

Effect of organic matters on CO₂ hydrate formation in Ulleung Basin sediment suspensions

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A manuscript submitted to *Environmental Science and Technology*

June, 2011

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Chemicals. The gas hydrate former used in this research was a commercial 99.9% grade compressed CO₂ (Sam-O Gas Co., Korea). All chemicals used in the research were ACS

reagent grade or higher; Methanol (100%, JT Baker), dichloromethane (99.9%, Merck), sodium hydroxide (97%, Sigma, USA), hydrochloric acid (35.0–37.0%, Jin chemical, Korea), potassium hydroxide (95.0%, Jin chemical, Korea), potassium chloride (99.0%, Jin chemical, Korea), silver nitrate (99.8%, Junsei, Japan), CDCl_3 (99.8%, Merck), DMSO-d_6 (99.9%, Sigma Aldrich), and humic acid (sodium salt, technical grade, Sigma Aldrich). The chemicals were used without any further purification.

UB Sediments. Marine sediments used in this research were sampled from a methane hydrate deposit site in Ulleung Basin (UB), East Sea of South Korea and their compositions were characterized by X-ray Diffraction (XRD). UB sediments were stored in a refrigerator at -4 °C before sample preparation. They were freeze-dried at -82 °C and 0.060 mBar (Labconco, USA) and stored in polypropylene bottles at room temperature (25 ± 0.5 °C) for geochemical analyses and hydrate formation experiments. No further purification of UB sediments was conducted for the sample preparation.

Characterization Methods to Identify Geochemical Properties of UB Sediments.

A. Mass loss on ignition

Organic matter fraction in UB sediments was approximately determined by the measurement of mass loss on ignition (MLOI). An exact amount of sediment (2 g) was weighed and added into pre-ignited ceramic crucibles and baked in a muffle furnace (Nabertherm, Germany) for 2 hours at 550 °C. The MLOI was expressed as $100(X-Y)/(X)$, where X and Y were the sample masses before and after ignition, respectively. Triplicate samples were prepared for MLOI analyses.

B. CHNS analysis

Elemental analyses (C, H, N, S) of UB sediments were conducted by a CHNS analyzer (EA110 Thermo Finnigan, Italy). No treatment for decarbonation of UB sediments was performed before the elemental analyses. After baking the samples (0.15 g) in the muffle furnace at 900 °C for 10 min, ash content in the samples was determined by percent dry solid weight. The results of CHNS analysis are shown in Table S1.

C. Dissolved organic carbon analysis

Dissolved organic carbon (DOC) was extracted from fine grain-sized UB sediment (~250 mesh). An exact amount of freeze-dried UB sediment (5 g) was placed separately in 50 mL centrifuge tubes and extracted with deionized water (30 mL). The sediment suspensions were sonicated in an Ultrasonic Processor (750 Watts) at 20 kHz for 10 min and centrifuged at 3000 rpm for 15 min. Supernatant was poured and collected to a new tube. This process was repeated three times for each sample. DOC analysis was carried out by a Teledyne Tekmar Apollo 9000 analyzer. The supernatant was acidified to pH 2 and sparged with CO₂ free air to remove dissolved inorganic carbon. The treated sample aliquots were introduced to the analyzer equipped with a non-dispersive infrared detector. Duplicate samples were prepared for the measurements of DOC.

D. Surface area and pore analyses

Surface area and pore properties of UB sediments were characterized by nitrogen adsorption/desorption isotherms obtained by an automatic adsorption instrument (ASAP 2000, Micromeritics) at 77 K. The surface area of UB sediments were analyzed with Brauner–Emmett–Teller method [1], while pore size calculations were carried out using Barret–Joyner–Halenda method [2].

E. Calculation of sediment core porosity from density log

Porosity was calculated from the following relationships [3]:

$$\rho_b = \frac{\rho_s (1 + W)}{1 + W \frac{\rho_s}{\rho_{pw}}}$$

$$\phi = \frac{\rho_s - \rho_b}{\rho_s - \rho_{pw}}$$

W: moisture content

ϕ = porosity

ρ_s = particle density

ρ_b = wet bulk density

ρ_{pw} = pore water density (1.024 g/cm³)

Table S1. Contents of carbon, nitrogen, hydrogen, and sulfur in UB sediments measured by an automated CHNS analyzer

UB sediment	
Element Name	Element %
Carbon	3.4354
Nitrogen	0.3485
Hydrogen	1.1125
Sulfur	1.7538
<i>Total</i>	<i>6.6502</i>

Table S2. Surface area and pore properties of UB sediments

Average surface are (m ² /g)	Average pore size (nm)	Average volume of pores (cm ³ /g)
29.8	11.3	0.09

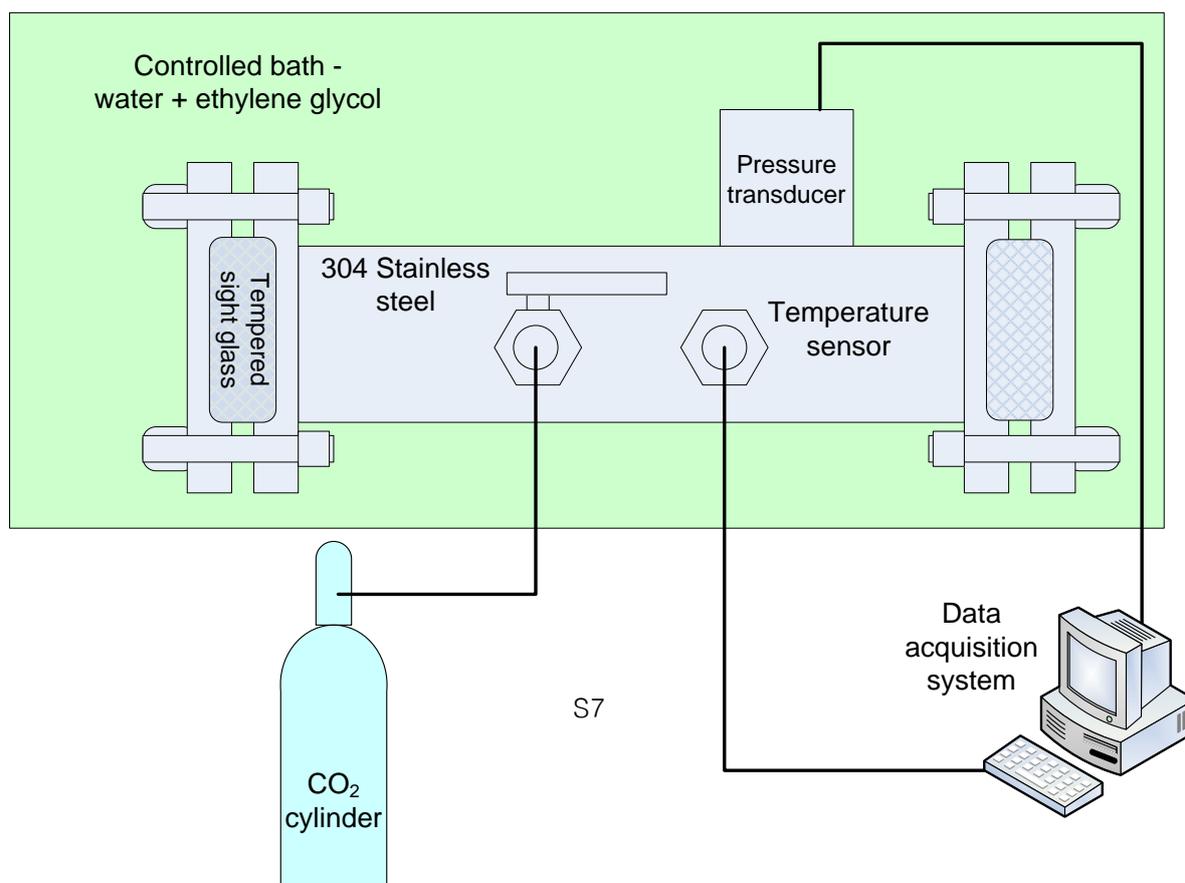


Figure S1. Schematic diagram of experimental setup

Experimental Setup. Hydrate formation experiments were conducted using a simple experimental system that has been previously described in detail [4]. A cylindrical 304 stainless steel pressurized vessel (50 cm^3) was used as a reactor. The vessel has two tempered sight glasses on opposite sides to allow visual observation during the hydrate formation experiments. Temperature and pressure sensors were connected to a data acquisition unit (Agilent 34970 A) with a response time of 20 sec.

Experimental Procedure. All solutions and suspensions were prepared with deionized water (DIW; $18\text{ M}\Omega\cdot\text{cm}$). Exact amounts of UB sediments (0.4 g, dried weight) were added to vessels with 30 mL DIW. The suspensions were continuously mixed until they reached equilibrium pHs. pH measurements were conducted in the suspensions using a combination electrode (9272 Orion Ross electrode, USA). Duplicate measurements were accurate in the pH error range of 0.01. Hydrate formation experiments were conducted in an isothermal, isobaric, and static (i.e., no stirring) environment. The pressurized vessel was filled with 25 - 30 mL of UB sediment suspension. It was placed into a temperature controlled bath at a constant temperature of 273.5 K. The vessel was purged with CO_2 to remove air from the headspace and dissolved air from the suspension. It was pressurized to 30 bar by introducing

CO₂ into the suspension until the formation of CO₂ hydrates. Hydrate formation was monitored by 300 min and then the experimental run was stopped. As reported previously [4], hydrate induction time was determined by the observation of temperature peak. All experiments were conducted in duplicates by renewing the sample preparation procedure at each run.

Sediment Organic Matter (SOM) Extraction Methods and Sample Preparation Procedures.

A. Extraction of UB sediments by solvents

SOMs were extracted using two different types of solvents [5-6]. A mixture of dichloromethane and methanol (5mL each) was used to extract polar organic matter fraction (P-extract), while dichloromethane (10 mL) was used for non-polar organic matter fraction (NP-extract). An exact amount of freeze-dried and fine grain-sized marine sediment (~250 mesh size, 1 g) was placed into a centrifuge tube and extracted by the solvents during a sonication. P- and NP-extracts were obtained from one sample sequentially. The tube was sonicated for 6 min and centrifuged at 1000 rpm for 5 min. The extraction procedure was repeated 6 times and extract from each procedure was collected for spectroscopic and spectrometric analyses. The extract was concentrated to ~10 mL in a rotary evaporator at 1 atm and 35 °C. An aliquot amount of concentrate (~3.3 mL) was transferred to a pre-weighed vial, evaporated to dryness in the rotary evaporator, and kept in a desiccator. The SOM characterization was conducted by infrared (IR) and/or nuclear magnetic resonance (NMR) spectroscopy and ultra performance liquid chromatography–electrospray ionization–mass spectrometry (UPLC–ESI–MS).

B. Extraction of UB sediments by alkaline solutions

An exact amount of sediment (1 g) was transferred into a centrifuge tube (50 mL) and equilibrated with 0.1 M HCl (10 mL). The tube was mixed at 200 rpm on an orbital shaker for 1 hr and centrifuged at 1000 rpm to separate supernatant with fulvic acids. The supernatant was decanted and 0.1 M NaOH (10 mL) was added into the tube in an anaerobic chamber. The tube was then mixed at 200 rpm on the shaker for 4 hr, allowed to stand overnight, and centrifuged at 3500 rpm. Its supernatant was decanted, acidified to pH 1 by adding 6M HCl, and allowed to stand for 12 hr. The supernatant was decanted and precipitates were dissolved by adding a mixture of 0.1 M KOH and 0.3 M KCl (5 mL each) in the anaerobic chamber. The tube was centrifuged at 3500 rpm to remove suspended solids. Humic acid fraction in the supernatant was precipitated again by adding 6.0 M HCl (2 mL) and allowing the suspension to stand for 12–16 hr (hereafter designated as H-extract [7-8]). The precipitated humic fraction was washed several times with deionized water until no chloride was observed in final washing. The precipitates were collected and used immediately for SOM characterization and hydrate formation test.

Analytical Procedures

A. XRD analysis

Sediment samples used in this study were analyzed by X-ray diffraction (XRD) to investigate their identity, purity, and crystallinity. We used Philips 1 XPert *Multipurpose X-ray Diffraction system* (MPD) equipped with Cu-K α radiation source (40 kV and 30 mA) and a wide angle goniometer (3-90°, 2 θ). X-ray scan was conducted at the step width of 0.02° 2 θ and scan step time of 0.5 sec under a continuous scan mode. Siroquant XRD software (Version 3) was used for quantitative analyses of the samples.

B. NMR spectroscopy

^1H -NMR spectra of SOM samples were measured to investigate general chemical composition of the extracts by Bruker Avance 400 spectrometer (400 MHz). NMR spectroscopic analyses were conducted at 90° pulse, 11 μsec pulse width, and -4.00 dB. Other acquisition parameters used for the analyses were: 16 scans, 16.02 ppm sweep width, and 3 sec relaxation delay. Solutions were prepared by dissolving SOM extracts in 0.5 mL deuterated-carbon tetrachloride (CDCl_3) or deuterated-dimethyl sulfoxide (DMSO-d_6) in 5 mm NMR tubes. Identification of functional groups in the NMR spectra was conducted by comparing their chemical shifts to those of tetramethylsilane (SiMe_4) at 0 ppm for ^1H . Chemical shifts at 0.4–1.0, 1.0–2.0, 2.0–4.2, and 6.0–8.0 ppm were assigned as terminal methyl protons of methylene chains, protons of aliphatic carbons removed from an aromatic ring, protons bound to aliphatic carbon (methyl and methylene groups), and aromatic protons, respectively.

C. FT/IR-ATR spectroscopy

SOM extracts were also analyzed to investigate the general chemical composition of the extracts by FT/IR-ATR. Infrared spectra of the SOM samples were obtained in an attenuated total reflection (ATR) mode using an IFS 66v/s Bruker spectrometer coupled with a Hyperion 3000 microscope equipped with a liquid nitrogen-cooled photovoltaic Mercury Cadmium Telluride-Focal Plan Array (MCT-FPA) detector and a 20x ZnSe ATR crystal objective. A reference spectrum of ZnSe crystal probe was obtained before sample analyses. Single-beam spectra of all SOM samples were obtained and normalized by single-beam background spectra of air and ZnSe crystal to produce the spectra in absorbance unit. ZnSe crystal probe of ATR unit provided constant pressure on the dried SOM sample. FT/IR-ATR analyses

were conducted at nominal resolution of 4 cm^{-1} and scanning wave length range between 600 and 4000 cm^{-1} . Scanned spectra (100 scans) were averaged before Fourier transform. Data processing was performed using a software package, Opus (Bruker Optics). The analyses were performed in an open atmosphere. Aliquot amounts of P- and NP-extracts (0.1 mL each) were placed under the optical crystal and scanned after applying the pressure (1 N) on the sample surface. The spectra of P- and NP-extracts were offset and normalized by a background spectrum.

D. UPLC/Q-TOF-MS/MS spectrometry

SOM extracts were analyzed to identify the chemical species and elemental compositions present in the extracts by ultra performance liquid chromatography–quadrupole time–of–flight mass spectrometer (UPLC/Q–TOF/MS/MS) system with an electrospray ionization (ESI) mode.

HPLC profile. All chromatographic separations were performed using an UPLC system (Waters). A C18 column (Acquity, 2.1 x 100 mm, 1.7 μm particle size, Waters) was used for the reversed phase separation at 25 °C. Acetonitrile/water (20/80, V/V) with 0.8% acetic acid was used as isocratic eluent. Total running time was different (20–40 minutes) depending on the extracts injected. Flow rate was 0.1 mL min^{-1} and the injection volume was 3–5 μL .

Q-TOF MS conditions. Eluates were directed to the Q–TOF MS/MS (MicroTOF–QII, Bruker, Daltonics). It was operated by a positive electrospray ionization mode at constant capillary voltage of 4.5 kV and end plate offset potential of –500 V. The scan range was between 50 and 1300 m/z. At constant temperature (210°C), nebulizing nitrogen gas flow rate and gas pressure were 8.0 L min^{-1} and 4.0 bar, respectively.

Data processing. Mass chromatograms and mass spectra data were processed by Compass Data Analysis system (Bruker). An m/z value with highest intensity was chosen as a representative value for a specific chemical compound of each peak. Its molecular formula was calculated using a built-in molecular formula generator in the Smart Formula software suite, which implements a SigmaFit approach to rank proposals according to mass deviation and isotopic pattern accuracy. We set up a mass tolerance at 10.0 ppm and maximum elemental composition at C=50, H=100, N=4, O=30, and S=2. Using the raw mass spectra data, the formula generator recommended a list of chemical compounds. Although we have a small m Sigma value, we selected several potential candidate compounds for each measured mass (m/z). An identification of the compounds was conducted by PubChem and ChemSpider database searches.

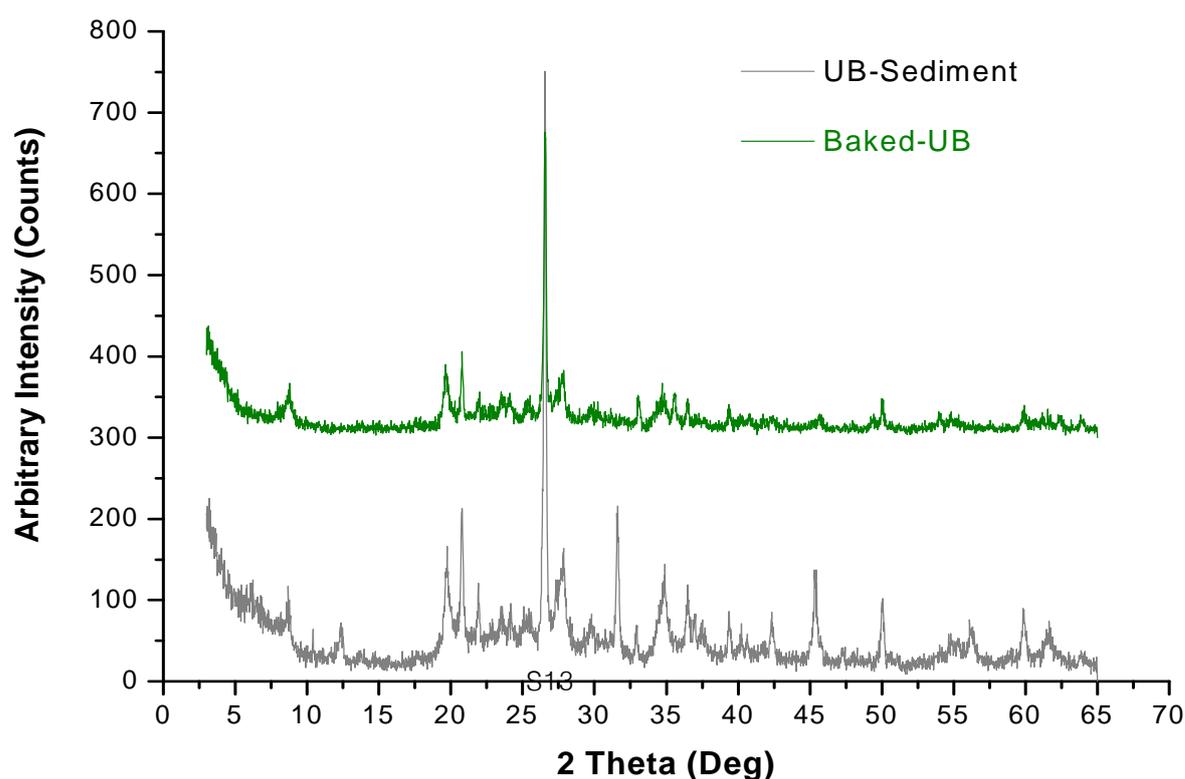


Figure S2: X-ray diffractograms of UB and baked UB sediments

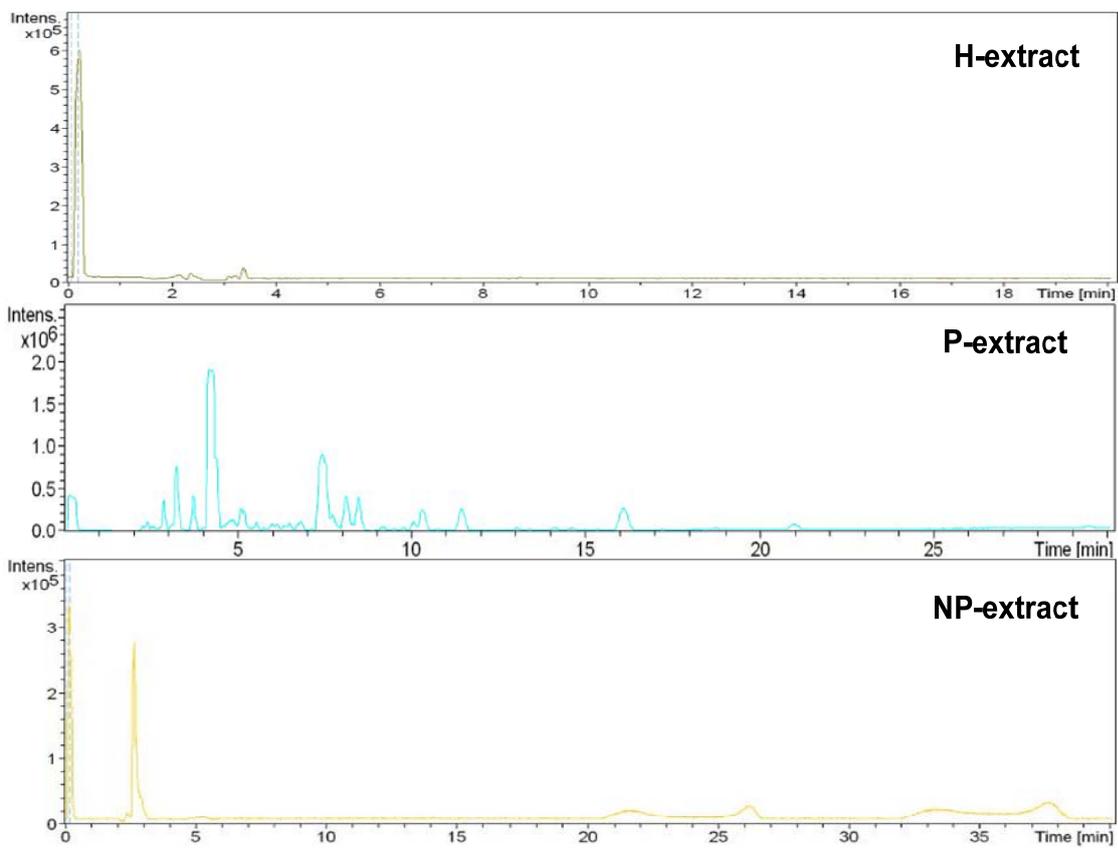


Figure S3(a): UPLC chromatograms for different extracts

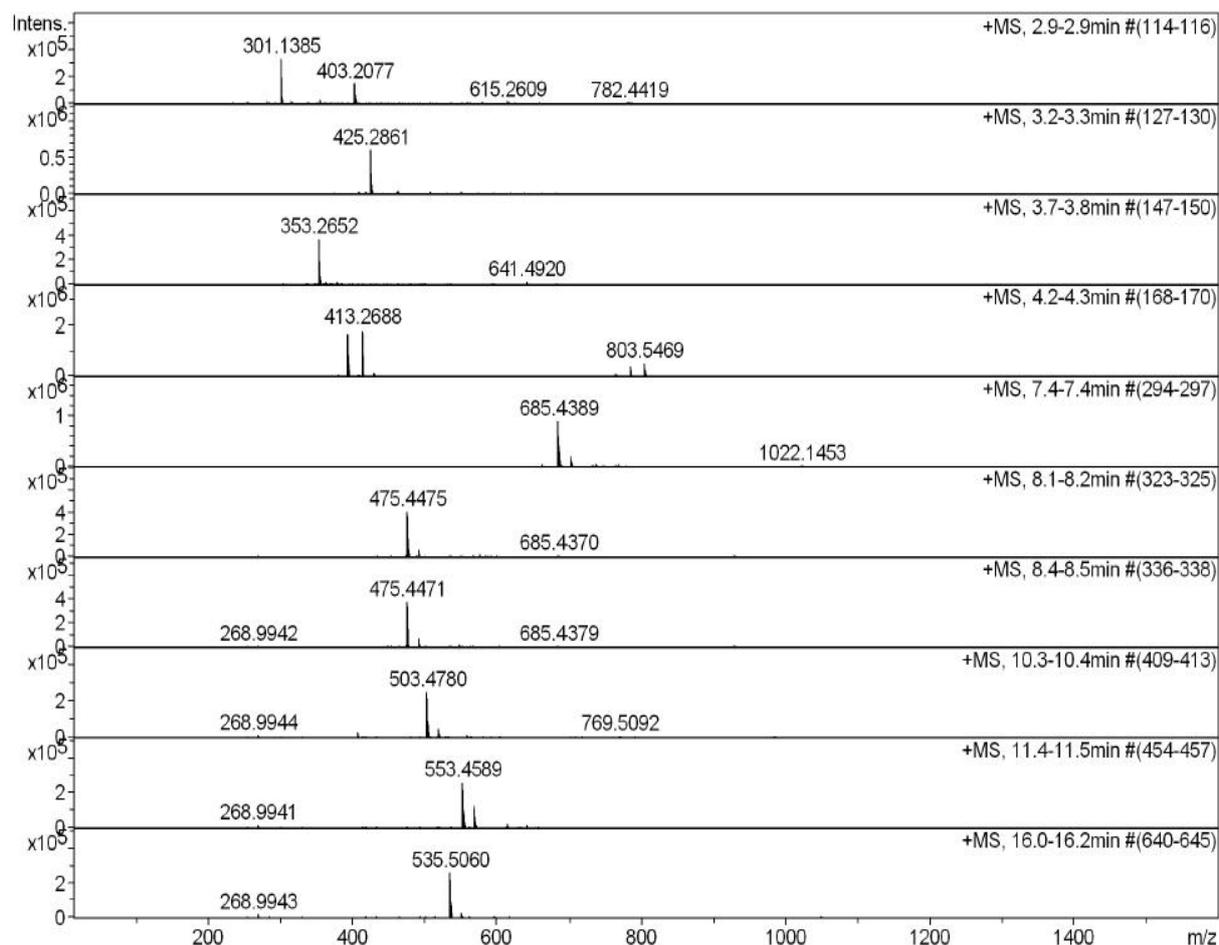


Figure S3(b). Positive electro-spray ionization mass spectrum for P-extract from UB sediment

Table S3. List of calculated mass values, elemental compositions, confirmation of compounds by ChemSpider and Pubchem, and number of isomers for peaks at different retention times in a chromatogram of P-extract.

R.T. (min)	Measured Mass (m/z)	Calculated Mass (m/z)	Formula [M]	Classification	No. of Isomers
2.9	301.1385	301.1394	C ₁₃ H ₂₀ N ₂ O ₆	Y*	117
		301.1369	C ₁₇ H ₂₀ N ₂ OS	Y	1000
		301.1403	C ₁₄ H ₂₄ N ₂ OS ₂	Y	31
	403.2077	403.2075	C ₁₈ H ₃₀ N ₂ O ₈	Y	9
		403.2050	C ₂₂ H ₃₀ N ₂ O ₃ S	Y	725
		403.2084	C ₁₉ H ₃₄ N ₂ O ₃ S ₂	Y	6
3.2	425.2861	425.2857	C ₂₃ H ₃₉ NO ₆	Y	19
		425.2832	C ₂₃ H ₄₀ N ₂ O ₃ S	Y	19
3.7	353.2652	353.2621	C ₂₀ H ₃₆ N ₂ OS	Y	9
4.2-4.3	413.2688	413.2686	C ₂₆ H ₃₆ O ₄	Y	208
		413.2655	C ₂₂ H ₄₀ N ₂ OS ₂	Y	1
7.4	685.4389	685.4323	C ₄₁ H ₅₆ N ₄ O ₅	Y	6
		685.4422	C ₃₉ H ₆₀ N ₂ O ₈	Y	2
		685.4364	C ₄₆ H ₅₆ N ₂ O ₃	NF [‡]	–
		685.4382	C ₃₄ H ₆₀ N ₄ O ₁₀	Y	1
		685.4369	C ₃₃ H ₆₄ O ₁₄	Y	1
		685.4404	C ₅₁ H ₅₆ O	NF	–
		685.4357	C ₃₈ H ₆₀ N ₄ O ₅ S	NF	–
		685.4456	C ₃₆ H ₆₄ N ₂ O ₈ S	NF	–
		685.4397	C ₄₃ H ₆₀ N ₂ O ₃ S	NF	–
		685.4391	C ₃₅ H ₆₄ N ₄ O ₅ S ₂	NF	–
		685.4416	C ₃₁ H ₆₄ N ₄ O ₁₀ S	NF	–
685.4431	C ₄₀ H ₆₄ N ₂ O ₃ S ₂	NF	–		
8.1-8.2	475.4475	475.451	C ₃₂ H ₅₈ O ₂	Y	7
8.4-8.5	476.4488	476.4462	C ₃₁ H ₅₇ NO ₂	Y	5
10.3-10.4	504.4807	504.4775	C ₃₃ H ₆₁ NO ₂	Y	1
11.4-11.5	553.4589	553.4575	C ₃₂ H ₆₀ N ₂ O ₅	Y	1
16.0-16.2	535.5060	535.5085	C ₃₅ H ₆₆ O ₃	Y	2

* Compound has a match in ChemSpider or PubChem [9-10]

[‡] Compound has no match in ChemSpider or PubChem

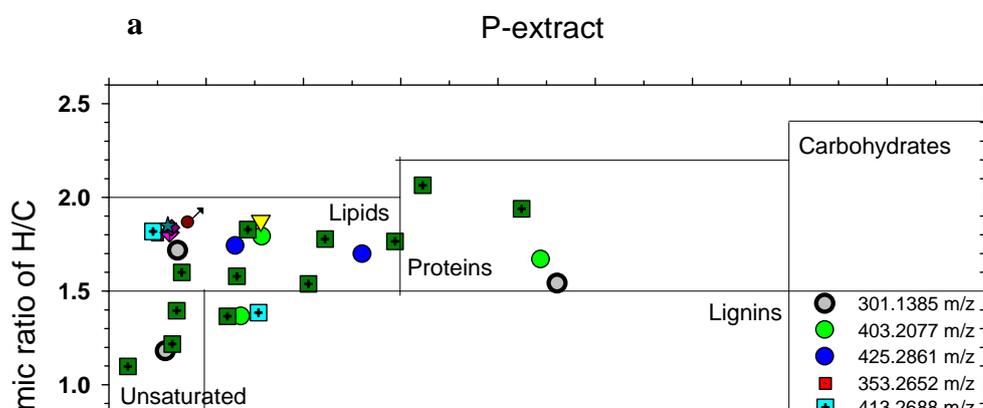
Table S4. List of calculated mass values, elemental compositions, confirmation of compounds by ChemSpider and Pubchem, and number of isomers for peaks at different retention time in a chromatogram of NP-extract.

R.T.	Measured	Calculated	Formula	Classification	No. of
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(min)	Mass (m/z)	Mass (m/z)	[M]		Isomers
2.6-2.7	409.1622	409.1605	C ₁₉ H ₂₄ N ₂ O ₈	NF ³	–
		409.1639	C ₁₆ H ₂₈ N ₂ O ₈ S	Y*	3
		409.1614	C ₂₀ H ₂₈ N ₂ O ₃ S ₂	Y	100
21.3-21.8	381.2946	381.2934	C ₂₂ H ₄₀ N ₂ OS	Y	6
37.5-37.9	413.2643	413.2646	C ₂₁ H ₃₆ N ₂ O ₆	Y	42
		413.2621	C ₂₅ H ₃₆ N ₂ OS	Y	18
		413.2655	C ₂₂ H ₄₀ N ₂ OS ₂	Y	1

* Compound has a match in ChemSpider or PubChem

³ Compound has no match in ChemSpider or PubChem



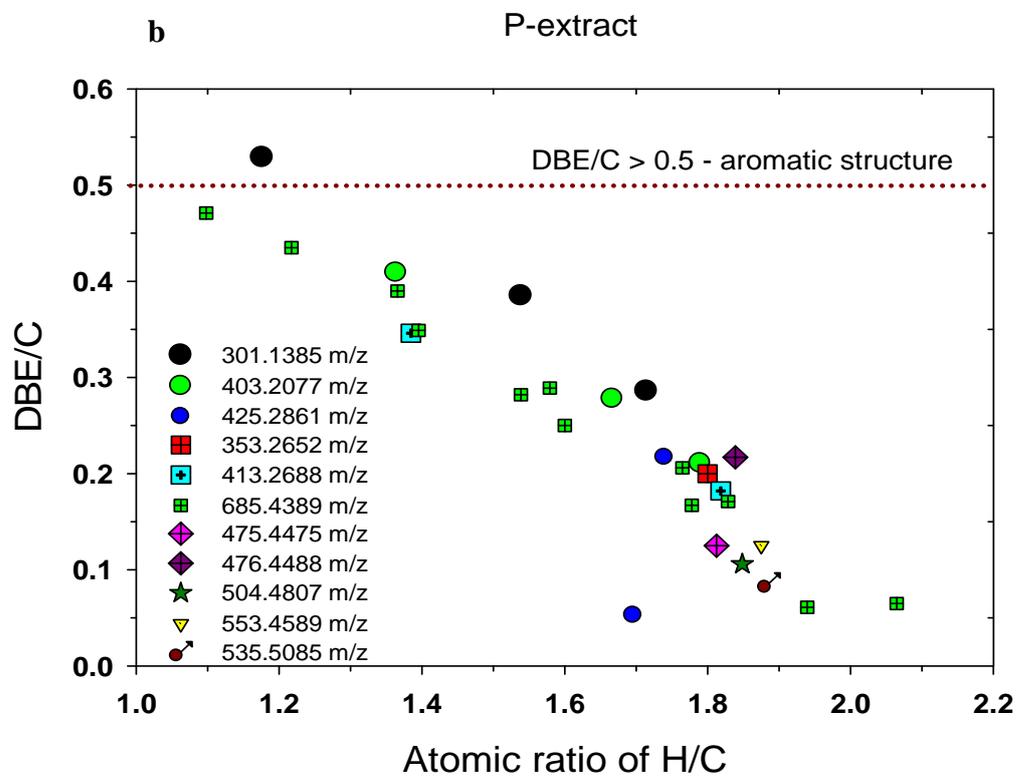
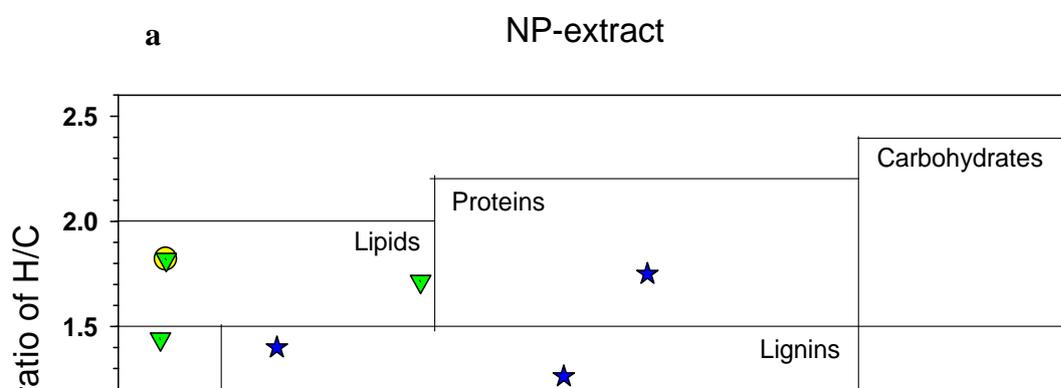


Figure S4(a) A van Krevelen diagram of P-extract, (b) Graphical representation of DBE/C demonstrating the aromaticity of P-extract.



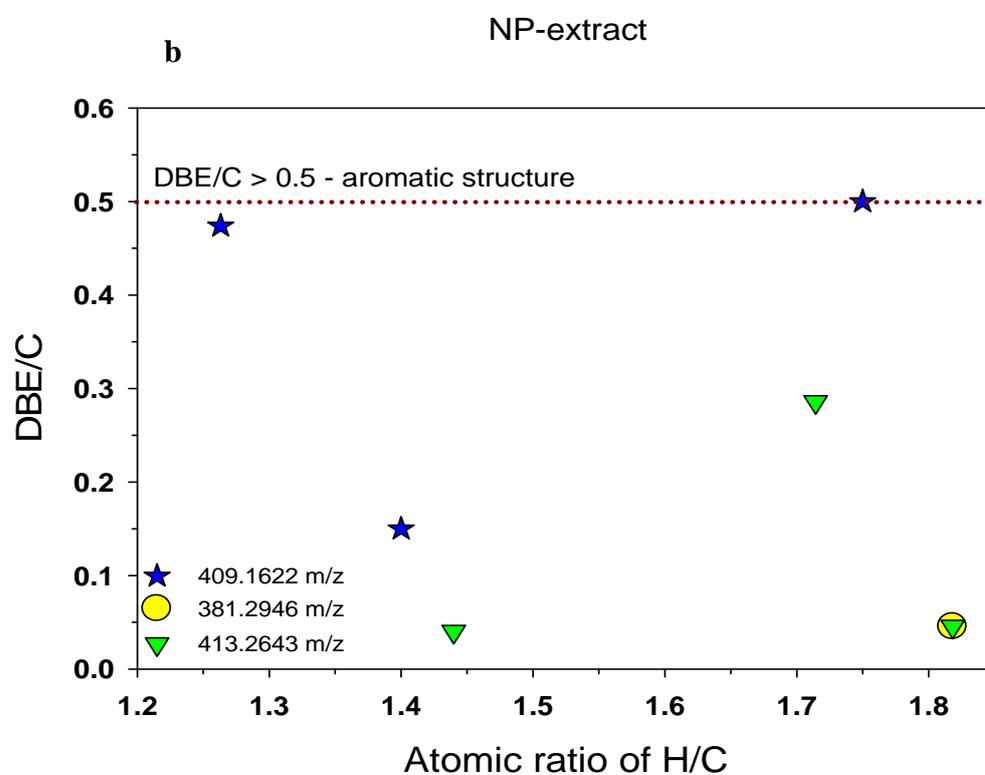


Figure S5(a) A van Krevelen diagram of NP-extract, (b) Graphical representation of DBE/C demonstrating the aromaticity of NP-extract.

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