

Evidence of chemical pathways of humification: a study of aquatic humic substances heated at various temperatures

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Abstract

The effects of thermal evolution on the composition and structure of aquatic humic substances (HS) heated at temperatures from 100°C to 500°C in presence of helium were investigated using elemental analysis, FTIR and solid state ¹³C CP/MAS NMR spectroscopy, and Py-GC/MS spectrometry. Elemental composition results showed that with increasing heating temperatures, there was an increase in the atomic H/C ratio and a decrease in the atomic O/C ratio in the HS. FTIR, solid state ¹³C CP/MAS NMR, and Py-GC/MS results showed decreases in amounts of oxygen-containing groups followed by their subsequent disappearance at higher temperatures. Formation of aromatics was observed at expense of aliphatics at higher temperatures. This study has given a substantial amount of information on mass loss, structural evolution, and the degree of maturity as the humic substances are heated with increasing temperatures, suggesting that thermal evolution may play an important role in the contribution of the HS as a precursor of coal like organic materials. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Aquatic humic substances are organic materials originating from both autochthonous (produced by aquatic organisms such as phytoplankton and microbes) and allochthonous (derived from terrestrial sources leached from soils and higher plants) sources.

As a major portion (approx. 70%) of the earth's organic materials, humic substances in all aquatic and terrestrial environments are transformed during geochemical processes. They are important starting materials for peat, coal, and petroleum deposits. The organic transformations associated with genesis of these fossils have been extensively studied. However, humification is the most important process during peatification and early stages of coalification (Teichmüller and Teichmüller, 1975). There are two main mechanisms, which have been proposed to interpret how humification takes place: the selective

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preservation of plant biopolymers, as reviewed by de Leeuw and Largeau (1993), and the polycondensation of small molecules (Bollag and Loll, 1983). During these processes, humic substances, gradually lose oxygen functional groups and by means of aromatization and condensation reactions, are incorporated into peat and coal as insoluble humins (Teichmüller and Teichmüller, 1975).

Artificial thermal techniques have been widely used to simulate maturation and diagenesis of natural organic matter, in particular, to determine the thermal degradation products, structural transformation, reaction mechanism, and kinetic parameters (Landais et al., 1989, 1993; Rose et al., 1992; Behar and Hatcher, 1996; Buchanan et al., 1997; Huang and Otten, 1998; Vane and Abbott, 1999) since it has been suggested by geological case histories that time and temperature are to some degree interchangeable. For example, hydrocarbons were generated when lignin was heated (Buchanan et al., 1997; Vane and Abbott, 1999). Oil was formed when kerogen was cooked (Rose et al., 1992; Huang and Otten, 1998). These observations allow artificial thermal techniques to study the role of temperature in the processes of natural organic matter maturation and hydrocarbon generation (Landais et al., 1993).

Artificial simulation of natural organic matter gives a good interpretation of structural changes, which may take place in the sedimentary basins during thermal maturation by temperature over the course of time. The most important information derived from artificial thermal simulation is transformations of aromatic rings from aliphatic carbons and oxygenated aliphatic carbons. However, caution is needed when this artificial simulation data is extrapolated to natural systems. It is never possible to compare quantitatively volatile products generated in the laboratory with those in the natural systems due to sampling problems (Behar et al., 1995). The reactions of some aliphatic structures, derived from the side-chain carbons of lignin are not simulated by such an artificial maturation process (Behar and Hatcher, 1996). This partially explains the deviations from the Van Krevelen diagram, which is observed in most artificial thermal maturation experiments (Behar and Hatcher, 1996). Nevertheless, the artificial simulation experiment is best way so far to obtain detailed information on maturity and early

coalification for natural organic substances. Artificial thermal evolution study for the immature natural organic substances is important in understanding the conversion of fossil materials derived from them.

Since humification is the most important process during early coalification, the aim of this study was to demonstrate an artificial thermal maturation process of the HS heated at different temperatures. The study could be interpreted in terms of the direct formation of lignite like materials from heating. In addition, structural changes of the HS during heating may help to understand the fate of depositional HS during humification.

2. Experimental

2.1. Chemicals

Chemicals were analytical grade from Sigma-Aldrich (Sigma-Aldrich, Castle Hill, New South Wales, Australia).

2.2. Isolation of swamp HS

Swamp water was collected from the Neranie swamp region, at the eastern shore of Myall Lake, New South Wales. The brown-colored swamp water is relatively unpolluted with a low pH of 4.2 and a low conductance of $\sim 130 \mu\text{S}/\text{cm}$. Isolation of the HS followed the procedure described by Aiken (1985). Full details of isolation procedure can be found elsewhere (Lu et al., 2000).

2.3. Simulated thermal maturation

Artificial maturation of the HS was carried out by heating about 100 mg of each sample for 15 min in a stream of helium gas at a flow rate of $100 \text{ cm}^3 \text{ min}^{-1}$ at temperatures ranging from 100°C to 500°C . The outline of the equipment is shown in Fig. 1.

2.4. Elemental analysis

The elemental composition (C, H, N, O) of the HS heated at different temperatures was determined by combustion analysis using a Perkin-Elmer 2400 CHN elemental analyzer. Elements of C, H, and N

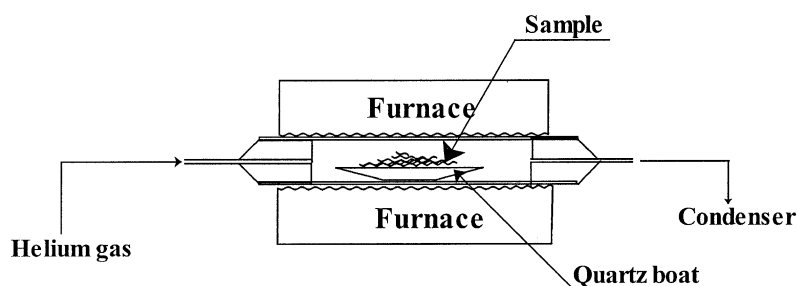


Fig. 1. Apparatus used for HS heated at different temperatures in the presence of a helium atmosphere.

were obtained directly from analysis, whereas oxygen was calculated by difference, i.e., $O\% = 100 - C\% - H\% - N\%$, and can include trace fractions of S and P elements. The chemical characteristics of the HS heated at different temperatures are summarized in Table 1.

2.5. FTIR spectroscopic measurement

FTIR spectra of HS heated at different temperatures were obtained on a Biorad SPC 3200 spectrometer. Approximately 16 scans were collected using 8-cm^{-1} resolution over the wave number range from 4000 to 400 cm^{-1} at room temperature. The HS samples (0.5 mg) were ground in a vibrating ball mill with 200 mg of dry KBr salt and the mixture was placed in an 11-mm pellet die. The pellets were pressed in a suitable die under vacuum at a pressure of $20,000\text{ lb/in.}^2$, producing a pellet suitable for FTIR spectroscopy measurement.

2.6. Solid state ^{13}C CP/MAS NMR characterisation

Humic substances heated at different temperatures were characterised by solid state ^{13}C cross-polarisation magic-angle-spinning nuclear magnetic resonance (^{13}C CP/MAS NMR). All solid state ^{13}C CP/MAS NMR spectra were recorded on a Bruker CXP-90 NMR spectrometer operating at a ^{13}C frequency of 22.65 MHz and at an MAS rate of 3.5 kHz. Pulse widths of $3.5\text{ }\mu\text{s}$ were used, with a 2-s recycle time and a 1-ms contact time. Spectra were collected in 1 K points, zero filled to 4 K, and Fourier transformed with a line broadening factor of 50 Hz. The relative quantities of each carbon type or functionality present in the HS were estimated by direct integration. The relative percent of each carbon type is expressed as percentage relative to peak at chemical shift of 130 ppm, which was detected in consistently high abundance in the HS heated at different temperatures. The results estimated for the ^{13}C CP/MAS NMR spectra are given in Table 2.

Table 1
Elemental composition of the HS heated at different temperatures

Temperature ($^{\circ}\text{C}$)	C (%)	H (%)	N (%)	O ^a (%)	H/C	O/C	N/C	Weight residue (%)
25	48.6	3.36	0.90	47.2	0.83	0.73	0.016	100
100	51.3	3.44	0.87	44.4	0.80	0.65	0.014	87
200	55.4	3.50	0.84	41.3	0.76	0.56	0.013	74
300	58.8	3.58	0.81	37.8	0.73	0.48	0.012	61
400	61.1	3.64	0.78	34.5	0.71	0.42	0.011	50
500	66.5	3.78	0.64	30.1	0.68	0.34	0.0082	44

H/C, N/C, and O/C are the atomic ratios.

^aBy difference.

Table 2
Estimation (%) of various carbon types from CP/MAS ^{13}C NMR spectra of the HS heated at different temperatures^a

Temperature (°C)	Alkyl (40 ppm)	Methoxy (56 ppm)	Carbohydrate (75 ppm)	Phenol (150 ppm)	Carboxylic (172 ppm)	Carbonyl (200 ppm)
– ^b	1.25	0.34	0.91	0.19	0.92	0.23
100	0.85	0.25	0.50	0.54	0.55	0.15
200	0.56	0.25	0.33	0.64	0.41	0.092
300	0.59	0.22	0.24	0.51	0.40	0.018
400	0.42	0.18	0.17	0.24	0.29	0.015
500	0.33	0	0.30	0.19	0	0

^aThe peak at chemical shift of 130 ppm is not listed in the table since the percent of all other peaks are expressed as percentage relative to the peak.

^bSamples were not heated.

2.7. Py-GC/MS spectrometry analysis

Py-GC/MS was performed on a Hewlett Packard 5890 gas chromatography and a HP 5970 mass selective detector. Chromatography was carried out on a fused silica column (30 m × 0.25 mm i.d., 0.25 μm film thickness) coated with a DB5MS (modified 5% phenyl, 95% methyl silicone). Samples of about 0.5 mg were introduced as solids using an injector to the SGE pyrojector and were pyrolyzed at a temperature of 550°C. The temperature of the column was kept at 5°C for 2 min, then raised at a rate of 5°C per min up to 290°C and kept there for 15 min. Helium was used as a carrier gas at a flow rate of 1 ml/min. The injection mode was approximately 1:5 split ratio. Mass spectra (1 scan/s) were recorded under electron impact at 70 eV.

The assignment of compounds released from the pyrolysis was based on a search of a mass spectral library by comparison with relative retention times of the HS, which had been run on the same instrument (Sihombing et al., 1996), and on other published collections of Py-GC/MS data of similar samples (Saiz-Jimenez and de Leeuw, 1986; Saiz-Jimenez et al., 1987). The abundance values are not absolute, since no internal standard was introduced, but are expressed as percentage relative to benzene, which was detected in consistently high abundance in all pyrolytic products of the HS heated at different temperatures.

Methylation of the HS heated at different temperatures was performed in Py-GC/MS analysis in order to overcome the problems derived from the loss of carboxylic groups during Py-GC/MS analysis. Small amounts (about 20 μl) of tetramethyl-am-

monium hydroxide (TMAH, 25% aqueous solution) was introduced to the HS and was mixed thoroughly with the samples. The samples were kept at the temperature of 40°C until they were completely dried. The samples were analysed for Py-GC/MS under the same condition as described above. The assignment of methylated compounds released from pyrolysis during Py-GC/MS analysis can be found elsewhere (Hatcher and Clifford, 1994).

3. Results and discussion

3.1. Mass loss during pyrolysis

The mass loss for the HS heated at different temperatures is shown in Table 1. Upon heating, the mass loss was evident. As expected, the mass loss increases with increasing temperatures, suggesting that the processes of maturity and degradation of the HS heated at different temperatures are similar to the long time process involved in organic matter maturation naturally occurring in depositional environments (Waples, 1993). The result suggests that there are a number of time–temperature pairs that could lead to the same level of maturation.

According to Wilson et al. (1999), in their thermogravimetric study of HS isolated from Bayer liquor, the mass loss at the temperature below 200°C could be attributed to water absorbed in the HS. However, based on FTIR and ^{13}C NMR results in this present study (see further in the text), besides water loss, decarboxylation and structural changes were also observed at temperatures below 200°C.

This may be due to the fact that the HS from Bayer liquor, which were subjected to a high temperature (150°C) caustic digestion (5 M NaOH) during the Bayer process before being isolated, are more thermo-resistant than the HS presented in this study. In fact, thermo-properties of humic substances are related to their structural characteristics and compositions (Gonet and Cieslewicz, 1998; Esteves and Duarte, 1999). In their study of thermogravimetric properties of aquatic HS isolated from different environments, Esteves and Duarte (1999) found that humic acids are more thermo-resistant than fulvic acids, which is in accordance with the higher aromaticity of humic acids compared to fulvic acids.

More mass loss occurred in the temperatures ranging from 200°C to 400°C because of loss of some volatile matter during heating (Van Krevelen, 1993). Our previous investigation (Lu et al., 1997) showed that carboxylic bonds were cleaved and decarboxylation occurred with a concomitant release of carbon monoxide when the humic substances were heated at temperatures from 200°C to 400°C. At higher temperatures ranging between 400°C and 500°C, less matter is lost during heating. This is probably due to the fact that aliphatics are converted to aromatics (Lu et al., 1997), leading to the formation of char, coke or semi coke like substances during heating (Van Krevelen, 1993).

3.2. Elemental composition

Table 1 shows the elemental composition and the corresponding atomic ratios of the HS heated at different temperatures. According to Steelink (1985), the index of atomic ratios (O/C, H/C, and N/C) is useful in the identification of structural changes and the degree of maturity of HS in different depositional environments. As shown in Table 1, O/C ratio decreases with increasing temperatures, indicating a decrease in the amounts of oxygen-containing functional groups such as methoxyl, carboxylic, and carbonyl functional groups in the HS with increasing temperatures (Ioselis et al., 1985; Campanella and Tomassetti, 1990; Lu et al., 1997). On the other hand, a decrease in O/C ratio of the HS could be also considered as an increase in the degree of aromatic condensation of the HS (Ibarra and Juan, 1985). In comparison to O/C ratio of the HS iso-

lated from different environments (Lu et al., 2000), O/C ratio of the HS heated at above 300°C was found to be lower than that of the HS extracted from brown coal, suggesting that the heating could accelerate the degree of maturity of the HS. In addition, H/C ratio decreases with increasing temperatures. The trend of H/C ratio of the HS suggests a progressive increase in aromaticity (Ibarra and Juan, 1985), and an increase in degree of maturity of the HS (Ibarra and Juan, 1985), as the heating temperature is elevated. The aromatic carbons are believed to be building blocks across HS (Schulten, 1995), and are the most stable organic components occurring naturally in environments.

In order to quantitatively provide reliable information about the early diagenesis evolution, Van Krevelen (1950) used the plot of H/C atomic ratio against O/C atomic ratio to reveal changes in properties associated with coalification reactions. Fig. 2 shows a linear relationship between H/C atomic ratio vs. O/C atomic ratio. The plot in Fig. 2 could be considered as a graphical–statistical representation of maturity and degradation processes such as dehydrogenation, dehydration, demethylation, and decarboxylation occurring during heating. In the natural system, the removal of chemically bound oxygen is

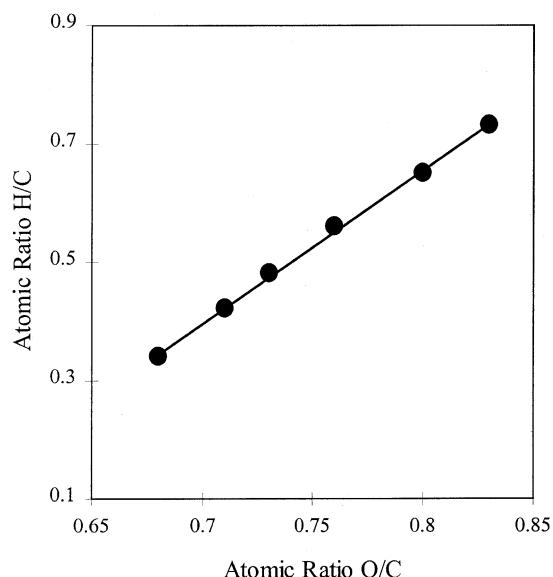


Fig. 2. Atomic ratio H/C against the ratio O/C for the HS heated at different temperatures.

one of the primary steps during the production of fuel and coal and this will increase its hydrocarbon content (Bennett and Abbott, 1999). In our previous study (Lu et al., 2000), the relationship between H/C and O/C revealed some useful information on maturity of the HS isolated from different depositional environments. Ibarra and Juan (1985) reported that H/C and O/C atomic ratios in humic acids isolated from brown coals decreased with increasing ranks. It, therefore, could be suggested that the evolution of HS associated with time and the temperature appears to proceed by similar reactions. Nevertheless, either with increasing burial depth, or with increasing temperature, loss of the oxygen containing functional groups is an important process during humification of these immature organic materials.

Table 1 also shows that the percent of nitrogen element decreases with increasing temperatures but the N element still remained at the temperature of 500°C. This is consistent with observation reported by Almendros et al. (1996), who claimed that the high N/C ratio in humin materials is due to the fact that resistant forms of N-containing compounds tend to remain during the humification process. Bearing in mind that N-containing compounds in the HS are most likely related to proteins, the high thermo-resistance of some N-containing compounds suggests that the thermal resistance at high temperature will not be due to N-containing amides in proteins but to heterocyclic N compounds such as those with pyrimidine and pyrazine structures, which are the remnants of pyrolysis products of proteins (Knicker et al., 1996). Furthermore, Wilson et al. (1999) found, in their studies of humic fractions isolated from Bayer liquor, that the N/C ratio increases with increasing molecular weights, suggesting that N-containing compounds concentrate in the higher molecular fractions and are less degradable. Gonet and Cieslewicz (1998) reported that the nitrogen content in humic acids is inversely proportional to the aromaticity of humic acid molecules and to their heat of combustion.

3.3. FTIR results

The FTIR spectra of the HS heated at different temperatures were recorded (spectra are not shown here). The detailed assignment of peaks observed in the spectra can be found in elsewhere in our in situ

thermal study for the HS using FTIR emission spectroscopy (Lu et al., 1997). Some changes observed because of heating are primarily related to two major reaction pathways: (1) the first reaction involves decarboxylation and (2) the second reaction transforms aliphatics to aromatics. Anhydrides were seen in the spectra of the HS heated at temperatures above 100°C due to formation of a small shoulder at 1850 cm^{-1} on the 1720- cm^{-1} peak, indicating that water molecules were eliminated from carboxylic groups and their adjacent hydroxyl groups in the HS to form anhydrides. Then decarboxylation was observed as the heating temperature was further increased since the intensity of peak near 1720 cm^{-1} decreased with increasing temperatures, suggesting that decomposition of carboxyl or carbonyl groups in the HS occurred with increasing temperatures. The spectra of the HS heated with increasing temperatures showed that the intensity of aliphatic peak at 2927 cm^{-1} decreased while the intensity of aromatic peak at 3100 cm^{-1} increased, implying that the aliphatics relative to aromatics decrease progressively with increasing maturity. Previous studies have shown that aromatic C–H is formed at the expense of aliphatic C–H through dehydrogenation of hydro-aromatics, or through dehydrogenative cyclisation (Vassallo and Attalla, 1992).

3.4. ^{13}C CP/MAS NMR results

It seems that ^{13}C CP/MAS NMR spectroscopy can provide additional information on the reaction pathway of the HS and the change of functional groups. Fig. 3 shows the CP/MAS ^{13}C NMR spectra of the HS heated at different temperatures. The spectrum of the HS without heating showed four regions, which are assigned to aliphatic carbons (0–50 ppm), oxygenated aliphatic carbons (50–110 ppm), aromatic carbons (100–160 ppm), and carboxylic/carbonyl carbons (160–220 ppm) (Lu et al., 2000). The peak at 40 ppm arises from the alkyl carbon components such as methyl, methylene, and methane carbons (Wilson, 1987). The peak at 22 ppm observed above 100°C may originate from methylenic C in a long chain aliphatic carbon compounds such as fatty acids, lipids, cutin acids, and other unknown aliphatic biopolymers. The peak at 56 ppm has been assigned to methoxy groups associated

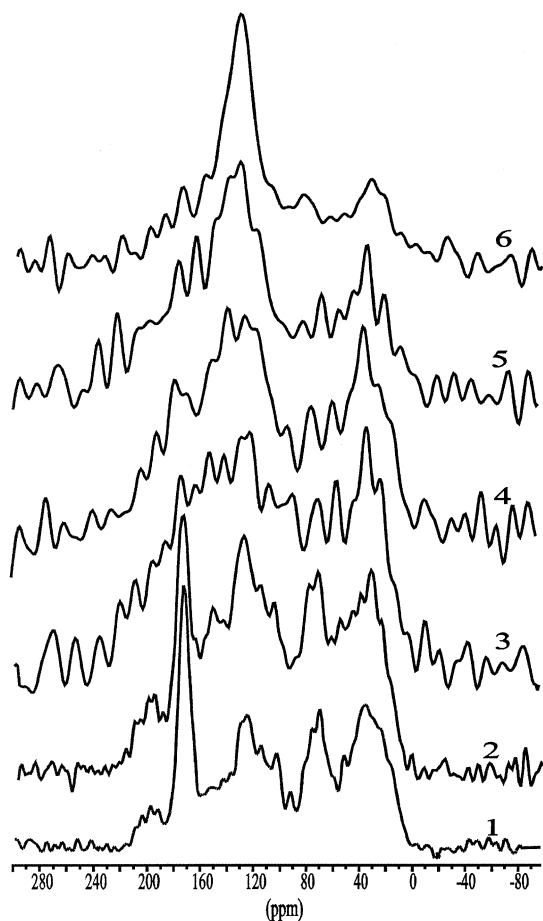


Fig. 3. ^{13}C CP/MAS spectra of the HS heated at different temperatures: (1) unheated; (2) 100; (3) 200; (4) 300; (5) 400; (6) 500.

with lignin and lignin-like products (Hatcher et al., 1980; Piotrowski et al., 1984; Malcolm, 1989). The peak at 75 ppm has been attributed to ring polysaccharides such as celluloses (Hatcher et al., 1980; Malcolm, 1989). The peak centered at 130 ppm is due to aromatic and unsaturated carbons or aryl-C with protonated aromatic C lying to the high field and the peak at 150 ppm may be assigned to both phenolic and methoxyl substituted aromatic rings (Liang et al., 1996). Finally, the peak at 172 ppm is attributed to carboxylic carbons including carboxylic acid, amide, and ester carbons and the peak at 200 ppm has been assigned as carbonyl, aldehyde, and ketone.

Increasing heating temperatures, some structural changes associated with maturity processes have been observed in the HS. In Table 2, the relative percent of each carbon type present in the HS is given. This is plotted vs. heating temperatures as shown in Fig. 4a and b. Fig. 4a shows that the relative percent of aliphatic carbons decreased from the HS (without heating) to the HS heated up to 200°C, suggesting

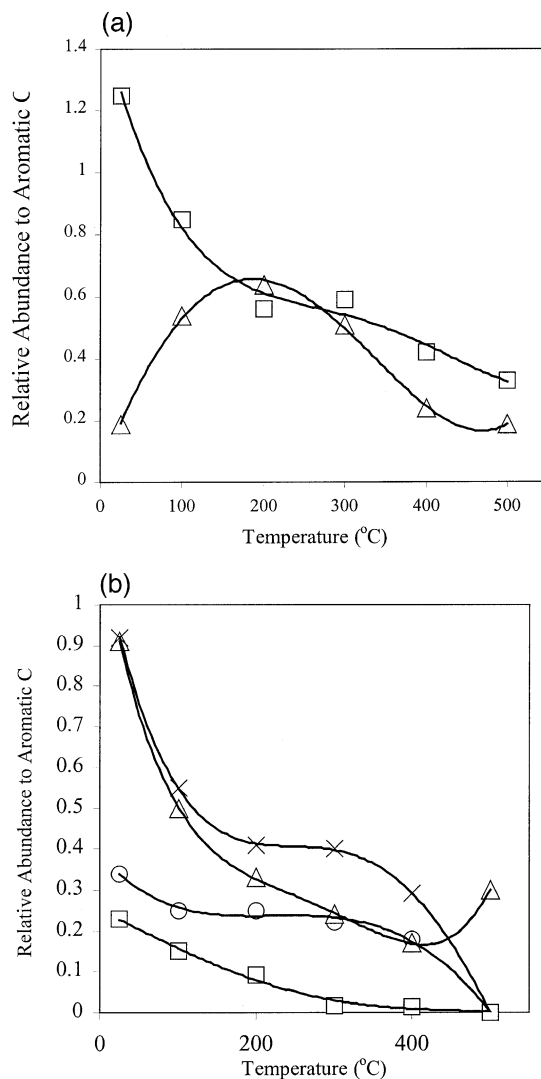


Fig. 4. (a) Relative abundance of various carbon type from CP/MAS ^{13}C NMR spectra of the HS heated at different temperatures: (□) alkyl; and (△) phenol. (b) Relative abundance of various carbon type from CP/MAS ^{13}C NMR spectra of the HS heated at different temperatures: (×) carboxylic; (△) carbohydrate; (○) methoxy; and (□) carbonyl.

that the HS may have undergone volatilization, during which small molecules and volatile alkyl compounds were progressively lost with increasing temperatures. Above 200°C, the relative percent of alkyl carbon decreases gradually, indicating gradual decarboxylation of some acids such as fatty acids and cutin acids and transformation of aliphatic protons to aromatic protons (Lu et al., 1997). However, preservation of alkyl carbon is evident since the peak is still observed at 500°C. The result suggests that the residue after heating at 500°C may contain rigid alkyl carbons, probable due to cross-linking of aliphatic polymers, which make up part of the humin like residue. This pathway could be also additional evidence for the hypothesis of Almendros et al. (1996) related to the preservation of aliphatic macromolecules in soil humins.

Fig. 4b shows that the relative percent of methoxy group at 56 ppm decreased rapidly with increasing temperatures from HS without heating to the HS heated up to 200°C. The methoxy group seems fairly stable from temperatures from above 200°C to 400°C since only relatively slight decreases in the relative percent of the peak vs. temperatures is observed. However, above 400°C, the peak decreases rapidly and has almost disappeared at 500°C. It will be seen further in the text that the relative intensity of phenolic peak at 150 ppm increases (Fig. 4a) with increasing heating temperatures up to 300°C. Using a solid-state lignin model compound, Buchanan et al. (1997) reported that cleavage of the prominent β -O-4 aryl ether linkages ($\text{Ar}'\text{-CH}_2\text{-CH}_2\text{-O-Ar}$) in lignin can occur in the solid state with the re-formation of 1,2-diarylethane linkages ($\text{Ar}'\text{-CH}_2\text{-CH}_2\text{-Ar-OH}$) at relevant maturation temperatures (150°C to 300°C) in the presence of clay materials. The results in this study indicated that fraction of phenolic carbon increases with increasing heating temperatures at the expense of lignin units in the HS. This loss of oxygen from lignin moieties is the first step in the thermal conversion of lignin-rich biomass to hydrocarbons (Vane and Abbott, 1999). Similar results were observed in the HS isolated from different environments (Lu et al., 2000), suggesting that time and temperature may have similar effects on diagenesis of lignin components in the HS. Above temperatures of 400°C, a rapid decrease in methoxy groups could be associated with the transformation of

ligninic carbons to aromatics. At temperature of 500°C, the methoxy group was not detected any more and this result is consistent with the observation of the HS isolated from high maturity of coals, which hardly contain any methoxy groups (Ibarra and Juan, 1985; Lu et al., 2000).

Fig. 4b shows that the relative percent of the carbohydrate group at 75 ppm decreases with increasing temperatures up to 200°C, indicating that some of carbohydrates in the HS are easily decomposed, forming smaller molecules. This seems to support the coalification theory that first step in coalification is the loss of carbohydrate units (Wilson et al., 1987). From 200°C to 400°C, the intensity of peak at 75 ppm decreases gradually, indicating that the group is progressively lost further with increasing temperatures. However, there was no increase in amount of polysaccharides (105 ppm), suggesting that the polysaccharides have undergone thermal decomposition or have been incorporated into the aromatic carbon during heating (Knicker et al., 1996). Similar results were reported in the humification process of the HS, in which polysaccharides and carbohydrates are lost during humification (Gonzalez-Vila et al., 1994; Lu et al., 2000). In natural soil, solid state CP/MAS ^{13}C NMR spectroscopy showed decreases of carbohydrates of soil organic matter during humification, mainly attributed to the preferential mineralization of polysaccharides (Zech et al., 1997). At temperature above 400°C, the peak shows an increase in relative intensity and is still observed at 500°C, suggesting that some of the carbohydrates show resistance to thermal decomposition. A similar phenomenon was observed by Almendros et al. (1996) in their study of soil humins. Some of carbohydrates left after heating at 500°C, which may contribute to thermal resistance, could be *O*-alkyl structures, similar to those suggested by Bayer et al. (1984).

In comparison to other groups, the relative percentage of aromatic carbons at 130 ppm was found to increase from 100°C to 300°C. As noted above, methoxy groups and carbohydrates decrease and the aromaticity of the HS increases during heating at the temperature up to 300°C. A decreasing area of oxygenated aliphatic carbon and an increasing area of aromatic carbon indicates an increasing stage of humification involved in thermal degradation

(Knicker et al., 1996). The aromatic carbon became predominant at 400°C due to the formation of aromatics at the expense of aliphatics above 400°C. The result was consistent with FTIR observation as discussed above. In addition, a similar result was also observed by Pang et al. (1989), who found that the humic acids isolated from three Australian brown coals contained a significant percentage of aryl ether, which may be due to polycondensed aromatics from wood tannin and lignin. Such evolution suggests that the humic substances have undergone transformation associated with humification and maturation during heating.

Some phenols are polymerized since the peak at 150 ppm increased up to 300°C. An increase in phenol carbon in this temperature range could be related to a decrease in the *O*-alkyl region at 56 ppm, suggesting that demethylation of lignins had occurred (Vane and Abbott, 1999). In this case, the humic substances become less reactive, more aromatic materials, largely through condensation of tannins, lignins, and consumption of their aliphatic compounds. A similar result was observed by Lu et al. (2000) in their characterization of the HS from different sources. This has been considered to be due to the high content of huminitic macerals, i.e., a wood pyrolysis product with a high content of polycyclic aromatic components (Wilson et al., 1987). Above 300°C, the peak decreases and only there is little phenol carbon left at 500°C, suggesting that most of those components (phenol and aliphatic–aromatic ether) have been decomposed by ring cleavage or polymerized to cyclic ether.

Finally, the relative percentages of the carboxylic group at 172 ppm and the carbonyl group at 200 ppm decreased with increasing temperatures (Fig. 4b) due to decarboxylation of the group upon heating. These groups have disappeared at 500°C. The result was consistent with FTIR observation as discussed above. Similar results were observed by Ibarra and Juan (1985), who, in their characterization of humic acids from different maturity of coals, reported that carboxyl groups decreased in humic acids with increasing maturity of coals. According to Ibarra and Juan (1985), the results of increasing carboxyl groups and decreasing phenolic hydroxyls suggest that humic acids increase maturation with their parent coals.

3.5. Py-GC/MS data

Py-GC/MS pyrograms for the HS without heating and those heated at 100°C and at 300°C are presented in Fig. 5 labeled with a, b, and c. More than 65 compounds identified for the HS are listed in Table 3. Full discussion of pyrolytic compounds released from the HS can be found elsewhere (Lu et al., 2000). The pyrolytic products of the HS without heating can be characterized by groups of N-containing compounds, sulfur-containing compounds, lignin products, carbohydrate products, aliphatic products, and aromatic products.

Pyrrrole, pyridine, and other N-containing compounds (N = 19, 20, 25, 26, and 28) were detected in the pyrogram of the HS without heating. The N-containing compounds (Fig. 6a) showed a correlation to elemental analysis results listed in Table 1 where nitrogen element decreased with increasing temperatures and was still detected at the temperature of 500°C. The fact that the N-containing compounds are resistant to thermal degradation during pyrolysis could be due to their strongly binding to the skeleton of the HS. Schulten (1995) suggested a three-dimensional structural concept for HS, where N-containing compounds are strongly incorporated into chemically stabilized HS and bind three major classes of organic constituents (N-containing compounds, aromatic, and aliphatics). The fact is further confirmed by the observation that the enrichment of heterocyclic N-containing compounds with time is remarkable in the soil organic matter (Schulten and Leinweber, 1996). Some nitrile compounds such as 4-methylbenzonitrile and 4-dimethylbenzonitrile were detected at heating temperature of 100°C. The intensity of those compounds decreased with increasing temperatures up to 300°C and was not detected at all above that temperature. The evidence implies changes of the nitrogen linkage in the HS since there were no nitrile compound signals detected in the HS without heating. A similar observation was reported by Woelki and Salzer (1995) in their thermal investigations of the structural changes in humic acid salts using *in situ* FTIR spectroscopy.

The sulfur-containing compounds such as thiophene (N = 13) and 2-dimethylthiophene (N = 22), which were previously reported by Saiz-Jimenez and de Leeuw (1986) in humic acid and fulvic acid

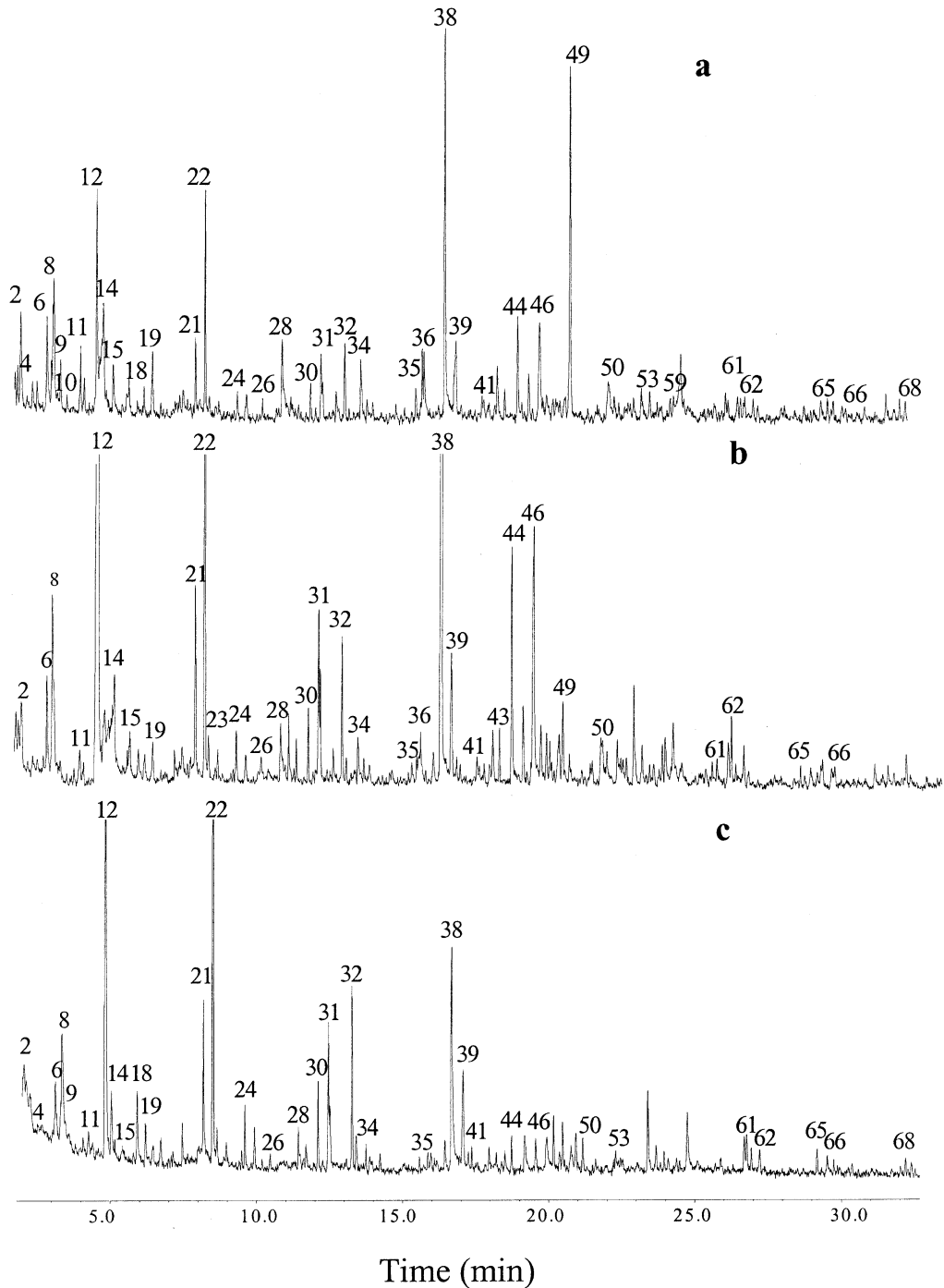


Fig. 5. Py-GC/MS profile of HS: (a) without heating; (b) heated at 100°C; and (c) heated at 300°C.

fractions of a soil, were detected in the HS without heating. Low abundance of these compounds reflects

the trace amount of the element in the HS. However, Fig. 6a shows that the relative percent of thiophene

Table 3

Typical products of the HS identified by Py-GC/MS. C = carbohydrate, P = protein, L = lignin, and Lp = lipid

Peak numbers	Compounds	Origin
1	Pentene	
2	Furan	C
3	Cyclopentene	
4	2-Methylpropenal	C
5	2-Methylpropanal	C
6	Hexene	
7	Hexane	
8	2-Butanone	Lp
9	3-Methylfuran	C
10	2,4-Hexadiene	
11	1,3-Methylcyclopentadiene	
12	Benzene	CPL
13	3-Methylbutanal	C
14	Thiophene	P
15	3-Methyl-3-buten-2-one	
16	Heptene	
17	Heptane	
18	Dimethylfuran	C
19	Vinylfuran	C
20	Pyridine	P
21	Pyrrrole	P
22	Toluene	PL
23	2-Methylthiophene	
24	Octene	Lp
25	Octane	Lp
26	1,3,5-Trimethylpyrazole	P
27	Methylpyridine	P
28	2-Furaldehyde	C
29	2-Methylpyrrrole	P
30	Ethylbenzene	PL
31	1,3-Dimethylbenzene	PL
32	Styrene	
33	Nonene	Lp
34	Nonane	Lp
35	Propylbenzene	
36	5-Methyl-2-furaldehyde	C
37	1,3,5-Trimethylbenzene	
38	Phenol	CPL
39	Benzofuran	C
40	Decane	Lp
41	2,4,6-Trimethylbenzene	
42	1-Ethyl-2methylbenzene	
43	Indene	
44	<i>o</i> -Cresol	L
45	Guaiacol	L
46	<i>p</i> / <i>m</i> -Cresol	L
47	Undecene	Lp
48	Undecane	Lp
49	2-Methylbenzofuran	C
50	Dimethylphenol	L
51	C ₄ -Alkylbenzene	
52	Ethylphenol	L

Table 3 (continued)

Peak numbers	Compounds	Origin
53	Methylguaiacol	L
54	Catechol	L
55	Naphthalene	
56	2,4-Dimethoxyphenol	L
57	Dodecene	Lp
58	Dodecane	Lp
59	Ethylguaiacol	L
60	C ₅ -Alkylbenzene	
61	Vinylguaiacol	L
62	Tridecene	Lp
63	Methylnaphthalene	
64	Tetradecane	Lp
65	Acetoguaiacone	L
66	Dimethylnaphthalene	
67	Methylvanillate	L
68	Pentadecene	Lp

decreased with increasing temperatures. It appears that some S-containing compounds are resistant to thermal degradation during heating since thiophene is still detected at temperature of 500°C. The reaction that may occur during heating is the condensation of lower molecular weight organic sulfur compounds into higher molecular weight substances and the subsequent stabilization of these sulfur compounds towards further degradation. This important information implies that thiophene is a useful biomarker for use as a thermal maturity indicator for natural organics in different depositional environments. Accumulation of organic sulfur into sedimentary humic acids occurs during early diagenesis (Francois, 1987). It has been generally accepted that pathway for incorporation of sulfur into HS is biological and chemical processes in anoxic sediments. Since the mechanism of reaction of S-containing compounds during heating is not clear (Saiz-Jimenez, 1995), further study is needed to understand the mechanism. If it could be confirmed, the mechanism would complement our knowledge of different reaction mechanisms involved in the incorporation of sulfur into coals (Casagrande and Ng, 1979).

Pyrograms showed that many aliphatic compounds were identified in the HS without heating. They are typical products arising from the pyrolysis of polymethylene structures, such as lipids with long aliphatic chain and paraffinic materials in the HS.

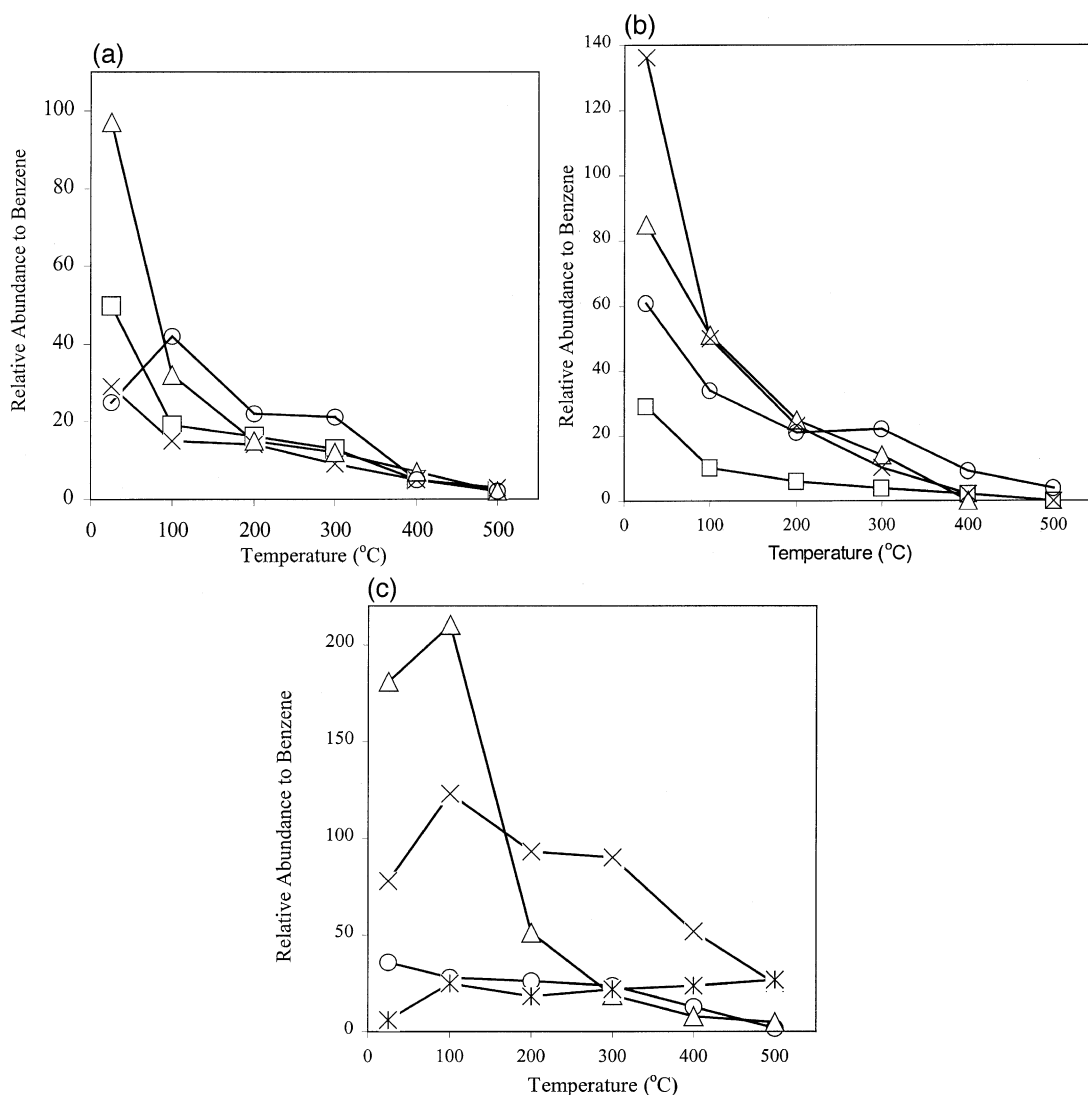


Fig. 6. (a) Relative abundance to benzene derived in the pyrograms of the HS heated at different temperatures: (□) hexene; (×) heptene; (○) pyrrole; and (△) thiophene. (b) Relative abundance to benzene derived in the pyrograms of the HS heated at different temperatures: (△) butanone; (○) benzofuran; (□) vinylfuran; and (×) 2-methylbenzofuran. (c) Relative abundance to benzene derived in the pyrograms of the HS heated at different temperatures: (△) phenol; (×) toluene; (○) styrene; and (✕) naphthalene.

Pyrograms showed that some of these compounds are unstable during heating since the relative intensities of some aliphatic compounds such as octene, nonane, and undecane decrease with increasing temperatures and were gone at a heating temperature of 300°C. This could be due to volatilization at lower temperatures and the transformation of aliphatics to aromatics at higher temperatures, as observed in

FTIR and ^{13}C CP/MAS NMR results. However, Fig. 6a shows that some of aliphatic compounds such as hexene and heptene are stable and they are still observed at pyrolysis temperature of 500°C. The result seems to be consistent with the behavior of aliphatic carbon observed in ^{13}C NMR results of the HS during heating. Due to the formation of highly aliphatic materials during heating, it has made the

polyaliphatic structures more resistant to thermal degradation.

Guaiacol ($N = 44$) and its derivatives ($N = 52, 54, 57, 59, 63, 65,$ and 69), the main constituent units of lignin, were detected in the pyrogram of the HS without heating. Upon heating, the intensities of these compounds decreased with increasing temperatures and were not detected at temperature above 300°C . This observation seems inconsistent with the ^{13}C CP/MAS NMR spectra of the HS in Fig. 3, which show that the chemical shift at 56 ppm assigned to methoxyl groups was observed at 400°C . This is probably due to the fact that the abundance of guaiacol is favorably formed at lower temperatures during Py-GC/MS analysis (Sihombing et al., 1996).

Furan and its derivatives ($N = 2, 8, 17, 18, 27, 35, 39,$ and 48) were detected in the HS without heating. It has been suggested that furan compounds arise from carbohydrates as well as probably being the product of microbial degradation of polysaccharides from plant cells (Hayes et al., 1989). Pyrograms showed that the trends of these pyrolysis products (e.g., methylfuran and furaldehyde) of the HS seemed to be correlated to the ^{13}C CP/MAS NMR results. The amount of furan and its derivatives decreased with increasing temperatures, indicating that carbohydrates were degraded at lower heating temperatures and led to aromatization at higher heating temperatures. A similar result was observed in the humification of the HS from different sources (Lu et al., 2000). However, compounds such as benzofuran, vinylfuran, and 2-methylbenzofuran are fairly stable and they are observed at 500°C . This may be due to the fact that some carbohydrates are incorporated into aromatic carbon during heating, as discussed in ^{13}C CP/MAS NMR result section.

Phenol ($N = 37$) and $\text{C}_1\text{--C}_2$ alkyl phenols ($N = 43, 45, 49, 51,$ and 52) were detected in the HS without heating. It seems that the pyrograms of these compounds show different thermal behaviors upon heating. Phenol was the major pyrolysis product up to heating temperature of 100°C . The relative amount of phenol decreased at temperatures greater than 100°C (Fig. 6c). The compound was still detected at 500°C . This could be due to the fact that the phenol has been continuously derived from lignin, which lost its methyl subunits during heating. However, $\text{C}_1\text{--C}_2$ alkyl phenols, such as *o*-cresol and *p/m*

cresol, are less stable. They decreased with increasing temperatures and were not detected at temperatures above 400°C . Dimethylphenolic compounds are relatively weak and they were not detected at temperatures above 200°C . The results agreed with solid state ^{13}C CP/MAS NMR results, which showed that at low heating temperatures, increases in phenol carbon was observed, while the most alkyl phenols were decomposed probably by ring cleavage or were polymerized to cyclic ethers during heating at high temperature.

Pyrograms showed aromatic compounds such as benzene ($N = 12$), toluene ($N = 22$), $\text{C}_1\text{--C}_2$ -alkylbenzenes ($N = 22, 30, 31,$ and 32), and naphthalene ($N = 54$), regarded as building blocks across the humic substances (Schulten, 1995), were detected in the HS without heating. Fig. 6c shows changes in the signal intensities of benzene, toluene, styrene, and naphthalene vs. heating temperatures. An increase in the relative intensity of toluene from 100°C to 300°C indicates an increase in aromatic structures during heating, which may imply, as observed by ^{13}C NMR spectra, the formation of aromatic carbons at the expense of methoxy and carbohydrate components during heating (Vane and Abbott, 1999; Knicker et al., 1996). Benzene and derivatives were detected to be dominant pyrolytic products at high heating temperatures, indicating either thermal resistance of these components in the HS or production of alkylbenzenes because of conversion of aliphatics during heating. A similar ^{13}C CP/MAS NMR result was observed for the HS heated at different temperatures due to polycondensed aromatics (Pang et al., 1989).

In contrast to ^{13}C CP/MAS NMR results, carboxylic acid was not detected for the HS without heating. This may be due to the decarboxylation process and to the formation of carbon monoxide as a pyrolytic product during Py-GC/MS analysis. When *in situ* methylation with TMAH was applied to the HS, benzenedicarboxylic acid dimethyl ester, benzenetricarboxylic acid trimethyl ester, and benzenetetracarboxylic acid tetramethyl ester, and benzenepentacarboxylic acid pentamethyl ester were detected as pyrolytic products of the benzenecarboxylic acids. In addition, butanedioic acid dimethyl ester was detected as a pyrolytic product of aliphatic acids. However, detailed measurements indicated that relative intensities of these compounds decreased with heat-

ing temperatures, suggesting that the results by Py-GC/MS analysis is consistent with the observation obtained by solid state ^{13}C CP/MAS NMR and FTIR spectroscopy. Decarboxylation, accompanying by the formation of carbon monoxide, is important step during thermal degradation of the HS heated at temperatures from 100°C to 400°C. At temperatures above 400°C, some polycarboxylic acids in the HS were converted to either phenol and methylphenols (Bracewell et al., 1980; Martin et al., 1994) or alkylbenzenes products during heating (Saiz-Jimenez, 1995), thus the intensities of benzenedicarboxylic acid dimethyl ester and its derivatives were detected relatively low at 400°C and no peaks of those compound were detected at 500°C.

4. Conclusion

In this study, humic substances isolated from swamp water were heated at temperatures from 100°C to 500°C in presence of helium and were characterized using variety of chemical, analytical, and spectroscopic techniques to monitor the chemical modifications and artificial evolution taking place in the HS. The study is summarized as follows.

(1) Elemental composition results showed that increasing carbon content and decreasing oxygen content in the HS with increasing heating temperatures. The change in atomic ratio of H/C against atomic ratio of O/C seemed to be consistent with the theory of maturation of the HS isolated from ranks of coals.

(2) FTIR spectra of the HS heated at different temperatures showed two reaction pathways: decarboxylation and the transformation of aliphatics to aromatics, suggesting that decomposition of the HS and changes in structure of the HS occurred during heating.

(3) Solid state ^{13}C CP/MAS NMR results showed that aliphatic carbons, oxygenated aliphatic carbons, and carboxylic carbons decreased, while the relative percent of aromatic carbon increased as the heating temperatures increased. Preservation of aliphatic carbon and carbohydrates and the complete loss of methoxy carbon and carboxylic groups were observed at 500°C. The results appeared to support the humification theory.

(4) Py-GC/MS results showed that oxygenated related components, such as furan and furan-derivatives lignin, and lignin-derived compounds were substantially removed with increasing heating temperatures. The aromatic compounds such as, benzene, toluene, and naphthalene were showed to be residues. The results obtained provide evidence that, during thermal evolution, the HS evolved in the direction closely resembling brown-like coal substances due to the condensation of aromatic rings in the solid residue of the HS, which had been heated at 500°C.

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