

## 湖底泥の有機酸の分析

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## Determination of Organic Acids in the Lake Sediment

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In order to study the detailed distribution and the composition of organic acids present in the lake sediments, a new analytical method was established and applied to the bottom sediments of Lake Biwa. Extraction of organic acids was carried out by the use of the method reported by Miyoshi and Neish. The ethyl ether extract was evaporated to dryness and converted to butyl esters by refluxing in butanol, H<sub>2</sub>SO<sub>4</sub> and anhydrous sodium sulfate by Yamashita's method. Using a Hydrogen-flame ionization detector and glass column (3 m×3 mm i.d.) of SE-52 on chromosorb W on a Shimazu GC-6A, nitrogen flow rate of 40 ml/min. and temperature programming between 45°C and 220°C at a rate of 6°C/min were selected as optimum condition of acid ester analysis. The average recovery of organic acids by the use of the above procedure was much higher than that of Koyama's silica-gel chromatography.

Identification and determination of organic acids in sediment samples from Lake Biwa were carried out and obtained:

In the bottom sediment, the organic acids detected were formic, acetic and regardless of the location and depth of the bottom sediments. In general Acetic acid was found in the highest concentration. The organic acids were not detected in the interstitial water of the sediment, but were found in adsorbed form on/in the sediment particles.

It is considered that the decomposition of organic matter in the lake sediments plays an important role on the metabolism in the lake. As is generally known, the lake sediment contains organic matter which is derived from undecomposed part of autochthonous organic matter produced in the lake water and allochthonous one from the outside of the lake.

Generally, the organic matter in the lake sediments decomposed under aerobic conditions is ultimately converted into carbon dioxide and mineral nutrient such as ammonia and phosphate.

While, in the mineralization process of organic matter under anaerobic condition, organic acids are produced as the intermediates by heterotrophic bacteria. With regard to utilization of organic acid in the anaerobic sediment, it is well known that methane producing bacteria utilize organic acids directly or indirectly as the substrate, while sulfate reducing bacteria utilize some restricted organic acids directly as the electron donor in the sulfate reduction. The concentration and composition of organic acids which are found in the sediments might reflect the bacteriological and biochemical feature of the environment.

From these point of view, the present paper deal

with the establishment of the more sensitive analytical method of organic acids, and applied to the bottom sediments of some fresh water region.

### Materials and Methods

#### *Preparation of Sediment Samples*

The bottom sediment samples were collected by the use of KK core sampler<sup>1)</sup> on July, 2, 1981. The sediment column was sliced into 1 cm or 0.5 cm thickness, and each slice was put into a plastic bottle, sealed tightly, kept cool in an ice box, and brought into the laboratory. In this experiment, two sampling stations, Station A (depth, ca. 80 m) and Station B (depth, ca. 3 m) were set up in each basin as shown in Fig. 1.

#### *Organic Acids in the Sediment*

Two hundred ml of 0.5 N H<sub>2</sub>SO<sub>4</sub>-80% ethanol solution were added to 50 g of the wet sediment sample and stirred vigorously for 48 h at room temperature. The supernatant fraction containing organic acids was separated by centrifugation (10,000×g, 15 min). The residual organic acids were further extracted from the precipitates by repeating above treatments for three times and all

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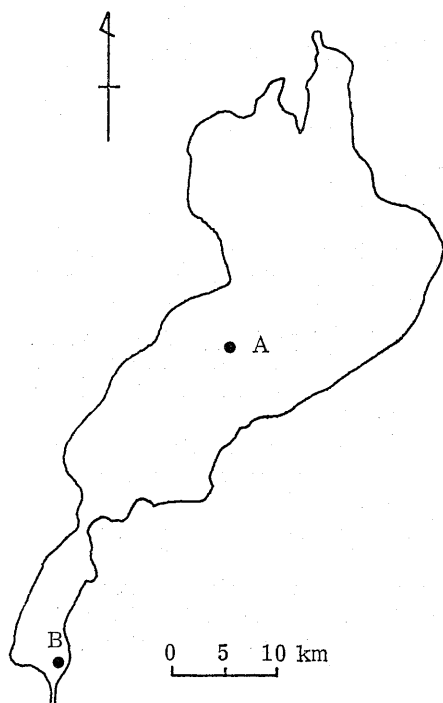


Fig. 1. Stations set up at the northern and southern basins of Lake Biwa.

of the supernatant fraction was put together, neutralized with NaOH, concentrated into 80 ml in vacuo at 55°C. This acidified solution (pH 1–2) was extracted again using Neish's continuous extraction method.<sup>2,3)</sup> The extract solution was evaporated to dryness under vacuo at 55°C and organic acids in it were estrified with *n*-butanol in the presence of H<sub>2</sub>SO<sub>4</sub>, anhydrous sodium sulfate, then shaken with hexane-water.<sup>4)</sup> The hexane fraction of the butyl derivatives, supplemented with *n*-dodecane as internal standard, was analyzed by gas chromatography under the condition shown in Table 1. Respective organic acid was identified by gas mass spectrometry shown in Table 2. For the comparison, the ether extract of organic acids obtained from same sediment samples described above was analyzed using the silica-gel chromatography.<sup>5)</sup>

#### Organic Acids in the Interstitial Water

Fifty ml of interstitial water of bottom sediment were adjusted to pH 2 by H<sub>2</sub>SO<sub>4</sub>, and organic acids in the solution were extracted with ether by Neish's continuous extraction. Identification and determination were carried out by gas chromatography as same as in the sediment samples.

Table 1. Instrument and operating conditions in the gas chromatography

Instrument	Shimadzu GC-6A Dual flame ionization detector
Column	3 m × 3 mm i.d., glass
Support	Chromosorb W, 60–80 mesh
Substrate	3% silicon SE-52
Column temperature	
Starting	45°C
Heating rate	6°C/min
Fishing	250°C
Injection port temperature	250°C
Detector temperature	250°C
Sensitivity	10t KY
Range	32 mV
Nitrogen flow rate	40 ml/min
Hydrogen flow rate	90 ml/min
Air flow rate	1000 ml/min

Table 2. Instrument and operating conditions in the gas mass spectrometry

Instrument	Shimadzu gas-mass LKB 9000S
Column	2 m × 3 mm i.d., glass
Substrate	3% Silicon SE-52
Accelerating voltage	3.5 KV
Ionizing electron energy	22 eV
Ion source temperature	310°C
Trap current	60 μA
Filters	240 Hz

Table 3. Comparison the gas chromatography with the silica-gel chromatography on organic acids analysis of sediment (0–10 cm)

Organic acid (μ moles/g wet mud)	Silica-gel chromatography		Gas chromatography	
	Station A	Station B	Station A	Station B
Formic acid	4	2	8.5	2.7
Acetic acid	40	25	50.5	27.8
Butyric acid	1	0	3.1	0.0

## Results and Discussion

### Comparison of the Gas Chromatography with the Silica Gel Chromatography in the Organic Acid Analysis

Miyoshi *et al.*<sup>2)</sup> and Nikaido<sup>3)</sup> analyzed organic acids in the sediments by use of silica gel chromatography. On the other hand, the gas chromatography by Harmon and Doelle<sup>7)</sup> and Horning<sup>8)</sup> have to determine volatile and non-

**Table 4.** Recovery of organic acids by silica-gel chromatography and gas chromatography using the sediment of station A (0~10 cm)

Organic acid ( $\mu\text{mol/g}$ wet mud)	Silica-gel chromatography			Gas chromatography		
	Found	Added	Recovered (%)	Found	Added	Recovered (%)
Formic acid	3	5.0	7 (80)	8.2	5.0	13.1 (99)
Acetic acid	42	20.0	57 (88)	51.3	20.0	72.1 (102)
Butyric acid	2	5.0	6 (80)	3.5	5.0	8.4 (97)
Propionic acid	0	5.0	4 (80)	0.0	5.0	4.9 (98)
Lactic acid	0	5.0	4 (80)	0.0	5.0	4.7 (94)
Citric acid	0	5.0	4 (80)	0.0	5.0	4.9 (98)

**Table 5.** The amount of organic acids in the bottom sediment and its interstitial water at station A and station B

Fraction	Organic acid ( $\mu\text{mol/g}$ wet mud)	Station A		Station B	
		(0-1 cm)	(9-10 cm)	(0-1 cm)	(9-10 cm)
Whole sediment	Formic acid	9.5	0.5	1.4	2.4
	Acetic acid	48.5	28.6	17.3	25.4
	Butyric acid	3.1	0.4	0.0	0.0
Interstitial water	Formic acid	0.0	0.0	0.0	0.0
	Acetic acid	0.0	0.0	0.0	0.0
	Butyric acid	0.0	0.0	0.0	0.0

volatile organic acids, separately. Accordingly, we adopted Yamashita's<sup>4)</sup> method in which both groups of organic acid can be determined at the same time. Table 3 shows the comparison of organic acid analysis of same sample, which were determined by the silica gel chromatography and by our gas chromatography.

The each value of the organic acids determined by gas chromatography was higher than that by silica gel chromatography. Table 4 shows the recovery of each organic acid supplemented in the sediment sample in the analysis by both methods. Also in the recovery test, the gas chromatography was far better than the silica gel chromatography.

From the facts presented above, it was found that gas chromatography is good enough to determine the organic acids present in the bottom sediments of water environments.

#### *Application of Method to the Bottom Sediment in Lake Biwa*

Table 5 shows the amount of organic acids in

the bottom sediments and its interstitial water at station A and B in Lake Biwa. In all the case, organic acid were not detected in the interstitial water. The fact suggests that organic acids are present in adsorbed form on/in sediment particles. The leading molecular species of organic acids occurring in the sediments of both stations were formic and acetic acids. Among these, acetic acid was found in the highest concentration in general. In the sea and lake environments, Miyoshi *et al.*<sup>2)</sup> has reported that the organic acids found in the bottom sediment are composed of butyric, propionic, acetic, formic, lactic and unidentified acids, and acetic acids is predominant. Koyama and Tomino<sup>9)</sup> and Koyama<sup>10)</sup> have also reported that the organic acids which are produced through the decomposition process of organic matter in the lake sediments are butyric, propionic, acetic and formic acids. In comparison with above reports, a major difference of the results obtained in this is absence of propionic and lactic acids in the sediments of Lake Biwa. Koyama<sup>11)</sup> pointed out that propionic and lactic acids participate in

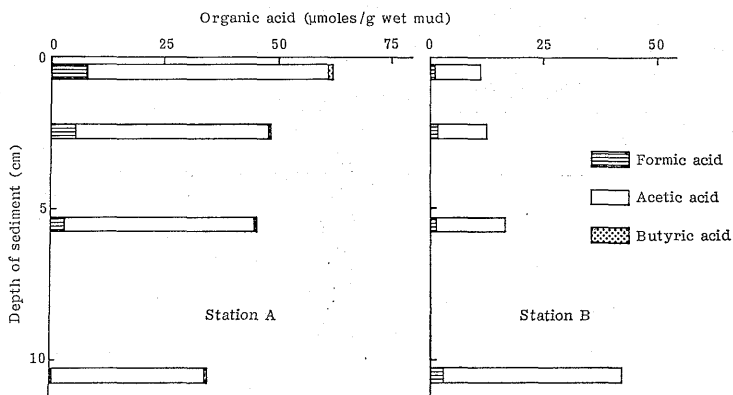


Fig. 2. Vertical distribution of organic acids in the sediments of station A and B.

the methane production in the mineralization process of organic matter in the lake sediments. Tezuka *et al.*<sup>12)</sup> demonstrated that sulfate reducing bacteria utilize lactic acids as the electron donor of sulfate reduction in the polluted river water. It may be considered that the absence of lactic and propionic acids in Lake Biwa may be due to the higher activity of bacterial utilization of these organic acids. The vertical distribution of organic acids in the sediments is shown in Fig. 2. At station A, the maximum concentration of total organic acids was observed at about 1 cm depth below the sediment surface. The similar pattern was also found in the vertical distribution at station B. However, the depth of the maximum concentration at station B was found about 7 cm depth below the surface. Further, the maximum concentration at station A was much higher than that at station B.

The accumulation of sulfides in the bottom sediment which was already shown in the previous paper seems to be fairly correlated with the distributions of organic acids in the both basins of Lake Biwa.<sup>13)</sup> The detailed relationship between sulfides and organic acids in the sediments will be made clear in the next step of the study based on the microbiological point of view.

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