Analysis of Malodorous Volatile Substances of Human Waste: Feces and Urine

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The amounts of volatile substances responsible for the malodor of human waste (feces and urine) obtained from the storage tank of a community waste-water treatment plant were determined. Thus far, there has been little systematic research on malodor-causing substances of human waste. These substances were collected using Tenax-TA, and their concentrations were determined by the usual thermal-desorption cold-trap injector/gas chromatography/mass spectrometry (TCT/GC/MS). About 90% of the malodor-causing substances were fatty acids: acetic acid, propionic acid and butyric acid. The proportion of ammonia was 6.5%. Other malodor-causing and minor substances detected were indole, skatole, pyridine, pyrrole, hydrogen sulfide, and methyl mercaptan. In addition, a small amount of paradichlorobenzene used as a deodorizer in household toilets was also recognized.

Key words —— human waste, malodorous substance, TCT/GC/MS

INTRODUCTION

Unpleasant odors in the environment attract public attention. They are emitted by facilities such as pig farms, chicken coops, sewage systems, and food-processing plants, as well as toilets, shoe closets and home kitchens.[1] The offensive odor control law[2] regulates the permissible amounts of twenty-two malodor-causing substances. In various places, concentrations of substances causing unpleasant odors are measured.

We have isolated a microorganism from nature[3,4] and examined its characteristics in terms of its ability to decompose hydrogen sulfide, methyl mercaptan, and skatole for its application in the degradation of substances causing unpleasant odors from human waste. Recently, malodorous substances emitted from a sewage system and a sewage disposal plant were degraded using a microorganism, and this is regarded as a promising method for eliminating unpleasant odors. Microbial deodorization is specific to the decomposition of malodorous substances. The chemical composition of substances causing unpleasant odors must be identified in order to eliminate them. However, no systematic studies have been carried out to determine the malodorous substances of human waste.

We therefore first evaluated methods of sampling and analyzing malodorous volatile substances of human waste. Then, we measured the concentrations of fatty acids, sulfide compounds and nitrogen compounds which are considered to be the main compounds[5–10] causing the unpleasant odors emitted from this waste, using the thermal-desorption cold trap injector method and the gas chromatography/mass spectrometry method.

MATERIALS AND METHODS

Materials and Chemicals —— Human waste samples were collected from a tank in the storage sewage disposal plant at West Port, Kitakyushu city. The samples were stored in a cooler box at 4°C. The commercial adsorbent filling agents Carbopack, Chromosorb (Supelco Inc., U.S.A.), and Tenax-TA (GL Sciences Inc., Japan), were used to collect sample gases. Standard reagents were purchased from Wako Pure Chemical Industries (Japan) and Tokyo Kasei Kogyo Co. (Japan). All of the other chemicals used were commercially available and of chemically pure grade. Distilled water, purified with a Milli-QSP system (Millipore, Milford, MA, U.S.A.), was used for all aqueous solutions.

Collection of Malodorous Components by the Head Space Method —— Figure 1 shows the three types of apparatus used for the collection of the malodorous substances. The substances were collected by passing ultrapure (99.9999%) helium gas (Japan Air Liquid Co., Ltd., Japan) through a purifier (packed with Molecular Sieve 3A) cooled with liquid nitrogen and then through a glass vessel in which

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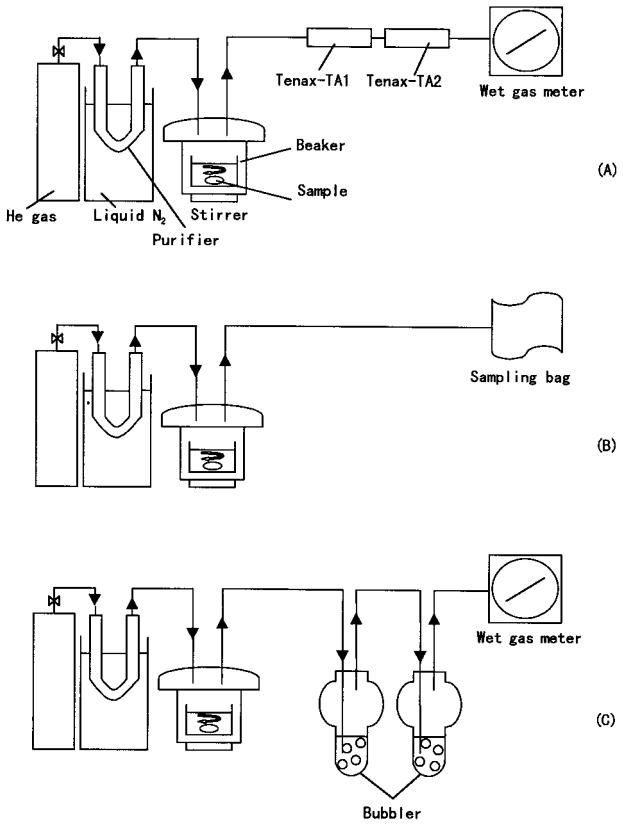

Fig. 1. Three Types of Apparatus to Collect Volatile Substances of Human Waste
(A): For fatty acids, aliphatic- and aromatic-compounds. (B): For sulfur compounds. (C): For ammonia and trimethylamine.

30 ml of human waste in a 100 ml beaker was stirred at about 300 rpm. One hundred microliters of 100 ppm benzene-d_6(C_6D_6) was added as an internal standard to 30 ml of the waste sample or ultrapure water. In the apparatus, Apiezon N (Apiezon Products, Ltd., U.K.) was applied to seal the grinding part of the glass vessel containing the human waste. The flow rate was adjusted to 1 l/min using a flow regulator (RK1350V, Kojima Seisakusyo). Teflon tubes 1/8 and 1/4 inch in diameter were used, and the cumulative flow was measured with a wet-gas meter (W-NK-1B, Shinagawa Keisokuki).

Measurements ——The qualitative analysis of malodorous substances from human waste was conducted simultaneously using thermal-desorption cold-trap injector/gas chromatography/mass spectrometry (TCT/GC/MS). Quantitative analyses were performed individually for fatty acids, aliphatic- and aromatic-compounds, sulfur compounds, and nitrogen compounds (*e.g.*, ammonia and amines), which are believed to be the malodorous substances involved.[5–10)]

Hydrocarbons, such as fatty acids, aliphatic- and aromatic-compounds, adsorbed with Tenax-TA at room temperature were analyzed using TCT/GC/MS, as shown in Fig. 1(A). Table 1 shows the conditions of TCT and GC/MS for hydrocarbons. Under these conditions, a polar DB-WAX analysis column (J & W Scientific, Folsom, CA, U.S.A.) was used, which detected all the target substances of short-chain fatty acids, sulfur compounds, pyridine, and pyrrole at high reproducibility. Identification was made by standard mass spectrum of a library research system. Confirmation was carried out by GC, retention time and mass spectrum with the reference substance, and quantity was determined by internal standard method.

Volatile sulfur compounds were analyzed by GC using a Flame photometric detector (FPD) as a detector after cold-trapping of the gas in a Tedlar bag(capacity 10 l). The gas collected in the bag shown in Fig. 1(B) was injected into the gas chromatograph using a gas-tight syringe and analyzed under the conditions listed in Table 2 according to the analytical methods[11,12)] of offensive odors specified by JIS K0092[13)] and K0108.[14)] Hydrogen sulfide and methyl mercaptan/nitrogen reference gas (100 ppm) were used, respectively, for the prepara-

Table 1. Analytical Conditions for Fatty Acids, Aliphatic- and Aromatic- Compounds

TCT	Chrompack
Sample injection method	Thermal heating desorption and reconcentration
Trap	DB-1(J & W), ϕ 0.25 mm $\times$ 30 m, film thickness 0.25 μm
Trap temperature	-130°C
Desorption temperature	240°C
Desorption time	10 min
Desorption flow rate	10 ml/min
Injection temperature	200°C
Injection time	3 min
GC/MS	Hewlett Packard HP5890+5970B
Column	DB-WAX (J & W), ϕ 0.25 mm $\times$ 30 m, film thickness 0.5 μm
Column temperature	16 min at 30°C, 5°C/min to 250°C
Injection temperature	200°C
Carrier gas	He, 1 ml/min
Separator temperature	280°C
Monitor	Scan range (m/z10–500), Selected ion monitoring

Table 2. Analytical Conditions for Sulfur Compounds

Gas chromatograph	Hewlett Packard HP5890II
Column	β, β'-oxydipropionitrile (GL Sciences Inc.)
	Glass ϕ 2 mm $\times$ 2.5 m
Column temperature	70°C (isothermal)
Injection temperature	200°C
Carrier gas	He, 30 ml/min
Detector	Flame photometric detector (FPD), 200°C

tion of calibration curves.

Ammonia was determined as follows. The helium gas flow rate was 0.5 l/min in the sample collection apparatus, and evaporated volatile substances were bubbled through 20 ml of a trapping solution (0.5% boric acid) contained in two absorption bottles (capacity 200 ml) connected in series [Fig. 1(C)]. This gas-trapping solution and the fluid used in washing were pooled and adjusted to a fixed volume; ten milliliters of this solution was placed in a test tube with a stopper and used as a sample for analysis. After 5 ml of 1% phenol 0.005% sodium pentacyano nitrosyl ferrate (III) solution was added to a sample and mixed well by shaking, 5 ml of 0.05% sodium hypochlorite solution was added, the stopper was applied, and the contents were mixed gently. The mixture was allowed to stand for 1 hr at 25–30°C, and the absorbance at 640 nm was measured. 10 ml of this solution was analyzed by absorptiometry ,the analytical method[11,12] for offensive odors (JIS K0099).[15] Ion chromatography was performed to confirm the measured values.

Amine was determined as follows. After helium gas was passed through the sample collection apparatus shown in Fig. 1(C) at a rate of about 0.5 l/min, evaporated volatile substances from human waste were trapped in 20 ml of a trapping solution (0.02 N H_2SO_4 in water) in two bottles connected in series. After the pH of this gas-trapping solution was adjusted to about 7 with 1 N NaOH, the solution was passed through a Millipore filter (pore size; 0.44 μm), and analyzed by ion chromatography under the conditions shown in Table 3.

RESULTS AND DISCUSSION

Collection of Malodorous Components from Human Waste and Evaluation of the Adsorbent

Since the concentrations of malodorous components from human waste are believed to be extremely low, sufficient evaluation including a reduction in the background value is needed. Therefore, we assessed the efficacy of the gas collection apparatus

Table 3. Analytical Conditions of Ion Chromatography for Trimethylamine and Ammonia

Ion chromatography	Yokogawa Analytical Systems IC7000
Injection volume	50 μl
Column	ICS-C25 (cation analysis)
Gird column	ICS-C2G
Eluate	5 mM tartaric acid/1 mM 2,6-pyridine dicarboxylic acid
Detector	Conductometric detector
Flow rate	1 ml/min
Column temperature	40°C

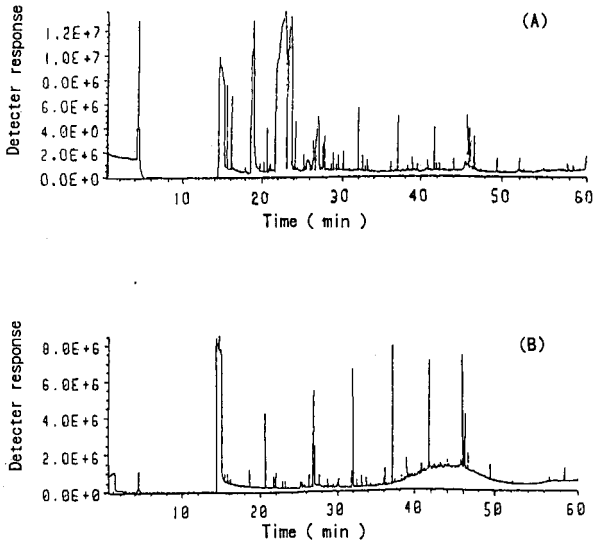

Fig. 2. TIC Chromatogram of GC/MS Analysis under Different Conditions

(A): Before improvement (using N_2 and a silicon tube). (B): After improvement (using He and a Teflon tube).

and the absorbent. In this case, a Tenax-TA adsorbent was preheated more than 3 hr at 300°C.

Pure nitrogen gas was flowed through a beaker without a sample and then passed through the adsorbent as shown in Fig. 1(A). Tenax-TA adsorbent tubes and the target substances were analyzed by GC/MS under the conditions listed in Table 1. In this experiment, silicon tubes and silicon grease were used. Many peaks were obtained and a low peak resolution was observed in the chromatogram in the case of the pure nitrogen gas in the beaker without a sample as shown in Fig. 2(A). The background values due to pure nitrogen gas, grease, silicon tubes, and adsorbent tubes were examined by GC/MS. These values were found to be greatly reduced by using 99.9999% ultrapure helium gas, Teflon tubes and Apiezon N grease with low contents of organic compounds, and by incorporating a purifier into the system [Fig. 2(B)].

Since a large amount of moisture is contained in the headspace gas of waste samples, the peak of water interferes with the GC/MS analysis. The trapping characteristics of Carbopack, Chromosorb, and Tenax-TA, which are commercial adsorbent filling agents, were determined to evaluate their appropriateness as trapping materials for malodorous fatty acids, nitrogen compounds, and aliphatic – and aromatic – compounds. Twenty mg of each adsorbent filling agent was packed in a glass tube, 5 mm in external diameter (3 mm in internal diameter) and 250 mm long, and then 10 l of the headspace gas of the waste samples was passed through the tube (0.5 l/min), and the target compounds were collected by adsorption. Carbopack and Chromosorb could not be used because they adsorbed water, and the baseline of the chromatogram produced using these adsorbents was unstable during GC/MS analysis. Tenax-TA (a porous polymer) was selected since its use produced a chromatogram with distinct peaks and a stable baseline.

Evaluation of Breakthrough Volume of Malodorous Substances Adsorbing Tenax-TA

Leakage from the adsorbent with odorous compounds is called a breakthrough, and the volume of gas passed at this point is defined as the breakthrough volume. The breakthrough volume was evaluated to determine the optimum volume of headspace gas of the waste samples which was captured by the adsorbent Tenax-TA. To determine the breakthrough volume of Tenax-TA for each target substance, a certain amount of the standard solution of fatty acid, pyridine and pyrrole were added to Tenax-TA1 and then helium gas was passed through two Tenax-TA trapping tubes connected in series. The compounds trapped in the first (Tenax-TA1) and second (Tenax-TA2) tubes were analyzed. No breakthrough for butyric acid, *i*-valeric acid, *n*-valeric acid and pyridine was observed in Tenax-TA1 after 30 l of helium gas was passed; for pyrrole, no breakthrough

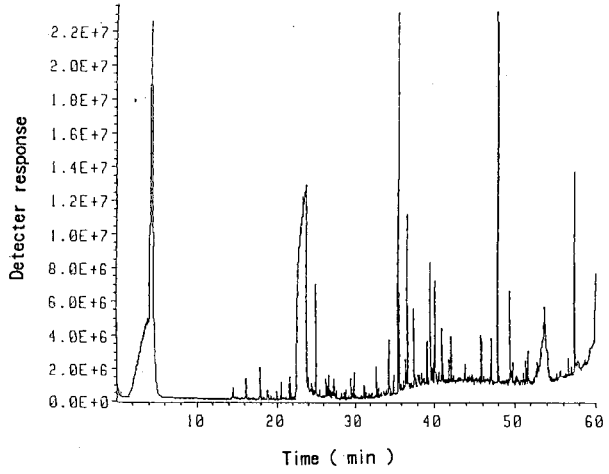

Fig. 3. TIC Chromatogram of Volatile Substances of Human Waste

was observed until 10 l of helium gas had been passed through (data not shown). Therefore, based on the detection limits, exposure of Tenax-TA to at least 10 l of each target substance is thought to be appropriate.

Simultaneous Identification and Determination of the Concentrations of Malodorous Components of Human Waste Using TCT

Malodorous substances of human waste were adsorbed onto Tenax-TA at room temperature, recondensed using TCT, and analyzed by GC/MS. Figure 3 shows the chromatogram, and Table 4 lists the various compounds identified by comparing their mass spectra with those of compounds in the National Institute of Standard Technology (NIST) library data. The recovery tests of 9 compounds were carried out. After spiking 0.1 μg of the target compounds into Tenax-TA, the rest of the procedure was performed according to the anaytical procedure. Recovery of the fatty acids, acetic acid, propionic acid, butyric acid, *i*-valeric acid, and *n*-valeric acid were 65.0%, 75%, 78.8%, 82.5%, and 85.0% respectively; those of the nitrogen compounds indole, skatole, pyridine, and pyrrole were 95.2%, 96.0%, 105.0%, 110.0%, respectively (data not shown). As listed in Table 5, the fatty acids acetic acid (65%), propionic acid (15%), butyric acid (6.5%), *i*-valeric acid (2.3%), and *n*-valeric acid (1.4%) accounted for about 90% of the malodorous substances from human waste. Therefore, elimination of these substances viewed as important to deodorize human waste. These fatty acids are thought to have been produced primarily by the microorganic decomposition of hydrocarbons in the excreta. In the malodorous volatile substances from human waste, nitrogen compounds such as indole, skatole, pyridine, and pyrrole were detected on the order of ppb; their contents were 0.31%, 0.55%, 0.14%, and 0.01%, respectively. These nitrogen compounds are believed to have been produced by the decomposition of proteins in human wastes by microorganisms. Phenol, methyl phenol (cresol) and ethyl phenol were also detected, and are thought to have been generated by the anaerobic decomposition of tyrosine, an aromatic amino acid, by microorganisms.[16,17] *p*-Dichrolobenzene, camphor, and naphtalene from aromatic preparations and insecticides used in toilets were also detected, indicating that these compounds are not easily degraded by microbes present in the storage tank or do not undergo spontaneous decomposition.

Determination of Sulfur Compounds

As listed in Table 5, sulfur compounds, namely, hydrogen sulfide and methyl mercaptan were detected at 19–50 ppm (five samples) and 0.7–1.1 ppm (five samples), respectively; their contents were 1.6% and 0.62%. These compounds are thought to have been produced by decomposition of proteins in human waste by microbes in the sewage tank. Yasuhara[17] identified methyl sulfide and methyl disulfide in volatile compounds contained in chicken feces, but they were not detected in our samples. The discrepancy in the results between chicken feces and human excretion may have been due to differences in composition between the two types of excretions or to decomposition of methyl sulfide and methyl disulfide by microorganisms in the sewage tank in this study.

Determination of Nitrogen Compounds

Ammonia was collected by bubbling it through a 0.5% boric acid solution [Fig. 1 (C)], and its concentrate was determined by both absorptiometry and ion chromatography on the basis of JIS K0099.[15] Its concentration was 18–34 ppm (10 samples) by either method as shown in Table 5, accounting for 6.5% or more of the total amount of malodorous components. The irritating odor of ammonia is considered to contribute greatly to the offensive odor of human waste,[18] and the results of our component analysis support this view. Therefore, the removal of ammonia is seen as important for the deodorization of wastes.

Trimethylamine was not detected in the 0.02 N

Table 4. Compounds Identified with GC/MS in the Head Space Gas of Human Waste

Retention Time (min)	Compound detected	Peak Area ($\times 10^6$)
14.43	Hydrogen sulfide	18.4
16.17	2-Propanone	62.1
17.84	1,2-Methyl propanal	79.3
18.81	2-Propanol	14.2
20.50	Decane	39.5
21.63	2-Butanol	49.7
21.81	Methyl benzene	38.7
23.94	2-Methyl propanol	98.8
24.44	Ethyl benzene	40.5
24.90	1,3-Dimethyl benzene	33.7
25.02	2-Methoxy ethanol	212.2
26.35	1-2-Dimethyl benzene	27.6
26.51	Dodecane	45.1
26.61	Pyridine	43.3
27.21	Cineole	33.5
27.34	Chloro benzene	76.4
27.67	1-Ethyl,2-methyl benzene	17.5
28.64	2-Methyl,1-propene	18.1
28.84	1-Ethyl,2-methyl benzene	20.5
29.45	1,2,3-Trimethyl benzene	40.7
29.85	Dodecane	53.9
31.15	1-Ethyl-4(2)-methyl benzene	46.3
31.40	8-Methyl heptadecane	22.7
32.77	Tritetracontane or Heptadecane	73.7
34.30	1,3(1,4)-Dichloro benzene	73.0
34.39	Acetic acid	145.4
35.64	1,2-Dichloro benzene	1548.3
35.95	2,3-Dihydro-5-methyl 1H-indene	26.0
36.26	1H-Pyrrole	22.5
36.47	Camphor	76.0
36.73	Propanoic acid	455.2
37.56	Dimethyl propanedioic acid	182.7
38.14	Hexadecane	32.4
39.12	Butanoic acid	91.8
39.88	1-Phenyl ethanone	48.6
40.16	3-Methyl butanoic acid	241.9
40.66	Heptadecane	35.2
41.83	Pentanoic acid	55.3
42.15	Naphthalene	101.0
42.42	N,phenyl formamide	14.3
47.00	Cyclododecane	41.5
47.88	Phenol	927.8
49.49	3-Methyl phenol	228.2
50.19	1,4,6-Trimethyl naphthalene	33.4
51.37	3-Ethyl phenol	65.0
51.66	2,5-Dichloro phenol	101.7
56.63	1H-Indole	64.4
56.64	Benzeneacetonitrile	37.4
57.46	3-Methyl 1H-indole	519.0
58.69	2-Heptadecanol	15.3

Table 5. Concentration of Malodorous Compounds from Human Waste

	Compound	Concentration (ppm)	Percentage (%)[a)]
Fatty acids	Acetic acid	40.00–120.00	65.00
	Propionic acid	5.30–27.00	15.00
	Butyric acid	1.50–9.20	6.50
	i-Valeric acid	0.53–2.60	2.30
	n-Valeric acid	0.41–1.60	1.40
S-containing compounds	Hydrogen sulfide	19.00–50.00	1.60
	Methyl mercaptane	0.70–1.10	0.62
N-containing compounds	Pyridine	0.03–0.23	0.14
	Pyrrole	0.01–0.02	0.01
	Indole	0.02–0.35	0.31
	Skatole	0.10–0.48	0.55
	Ammonia[b)]	18.00–34.00	6.50
	Trimethylamine[b)]	0.80–1.20	0.60

a) Percentage was calculated on the basis of the average value of each concentration. b) Concentration of ammonia and trimethylamine was determined by ion chromatography. Concentration of S-containing compounds in 5 samples and concentration of other compounds in 10 samples were measured.

H_2SO_4 solution [Fig. 1 (C)] bubbled with the sample gas, even by direct analysis using ion chromatography (data not shown). However, its analysis became possible when the pH of the sample solution was adjusted to about 7 with 1 N NaOH, and its concentrate was determined to be 0.8–1.2 ppm. Although this value is 10% or less of the ammonia concentration, the odor-threshold concentration of trimethylamine is one thousandth, or less that of ammonia, so that its contribution to the smell of urine is believed stronger than that of ammonia.

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