C) but can be explained if the main phase of iron sulfide in R1 has an atomic percentage of iron

greater than 48% (Schwarz and Vaughan, 1972). Even though hexagonal pyrrhotite is also a non-ferrimagnetic iron sulfide, the pyrrhotite in R1 is likely a monoclinic phase because of a small but sharp decrease in the susceptibility at 320°C (the Curie temperature of monoclinic pyrrhotite) and the absence of a sharp increase in the sus-
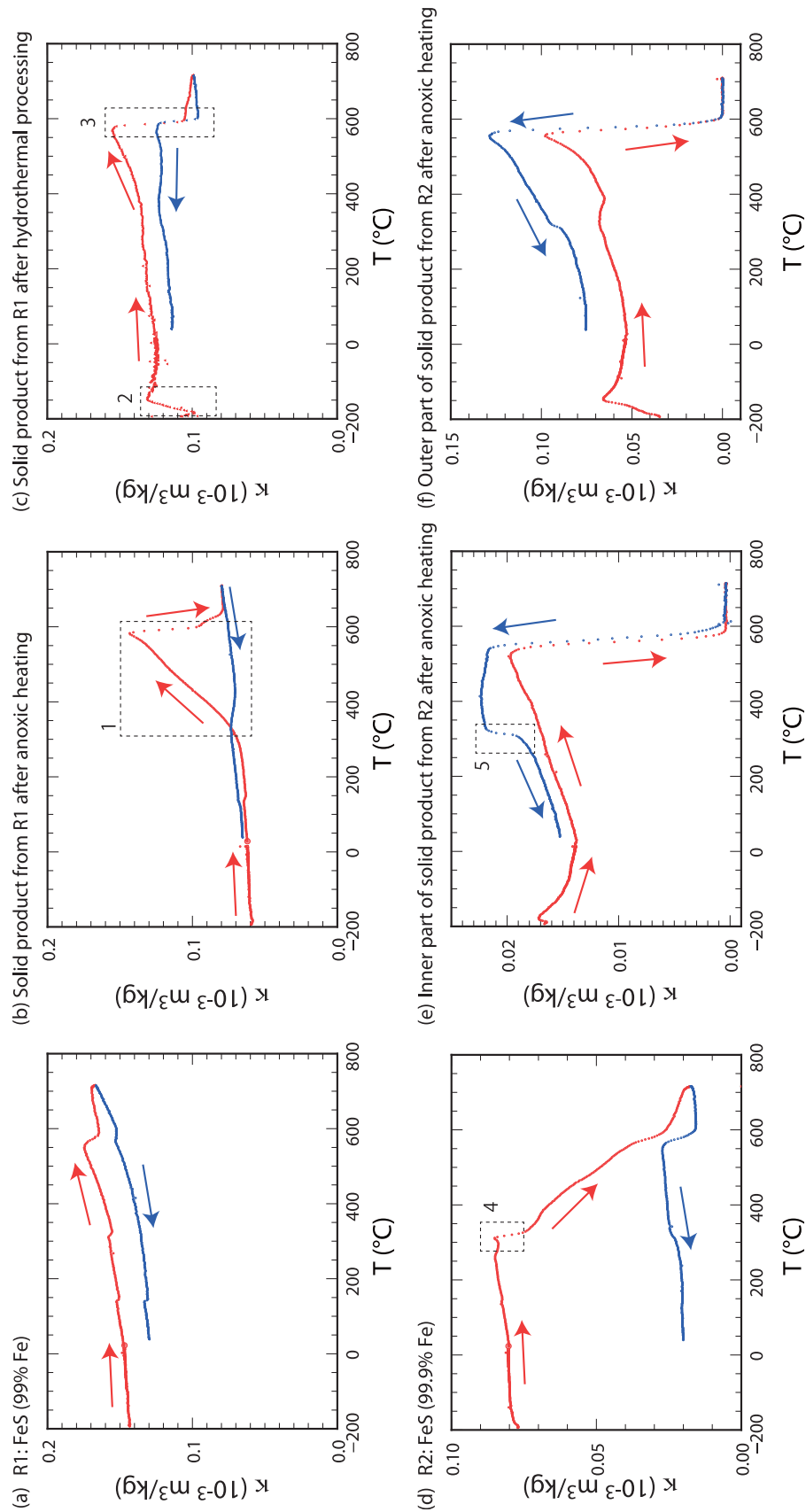
Fig. 2. Magnetic susceptibility curves of the FeS reagents and their solid products after anoxic heating or hydrothermal processing. The main magnetic features are highlighted in the dotted boxes and are interpreted as 1) the formation of magnetite upon heating, 2) the Verwey transition of magnetite at approximately −153°C, 3) the susceptibility change at the Curie temperature of magnetite (580°C), 4) the susceptibility change at the Curie temperature of monoclinic pyrrhotite (320°C), and 5) the β-transition of FeS at 315°C.

ceptibility near 200°C (the λ-transition of hexagonal pyrrhotite) (Fig. 2a; Supplementary Materials). Collectively, the iron sulfide phase in R1 is primarily hexagonal troilite with monoclinic pyrrhotite as a minor phase. The main iron sulfide phase in R2 is also hexagonal troilite with monoclinic pyrrhotite as a minor phase (Figs. 1b and 2a; Appendix).

The main phases of iron sulfide in the solid products after the experiments with anoxic heating or hydrothermal processing of R1 were the same as those of the starting reagent (troilite with space group P-6 2c), as evidenced by a distinct XRD peak at 47.1° (Fig. 1a). By contrast, the main phases of iron sulfide in the solid products after the anoxic heating of R2 were likely different from those of the starting reagent as suggested by a slight shift in the peak position at 43.5° (Fig. 1b) and the lack of a peak at 47.1° (Fig. 1b). These XRD features best correlate with 1C-pyrrhotite with a NiAs-type unit cell of a = b = 3.456 Å and c = 5.779 Å (space group: P 63 mc, No. 186), which is stable above 100–150°C (e.g., Nakazawa and Morimoto, 1970). The differences in the crystal structures of the solid products after anoxic heating of R1 and R2 are unclear but may originate from the sluggish kinetics of structural change in the coarser grained troilite (R1) (Supplementary Figs. S1 and S2).

An additional mineralogical characteristic of the FeS reagents was the existence of metallic iron (Fe(0)) (Figs. S1 and S2), a reactant to synthesize troilite (Fe + S $\rightarrow$ FeS). The mass-normalized magnetic susceptibility κ at 700°C can be used as a measure of the α-Fe(0) concentration because the Curie temperature of bcc α-Fe(0) is 765°C (Dunlop and Özdemir, 1997). Using this index, the α-Fe(0) concentration in R2 was estimated to be 13% of the corresponding value for R1 (i.e., κ_{R2}/κ_{R1} at 700°C = (0.18 × 10^{-3} m^3/kg)/(1.7 × 10^{-3} m^3/kg); Figs. 2a and 2d). Notably, α-Fe(0) was nearly absent in the solid product after the anoxic heating of R2 (<0.1 × 10^{-3} m^3/kg at 700°C). By contrast, α-Fe(0) likely remained in the solid products after the anoxic heating and hydrothermal processing of R1. The α-Fe(0) concentrations in those solids were estimated to be 50% and 60% of the corresponding values in R1, respectively (Figs. 2a–c). The decrease in the α-Fe(0) concentration resulting from anoxic heating and hydrothermal processing likely originates in the oxidation of Fe(0) to magnetite or wüstite, as suggested by the XRD peak at 35.5° and the magnetic features of the solid products (Figs. 1, 2b, 2c, 2e, and 2f; Appendix).

For the pyrite sample R3, anoxic heating at 450°C did not induce the decomposition of pyrite; however, anoxic heating at 650°C induced the complete decomposition of pyrite to pyrrhotite. The contrasting results in the anoxic heating experiments likely arose from the sluggish kinetics of the thermal decomposition of pyrite below 500°C

in O$_2$-free conditions, as suggested by the experimental evidence that the rate constant of the reaction FeS$_2$ $\rightarrow$ FeS + 1/n S$_n$ at 450°C was below 1% of that at 650°C (Schwab and Philinis, 1947). A sequential rinse with 6N HCl, pure water, and organic solvents (methanol and hexane) of R3 and R4 in air did not induce the change in the mineral composition of the resultant solids (i.e., pyrite with a = b = c = 5.417 Å, Pa-3), even though a part of the pyrite might have dissolved to form ferrous iron (Fe^{2+}) and sulfate during the acid wash.

Carbon and nitrogen concentrations

The average concentrations of carbon and nitrogen in the FeS reagents prior to the experiments were 27 ppm and 2 ppm respectively, for R1 and 59 ppm and 3 ppm respectively, for R2 (Table 1). The carbon isotope ratios of R1 and R2 were –26‰ and –30‰, respectively, suggesting that the main carbon phase in R1 and R2 was organic matter. The hydrothermal processing of R1 increased the carbon concentration in the solid phase by 100% compared to the starting material (R1 before the experiment). The reason for this increase in the carbon concentration may arise from the contamination of nonvolatile organics potentially dissolved in organic solvents (hexane and acetone), which were used for the rinse after the hydrothermal processing and remained on the FeS surface during drying in air or a vacuum. This study used high purity hexane (HPLC analysis grade) from a glass container that was freshly opened, and therefore dissolution of the organic contaminants might have occurred during the rinse of the samples. However, when the solid phase from the anoxic heating was not washed with the organic solvents, the carbon concentration in the solid decreased to averages of 66% and 40% in comparison to that of the initial concentrations of R1 and R2, respectively, before the experiments.

The hydrothermal processing of R1 reduced the nitrogen concentration in the solid phase by 53% compared to the starting material. The amount of nitrogen decrease in the solid phase (1.7 μmol N) was comparable to the amount of ammonium released to the aqueous fluid during the hydrothermal processing (2.6 μmol N). The anoxic heating of R1 and R2 also reduced the nitrogen concentration in the solid phase by 62% and 80%, respectively, compared to the starting materials. Collectively, anoxic heating and hydrothermal processing promote the decomposition of the organic contaminants of FeS.

The carbon and nitrogen concentrations in pyrite were 1443 ppm and 25 ppm, respectively, for pyrite R3. The carbon isotope ratio of R3 was –29‰ and, therefore, indicates the existence of organic matter in R3. Both the rinse with pure water and the sequential rinse with 6N HCl, pure water, and organic solvents (methanol and hexane) increased the carbon concentration of R3 by 8%
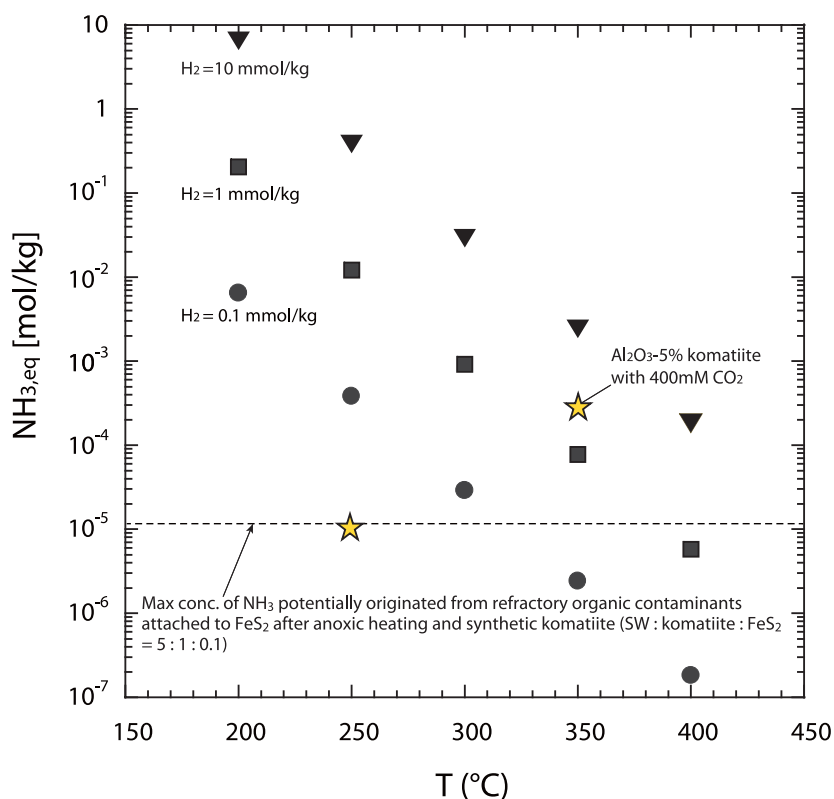
Fig. 3. Relation between the temperature and calculated NH_3 concentration in chemical equilibrium with N_2 and H_2 in a hydro-thermal fluid. The N_2 concentration is set to 500 µmol/kg.

(+117 ppm) and 8% (+117 ppm), respectively, whereas the anoxic heating of R3 at 450°C reduced the carbon concentration in the solid phase by 25% (–360 ppm). These results indicate that R3 likely associates organic compounds that are resistant to acid hydrolysis and are insoluble to water and organic solvents (e.g., kerogen), consistent with its high C/N atom ratio (69). By contrast, the anoxic heating of pyrite partly decomposed such persistent organics, as in the case of the FeS reagents. The nitrogen concentration of R3 was not significantly changed by the wet chemical treatments and anoxic heating at 450°C, suggesting that nitrogen exists as persistent moieties.

The carbon and nitrogen concentrations in a powder sample made from large pyrite crystals (R4) were 35 ppm and 2 ppm, respectively. The carbon isotope ratio of R4 was –32‰. These results indicate the existence of organic matter in R4; however, the abundance of organic matter in R4 is much lower than that in R3.

DISCUSSION

Iron sulfides are key materials to consider the origin of life. Therefore, the removal of organic contaminants attached to iron sulfides is a key pretreatment in a variety of experiments to test the role of iron sulfides in prebiotic chemistry. This study shows that commercially available iron sulfide reagents contain considerable amounts of organic contaminants (up to 50 ppm carbon and 6 ppm nitrogen for troilite and up to 1560 ppm carbon and 30 ppm nitrogen for pyrite). Such organic contaminants were difficult to completely remove from iron sulfides under the experimental conditions of this study, suggesting that most of them exist as persistent organic components that don't thermally decompose or dissolve with non-polar and polar solvents. Clearly, future work is needed to establish more efficient protocols to substantially remove persistent organics. The iron sulfides prepared in this study, however, could be used in some experiments as discussed below.

Of the cleaning protocols tested in this study, the anoxic heating of iron sulfides robustly decomposed a relatively labile fraction of organic contaminants (Table 1). Therefore, it is expected that organic contaminants surviving anoxic heating may be kerogen-like components that are unreactive in anoxic conditions at 1000°C for troilite and 650°C for pyrite. Therefore, after anoxic heating, troilite could be used to test the extraterrestrial synthesis of biomolecules (e.g., peptides) in experiments under anoxic conditions at 150°C (the temperature of

aqueous alterations of carbonaceous chondrites). In such experiments, the solid product after the anoxic heating of R1 is better, at present, to use as a starting material, because the solid product is primarily composed of hexagonal troilite found in chondrites (Fig. 1a; Skala *et al.*, 2006). Even though the solid product contained a small amount of magnetite, wüstite, and metallic iron, magnetic separation of these minerals would enable us to concentrate the troilite fraction with a small amount of refractory organic contaminants (≤30 ppm C).

Further, prebiotic nitrogen fixation in early Earth hydrothermal environments could be tested using the pyrite samples prepared in this study. In the hydrothermal experiments under simulated Hadean conditions, the steady-state H_2 concentrations of fluids from the reaction of synthetic komatiite (synthetic temperature 1450°C) and CO_2-rich seawater were 24 μmol/kg and 3 mmol/kg at 250°C and 350°C, respectively (water/rock mass ratio = 5; Ueda *et al.*, 2016). Assuming that FeS promotes the N_2 reduction by reacting with H_2 originating from the serpentinization of komatiite toward chemical equilibrium ($N_2 + 3H_2 \rightleftarrows 2NH_3$), it is estimated that the ammonia concentration in a fluid can be up to 10 μmol/kg and 280 μmol/kg at 250°C and 350°C, respectively, in the closed system of CO_2-rich seawater : komatiite : pyrite = 5:1:0.1 with 500 μmol/kg of N_2 in the unreacted fluid (equation (1); K = $10^{-4.236}$ at 250°C and $10^{-2.038}$ at 350°C) (Fig. 3). By contrast, when using the pyrite sample R4 (1.6 ppm N), the maximum concentration of ammonia that potentially originates in the deamination of organic contaminants from pyrite plus that which originated in the synthetic komatiite (0.6 ppm N; Table 1) is calculated to be 11 μmol/kg in the experiment of the same system. Therefore, when using isotopically labeled $^{15}N_2$, the relatively low nitrogen blank of the starting materials enables the distinction of ammonia produced from the N_2 reduction ($^{15}NH_3$) and that produced from the refractory organic contaminants ($^{14}NH_3$) in the experiment at 350°C. Further, if the experiment is designed to evaluate the pyrite-mediated N_2 reduction more strictly in the system of CO_2-rich seawater and pyrite without komatiite but with pure H_2 and N_2 gases, the maximum concentration of ammonia contaminants from the staring material is reduced to 3 μmol/kg (seawater : pyrite R4 = 5:0.1), which enable us to test the pyrite-mediated N_2 reduction even at 250°C. Because it is likely that large euhedral pyrite deposited from hydrothermal activity (e.g., R4) only contains relatively small amounts of organic matter within its crystal, such pyrite is better to use in the experiment for the pyrite-mediated synthesis of ammonia.

CONCLUSIONS

Based on the mineralogical (XRD, magnetic suscep-

tibility) and chemical analyses, this study provides the criteria for the selection of troilite and pyrite reagents and for the evaluation of the anoxic heating, hydrothermal processing, and wet chemical treatment to remove organic contaminants. The complete removal of organic contaminants from iron sulfides is difficult under the experimental conditions of this study, and future effort is needed to establish more efficient protocols to substantially remove organic contaminants (e.g., >90%). In addition, anoxic heating and hydrothermal processing of troilite reagents reduced the metallic iron impurity contents but produced small amounts of iron oxides. Nonetheless, appropriate sample selection combined with anoxic heating and mineral separation would allow the preparation of troilite or pyrite fractions with a relatively small amount of refractory organic contaminants (≤40 ppm C, ≤2 ppm N) that could not contribute to the synthesis of ammonia and polymeric biomolecules in simulated extraterrestrial or prebiotic earth hydrothermal conditions.

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APPENDIX: INTERPRETATION OF THE MAGNETIC SUSCEPTIBILITY CURVES

99%-Fe samples (R1)

The magnetic susceptibility curves of R1 exhibit little change and remain high even at 700°C (Fig. 2a). This is not expected for ferrimagnetic iron sulfides or iron oxides that have Curie temperatures below 700°C, indicating that the main carrier of the susceptibility signal is bcc α-Fe, which has a Curie temperature of 765°C (Schwarz and Vaughan, 1972; Dunlop and Özdemir, 1997). In other words, the iron sulfide phase in this sample is primarily non-magnetic. The candidate phases are troilite and hexagonal pyrrhotite. The absence of the λ-transition of hexagonal pyrrhotite, a sharp increase in the susceptibility near 200°C, suggests that troilite is more likely (Schwarz and Vaughan, 1972). The solid products after the anoxic heating and hydrothermal experiments also reveal the dominance of α-Fe(0) in the magnetic susceptibility (Figs. 2b and 2c). The solid product after the anoxic heating shows a gradual increase in the susceptibility from 300°C followed by a sharp decrease at 580°C (Fig. 2b), suggesting the formation of magnetite upon heating. Indeed, a sister sample heated to 600°C exhibited the Verwey transition of magnetite at approximately −153°C. The magnetite signal was not detected in the cooling curve after 700°C, indicating the destruction of magnetite between 600°C and 700°C. To explain this behavior, we speculate that the sample contained metastable FeO wüstite due to quenching. Below 550°C, wüstite + α-Fe decompose to magnetite + α-Fe. Nonetheless, the room temperature susceptibilities before and after the heating in the Kappabridge are comparable. This suggests that most of the α-Fe(0) was not involved in the formation and destruction of magnetite and that the susceptibility values at room temperature and 700°C simply reflect the amount of α-Fe(0). After the hydrothermal experiment, the sample showed sharp susceptibility changes at the Curie temperature and the Verwey transition of magnetite (585°C and −153°C, respectively) (Dunlop and

Özdemir, 1997), suggesting the production of magnetite during the hydrothermal experiment.

99.9%-Fe samples (R2)

The heating magnetic susceptibility curve of R2 exhibits a sharp decrease at 320°C, which we interpret as the Curie temperature of monoclinic pyrrhotite (Fig. 2d). The susceptibility at 700°C is approximately 12.5% the corresponding value for R1 (Figs. 2a and 2d). The solid products after the anoxic heating experiment do not show sharp changes at 320°C. The primary feature of those samples is a sharp drop at the Curie temperature of magnetite (580°C). Of the materials from the inside and outside of the solid product after the anoxic heating, the latter showed larger susceptibilities and sharper decreases at ~580°C and a more pronounced Verwey transition (Fig. 2f). These observations indicate that the outside material contains more abundant, near-stoichiometric magnetite, likely formed by oxidation during quenching. After heating to 700°C in the Kappabridge, all samples exhibited a decrease in their susceptibility at 315°C upon cooling. This is likely the β-transition of FeS. The absence of the β-transition in the heating susceptibility curve is perplexing; however, quenching after the anoxic heating experiment may preserve the metastable high temperature phase. The absence of the Curie temperature of monoclinic pyrrhotite or the λ-transition of hexagonal pyrrhotite in the susceptibility curves indicates that the iron sulfide in these samples is troilite.