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Note

Determination of cellulose and non-cellulose carbohydrates in recent sediments by gas chromatography

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Various kinds of polysaccharides are present in recent sediments. As cellulose is the principal constituent of cell walls in higher plants, the determination of cellulose in sediments is expected to provide information about the contribution of higher plants to sedimentary organic matter.

Existing methods for quantifying carbohydrates in recent sediments generally include treatment with concentrated sulphuric acid and then hydrolysis with dilute acid, followed by colorimetric¹, paper chromatographic^{2,3} or gas chromatographic quantification⁴⁻⁷. However, in these methods cellulose and non-cellulose carbohydrates have not been determined separately. These methods may also be liable to decomposition of non-cellulose carbohydrates on treatment with concentrated sulphuric acid.

There have been few reports of the determination of cellulose in recent sediments. Rogers⁸ hydrolysed with 50% sulphuric acid and determined cellulose colorimetrically in lake sediments. Hemicellulose and cellulose in peat samples were determined by proximate analysis^{9,10}, consisting in the extraction of hemicellulose by hydrolysis with dilute hydrochloric acid and the extraction of cellulose by treatment of the pre-hydrolysed sample with concentrated sulphuric acid, followed by a colorimetric quantification. However, in these methods optimum conditions for the extraction of the carbohydrate fractions were not investigated.

A gas chromatographic (GC) method for the determination of cellulose and non-cellulose carbohydrates would be advantageous in providing more accurate molecular information than a colorimetric method.

In this study we examined the optimum conditions for the quantitative extraction and GC determination of cellulose and non-cellulose carbohydrates in recent sediments.

EXPERIMENTAL

Apparatus

Alditol acetates were separated on a 2 m × 3 mm I.D. glass column packed with 5% silicone OV-275 coated on 80-100-mesh Chromosorb W AW DMCS (Gaschro Kogyo Co.). The chromatograph was a Shimadzu Model GC-6AM equipped with a flame-ionization detector. Nitrogen gas was used as the carrier at a flow-rate of

40 ml/min. The column oven was programmed from 170 to 245°C at 2°C/min. Peak areas were measured with a Shimadzu Model C-R1A integrator.

The hydrolysis temperature was controlled ($\pm 0.2\%$) by a dry oven (Shimizu Rikagaku, Model PS-830 H).

A Branson Model Branson 220 was used for ultrasonic dispersion.

Materials

A sediment sample was taken from the central part of Lake Haruna, which is one of the representative mesotrophic freshwater lakes in Japan, with a Eckman dredge, and then was freeze-dried. Whatman CF-11 cellulose powder (ash contents 0.015%, medium length fibre powder) was used as a standard cellulose without further purification.

Procedures

Determination of non-cellulose carbohydrates in a sediment sample. A freeze-dried sediment sample (50–100 mg) was weighed in a 10-ml glass ampoule. A 5-ml volume of 1.0 *N* hydrochloric acid was added to the ampoule, which was then sealed under nitrogen. The sediment sample in the ampoule was hydrolysed by heating at 100°C for 7 h in a dry oven.

After hydrolysis, the suspension sample was transferred into a 10-ml glass centrifuge tube and centrifuged. About 2% hydrochloric acid was added to the tube and the suspension was centrifuged again. This procedure was repeated twice. The combined supernatant solution was used for non-cellulose carbohydrates analysis and the precipitate for cellulose analysis.

Reduction and acetylation of monosaccharides were achieved by using a modification of a method reported by Ochiai¹¹. To the supernatant solution, 50 μg of inositol were added as an internal standard and the solution was evaporated just to dryness with a rotary evaporator. After drying, sodium borohydride solution (about 50 mg in 7 ml of distilled water) was added and monosaccharides in the solution were reduced to alditols by heating at 60°C for 2 h. The dark precipitate produced during reduction was removed by centrifugation. The supernatant was applied to the top of a column (15 cm \times 0.8 cm I.D.) of Dowex 50W-X8 (H^+) cation exchange resin. Alditols were eluted with 25 ml of distilled water. The eluate containing alditols was evaporated to dryness with a rotary evaporator. After drying, about 10 ml of methanol were added to the residue and the solution was evaporated to dryness to free it from borate ion. This procedure was repeated twice. The dried residue was transferred into a 1-ml glass ampoule with methanol and the solvent was completely removed under vacuum. After addition of 100 μl of acetic anhydride-pyridine (1:1), alditols were acetylated by heating at 100°C for 2 h. The solvent mixture was evaporated to dryness and the residue was dissolved in 50 μl of chloroform. An aliquot of this solution was injected on to the GC column.

Determination of cellulose in a sediment sample. The precipitate that was obtained by centrifugation after hydrolysis with 1.0 *N* hydrochloric acid was completely dried under vacuum in a 10-ml centrifuge tube. After addition of 0.3 ml of 72% sulphuric acid to the tube, the contents were dispersed ultrasonically and heated at 40–45°C for 1 h to dissolve cellulose. Immediately after heating, distilled water was added to dilute the sulphuric acid to 1.0 *N*. This suspension sample was transferred

into a 10-ml glass ampoule and then sealed under nitrogen. The sample was hydrolysed by heating at 100°C for 7 h in a dry oven. After 50 μ g of inositol had been added to the hydrolysed sample as an internal standard, the suspension was neutralized by addition of 1.1 g of barium hydroxide. Immediately after neutralization, the suspension was adjusted to about pH 5 by addition of 2 N hydrochloric acid. The lead sulphate produced was removed by filtration through a glass-fibre filter (Whatman GF/C) that had been baked in a furnace at 500°C for 4 h.

The monosaccharides in the filtrate were analysed by the method described above for non-cellulose carbohydrates analysis.

RESULTS AND DISCUSSION

The method that we wanted to establish for the determination of non-cellulose and cellulose carbohydrates in sediments consists of two major steps: (1) hydrolysis of non-cellulose carbohydrates with dilute hydrochloric acid and (2) dissolution of cellulose with concentrated sulphuric acid followed by hydrolysis of the dissolved cellulose with dilute sulphuric acid. In order to establish the method, we examined the optimum conditions for these two steps.

Hydrolysis of non-cellulose carbohydrates in a sediment sample

Fig. 1 shows a typical gas chromatogram of monosaccharides obtained by hydrolysis of a surface sediment of Lake Haruna with dilute hydrochloric acid. The following eight monosaccharides were found: glucose, galactose, mannose, xylose, arabinose, ribose, fucose and rhamnose.

In order to establish the optimum conditions for the hydrolysis of non-cel-

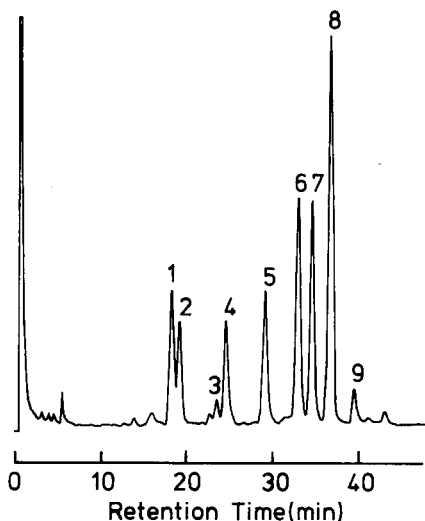


Fig. 1. Gas chromatogram of alditol acetates of monosaccharides obtained by dilute hydrochloric acid hydrolysis of a surface sediment from Lake Haruna (non-cellulose fraction). Peaks: 1 = rhamnose; 2 = fucose; 3 = ribose; 4 = arabinose; 5 = xylose; 6 = mannose; 7 = galactose; 8 = glucose; 9 = inositol (internal standard).

TABLE I

EFFECT OF HYDROCHLORIC ACID CONCENTRATION ON THE HYDROLYSIS OF NON-CELLULOSE CARBOHYDRATES IN A SEDIMENT SAMPLE AT 100°C FOR 7 h

Rh = rhamnose; F = fucose; Ri = ribose; A = arabinose; X = xylose; M = mannose; Ga = galactose; Gl = glucose. The values were calculated by taking assigning a value of 100 to the amounts of monosaccharides obtained by 1.0 *N* HCl hydrolysis at 100°C for 7 h.

HCl concentration (<i>N</i>)	Total	Rh	F	Ri	A	X	M	Ga	Gl
0.50	67	80	76	78	71	74	59	66	63
1.00	100	100	100	100	100	100	100	100	100
1.25	93	93	96	84	90	88	93	92	95
1.50	89	93	92	72	84	81	86	87	96
2.00	63	71	68	28	58	51	64	61	69

lulose carbohydrates, a sediment sample was hydrolysed with various concentrations of dilute hydrochloric acid and at different temperatures. Tables I and II give the results. The amounts of each monosaccharide given in Tables I and II represent values obtained by arbitrarily taking the amounts of the corresponding monosaccharide obtained by 1.0 *N* hydrochloric acid hydrolysis at 100°C for 7 h as 100. Hydrolysis under these conditions yielded largest amounts of individual monosaccharides.

These results are in good agreement with the optimum conditions reported by Ochiai¹¹ for dissolved carbohydrates in a lake water.

Hydrolysis of cellulose with dilute hydrochloric acid

Hydrolysis of cellulose with dilute hydrochloric acid was carried out to test whether or not cellulose were hydrolysed under the conditions where non-cellulose carbohydrates were hydrolysed. Cellulose powder (Whatman CF-11) was hydrolysed with 1.0 *N* hydrochloric acid at 100°C for 3, 5 or 7 h. Only 2.7–5.6% of the initial amounts of the cellulose was hydrolysed to glucose under these conditions, which indicates that non-cellulose carbohydrates can be extracted by 1.0 *N* hydrochloric acid hydrolysis at 100°C for 7 h with negligible extraction of cellulose.

TABLE II

EFFECT OF TEMPERATURE ON THE HYDROLYSIS OF NON-CELLULOSE CARBOHYDRATES IN A SEDIMENT SAMPLE WITH 1.0 *N* HYDROCHLORIC ACID FOR 7 h.

Abbreviations and calculation of results as in Table I.

Hydrolysis temperature (°C)	Total	Rh	F	Ri	A	X	M	Ga	Gl
90	86	96	101	88	92	96	79	84	77
100	100	100	100	100	100	100	100	100	100
110	76	78	90	19	66	50	78	78	85

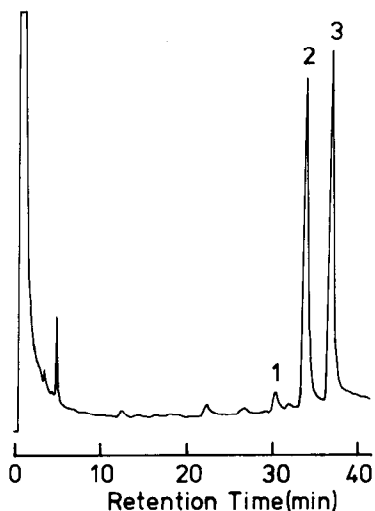


Fig. 2. Gas chromatogram of alditol acetates of monosaccharides obtained from a surface sediment from Lake Haruna (cellulose fraction). Peaks: 1 = mannose; 2 = glucose; 3 = inositol (internal standard).

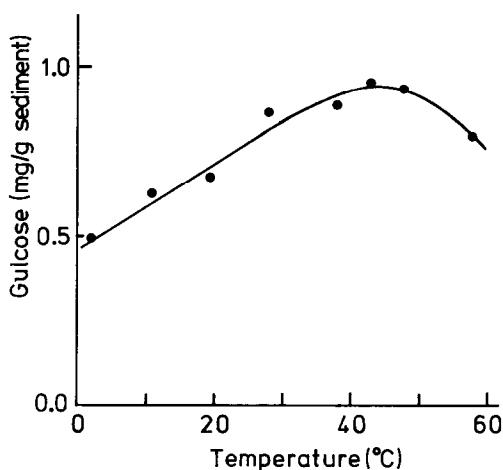


Fig. 3. Amounts of glucose obtained from a surface sediment from Lake Haruna by 72% sulphuric acid treatment at different temperatures.

Determination of cellulose in a sediment sample

The cellulose in a sediment sample was dissolved with 72% sulphuric acid after removing non-cellulose materials by hydrolysis with 1.0 *N* hydrochloric acid. The dissolved cellulose were hydrolysed with 1.0 *N* sulphuric acid and then analysed by GC as alditol acetates. Fig. 2 shows a typical gas chromatogram of monosaccharides obtained after hydrolysis of cellulose in a sediment from Lake Haruna. As expected, the most abundant monosaccharide was found to be glucose. A very small amount of mannose was also detected in the hydrolysates, but mannose was found to be contaminant during the analytical procedure on the basis of a blank experiment. Consequently, the above result indicates that the carbohydrate remaining after hydrolysis with 1.0 *N* hydrochloric acid of a sediment sample is cellulose.

In order to establish the optimum temperature for extraction of cellulose, a pre-hydrolysed (1.0 *N* hydrochloric acid) sediment sample was heated in 72% sulphuric acid at different temperatures (3–60°C) and hydrolysed with 1.0 *N* sulphuric acid.

As shown in Fig. 3, the yield of glucose was maximum at an extraction temperature of 40–45°C.

Total carbohydrates in sediments have generally been determined after treatment with 72% sulphuric acid at room temperature^{1,5} or below room temperature^{2-4,6,7,12}. Fuchsman¹³ claimed that treatment with concentrated (72–80%) sulphuric acid above 20–30°C had a risk of side reactions occurring. The present study, however, showed that heating in 72% sulphuric acid at 40–45°C was optimum for the extraction of cellulose from sediments.

In order to calculate the efficiency of the extraction of cellulose, cellulose powder (Whatman CF-11) was heated in 72% sulphuric acid at 35–40°C followed by

TABLE III

ANALYSIS OF NON-CELLULOSE AND CELLULOSE CARBOHYDRATES IN A SURFACE SEDIMENT OF LAKE HARUNA BY THE PROPOSED METHOD

Carbohydrates	Number of analyses	Mean (mg/g sediment)	Standard deviation (mg/g sediment)	Relative standard deviation (%)
Non-cellulose	4	13.2	0.9	6.9
Cellulose	3	0.95	0.02	1.7

hydrolysis with 1.0 *N* sulphuric acid. The results showed that $90.1 \pm 1.7\%$ of the initial weight of cellulose was recovered as glucose, and that the side reactions during treatment with 72% sulphuric acid occur to only a very small extent.

Application to a sediment sample

As a result of the experiment described above, we concluded that hydrolysis of sediments with 1.0 *N* hydrochloric acid at 100°C for 7 h is optimum for the extraction of non-cellulose carbohydrates, and heating of pre-hydrolysed (1.0 *N* hydrochloric acid) sediment in 72% sulphuric acid at 40–45°C and hydrolysis of the heated sample with 1.0 *N* sulphuric acid at 100°C for 7 h is optimum for the extraction of cellulose.

Non-cellulose and cellulose carbohydrates in a surface sediment of Lake Haruna were analysed by the proposed method. Table II gives the results and shows that the reproducibility of the method is within $\pm 7\%$ (relative standard deviation).

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