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Odor and Volatile Compounds in Liquid Swine Manure. III. Volatile and Odorous Components in Anaerobically or Aerobically Digested Liquid Swine Manure

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Volatile and odorous components were isolated from anaerobically or aerobically digested liquid swine manure by direct solvent-extraction and flash-distillation under reduced pressure. The concentrations of indole, oxindole, dimethylsulfoxide, phenol, and a few carboxylic acids increased, and *o*-aminoacetophenone decreased during digestion. Alkaline components were not so important for the odor. Offensive odor was formed by mixing phenols and carboxylic acids. Complete aeration resulted in a remarkable decrease of lower carboxylic acids.

Research on malodor has attracted only few researchers in contrast to studies concerned with flavor and perfume, and therefore, it is retarded. In Japan a piggery is one of the most malodor-evolving sources. Measurements have been carried out for ammonia, trimethylamine, hydrogen sulfide, methyl mercaptan, dimethyl sulfide, *etc.* which are controlled by the Japanese law on malodor, but there are only a few studies that have investigated other compounds or have considered the nature of the malodor. In research on malodor, it is very important and difficult to isolate odorous substances from samples effectively. Broadly speaking, there are three malodor-evolving sources in a piggery, that is, pig itself, solid manure, and liquid manure. A series of past studies have been concerned with liquid manure which had accumulated, rotted, and evolved a strong malodor.[1,2]

Carboxylic acids and many other compounds have already been detected in liquid swine manure by steam distillation.[1,2] It may be true that some substances decompose thermally during steam distillation, although it is a very useful method to separate volatile compounds from nonvolatiles. In this study direct solvent-extraction and flash-distillation under reduced pressure were used for isolation of odorous compounds in order to avoid thermal decomposition.

This paper deals with the isolation and the identification of odorous compounds having a medium or high boiling point in liquid swine manure and with the change of these odorous compounds under anaerobic or aerobic digestion.

Experimental

Material and Procedure. Liquid swine manure was collcted from a drain under pigsties near Takezono, Tsukuba New Town, Ibaraki Prefecture, at an inlet of a pit.

Two experiments were carried out. In the first experiment the air-flow rate was adjusted so that liquid manure was not digested completely in order that information on intermediate changes of some components due to chemical or microbial action was obtained. An aim of the second experiment was to obtain information on the relative ratio of typical odorous components between anaerobic digestion and completely aerobic digestion. The latter experimental conditions were similar to situation of odor control for liquid manure in pigsty.

In the first experiment the liquid swine manure was divided into three parts. One part (2.5 l) was shaken with dichloromethane (400 ml) for 15 min and the lower slurry containing the organic layer was separated. The other two parts (each 2.5 l) were digested at 35 °C for a week under bubbling of nitrogen (100 ml/min) or air (100 ml/min) respectively. Then the respective digested liquid manure was shaken with dichloromethane (400 ml) for 15 min, and the lower slurry was separated. Each slurry was centrifuged at 10000 rpm for 10 min and the organic layer was separated. The extract was fractionated into three fractions as described in Fig. 1.

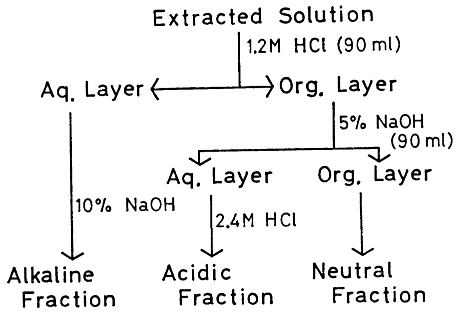

Fig. 1. Fractionation of liquid swine manure extract.

In the second experiment the liquid swine manure was divided into two parts. One part (50 l) was kept in glass bottles and digested at 20—25 °C for a week. The liquid manure became dark green and the surface was covered with a black formy film after a day, so the manure corresponded to anaerobic digestion. In the case of anaerobic digestion an inert gas such as nitrogen was not passed, when the experimental conditions approached the same situation where piggery liquid manure was stored in an anaerobic situation. The other part (each time 2 l, total 18 l) was aerobically digested by bubbling air at a rate of 2 l/min in a glass vesel at 20—25 °C for a week and then, malodor became very weak. Both parts of the rotten swine manure were flash-distilled under reduced pressure by means of the apparatus shown in Fig. 2. The receiver was chilled with ice, the two traps were cooled with Dry Ice and methanol to condense water vapor and volatiles completely, and the third trap chilled with liquid nitrogen was used

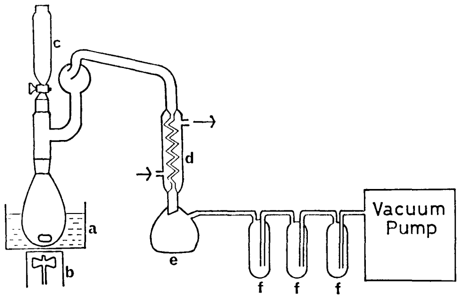

Fig. 2. Apparatus for flash-distillation under reduced prssure.
a, Water-bath; b, magnetic stirrer; c, funnel; d, Graham condenser; e, receiver; f, trap.

to prevent contamination from oil of the vacuum pump. The distillate was extracted continuously for 24 h with ethyl ether after saturation with sodium chloride. The extract was fractionated into three fractions as shown in Fig. 1.

Each fraction in both experiments was evaporated, after drying with anhydrous sodium sulfate, by means of a Kvrderna-Danish concentrator under atmospheric pressure.

Gas Chromatography. A Shimadzu Model GC-5A gas chromatograph was used. The flame detectors were operated using a hydrogen-flow rate of 50 ml/min and an air-flow rate of 0.5 l/min. Thermoconductivity detectors were used for the organoleptic tests. For the acidic fraction the gas-chromatographic conditions were as follows: injector and detector temperatures, 250 °C; column, 3 m×3 mm i.d. glass column packed with 2% DEGS+0.5% H_3PO_4 on 60- to 80-mesh, acid-washed, DMCS-treated Chromosorb W; column temperatures, 50 to 190 °C by an increase at a rate of 8 °C/min; carrier-gas (nitrogen) flow rate, 30 ml/min at 5 kg/cm^2. For alkaline and neutral fractions the gas-chromatographic conditions were as follows: injector and detector temperatures, 300 °C; column, 3 m×3 mm i.d. glass column packed with 2% OV-17 on 60- to 80-mesh, acid-washed, DMCS-treated Chromosorb W; column temperatures, 100 to 250 °C with temperature increase of 10 °C/min; carrier-gas (nitrogen) flow rate, 30 ml/min at 5 kg/cm^2.

Organoleptic Test for Odor. This test was carried out by means of smelling the odor of compounds progressively eluted from the outlet of the gas chromatograph.

Mass Spectrometry. A JEOL Model JMS-D 100 mass spectrometer was connected with a JEOL JGC-20 K gas chromatograph and a JEOL JMA-2000 mass data analysis system. The gas-chromatographic conditions were the same as above except for the size of the column. The size of the column was 3 m×2 mm i.d. The mass-spectrometric conditions were as follows: ion-source temperature, 140 °C; ion-source pressure, 1.2×10^{-6} Torr; ionizing corrent, 3×10^{-4} A; ionizing energy, 75 eV; accelerating voltage, 3 kV; mass range, m/e 10 to 450; scan time, 2.7 s; scan interval, 5 s. The mass data analysis procedures were subtraction of the background mass spectra, normalization by the most intense peak, and a search of mass spectra catalogue.

Results and Discussion

Digested liquid swine manure gave a pH of 8—9 regardless of anaerobic or aerobic digestion. The odors from digested liquid manure under anaerobic and aerobic conditions were very different each other. Though sulfur-containing compounds would be assumed to contribute to the difference of odor, studies on sulfur-containing components are in progress and the results will be reported at a later date. Many odorous compounds were isolated and identified from anaerobically stored piggery liquid manure by steam distillation,[1,2] but the odor of the steam-distillate has been different from the original odor. The reason for this is that some compounds are thought to decompose during steam distillation. A direct extraction has the benefit of minimizing those losses and decomposition and to keep the content ratio of many components identical to the original manure. Centrifuging above 5000 rpm was very effective for separating the organic layer of the slurry formed by shaking liquid manure with dichloromethane. The odor of the organic layer resembled that of the original manure. Typical gas chromatograms of fractions in the first experiment are shown in Fig. 3 to Fig. 5. The identification of each peak in the gas chromatogram was carried out by gas chromatography-mass spectrometry and the quantification was done by gas chromatography. The results of identification and quantification of the components related to the odor are shown in Table 1. In performing fractionation, removal of the alkaline fraction did not change the odor of the extracted solution, but removal of the neutral or acidic fraction, particularly the latter fraction, greatly altered the odor. The organoleptic test showed that the alkaline fraction did not contribute so much to the odor of swine liquid manure. Of course, ammonia and trimethylamine play an important role on the malodor,[3] but they cannot be detected by the method used in this study.

Indole and di-2-ethylhexyl phthalate decomposed easily, and skatole decomposed slowly under aerobic conditions. Indole concentration increased but for skatole the concentration hardly changed under anaerobic conditions. The results show that indole was formed from protein or its decomposed products under

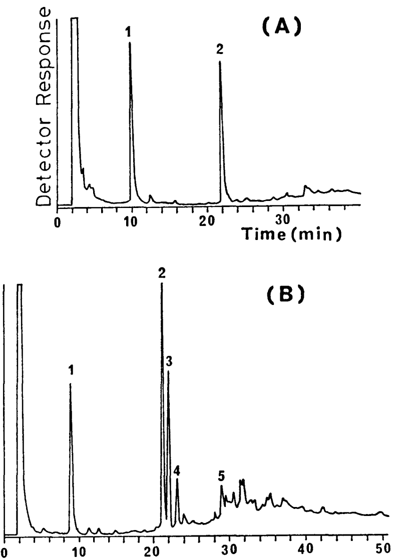

Fig. 3. Gas chromatograms of alkaline fractions.
(A), Raw liquid swine manure; (B), aerobically digested liquid swine manure.

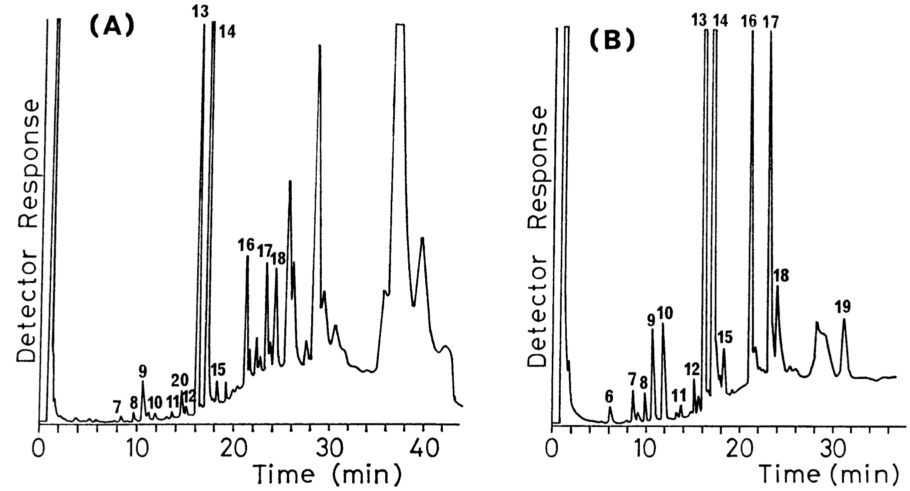

Fig. 4. Gas chromatograms of acidic fractions.
(A), Raw liquid swine manure; (B), aerobically digested liquid swine manure.

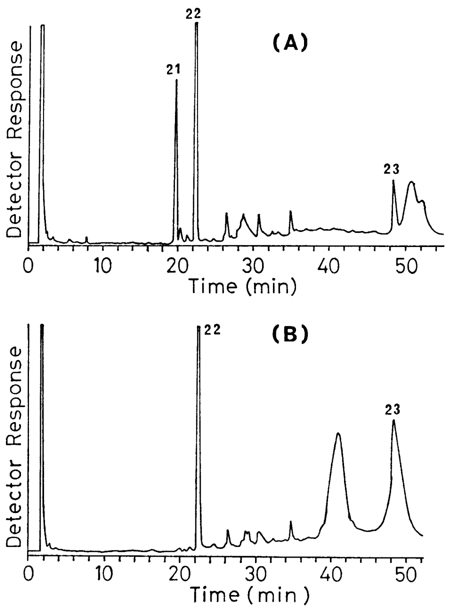

Fig. 5. Gas chromatograms of neutral fractions.
(A), Raw liquid swine manure; (B), aerobically digested liquid swine manure.

TABLE 1. IDENTIFICATION AND QUANTIFICATION OF VOLATILE AND ODOROUS COMPONENTS[a)]

Peak No.	Compound	Raw wt/l	Anaerobic wt/l	Aerobic wt/l
1	Aniline	8.2 μg	16 μg	15 μg
2	*o*-Aminoacetophenone	294 μg	20 μg	30 μg
3	Methylquinazoline	—	trace	16 μg
4	Dimethyl- or ethylquinazoline	—	—	4.8 μg
5	Isatin	—	—	3.0 μg
6	Acetic acid	—	1.3 μg	2.6 μg
7	Isobutyric acid	—	5.2 μg	5.6 μg
8	Butyric acid	—	8.4 μg	5.2 μg
9	Isovaleric acid	28 μg	28 μg	16 μg
10	Dimethylsulfoxide	3.8 μg	32 μg	17 μg
11	Valeric acid	3.2 μg	4.8 μg	1.9 μg
12	Hexanoic acid	6.4 μg	9.2 μg	6.0 μg
13	Phenol	1.23 mg	2.5 mg	0.44 mg
14	*p*-Cresol	28.4 mg	26 mg	16 mg
15	*p*-Ethylphenol	2.22 mg	2.22 mg	2.08 mg
16	Benzoic acid	63 μg	39 μg	48 μg
17	Phenylacetic acid	50 μg	42 μg	45 μg
18	3-Phenylpropionic acid	48 μg	27 μg	10 μg
19	Oxindole	—	41 μg	7.6 μg
20	*o*-Methoxyphenol	4.1 μg	—	—
21	Indole	0.87 mg	1.63 mg	trace
22	Skatole	2.05 mg	1.89 mg	1.46 mg
23	Di-2-ethylhexyl phthalate	0.24 mg	0.14 mg	0.06 mg

a) These values are not corrected with extraction coefficients.

anaerobic conditions and agreed with the results reported by Spoelstra.[4)] Oxindole may be supposed to be formed from indole by microbial action. Dimethylsulfide may also be supposed to change to dimethylsulfoxide. Indole, skatole, and dimethylsulfoxide were malodorous among neutral components.

In alkaline fraction, aniline, methylquinazoline, dimethyl- or ethylquinazoline, isatin, and *o*-aminoacetophenone were detected. Quinazoline derivatives and isatin were assumed to be formed during aerobic digestion. *o*-Aminoacetophenone which existed in relatively large quantities at first decreased in the same way for both digestion conditions. The odor qualities of aniline, quinazoline derivatives, and *o*-aminoacetophenone were significantly less than the odor qualities of other components.

It has been reported that concentrations of phenol, *p*-cresol, and *p*-ethylphenol have increased in anaerobically stored piggery wastes.[4)] The change of phenol in this study was identical with the above result. Acetic acid, isobutyric acid, and butyric acid were not detected in raw manure, but they were found at almost the same concentrations in both anaerobically and aerobically digested manure. In this experiment there was little difference in contents of carboxylic acids among raw, anaerobically digested, and aerobically digested manures. McGill and Jackson[5] reported the content ratio of carboxylic acids in pig slurry filtrates that showed acetic acid as a major component (65.5%). This experiment gave different results, but an exact comparison was not possible since the above study[5] did not report quantitative values, but relative values. In this experiment the acidic fraction did not have an irritating odor such as acetic acid and propionic acid, but had a sweaty and putrid odor. This odor was a little different from the odor of each carboxylic acid such as butyric or isovaleric acid and phenol. The organoleptic test showed that C_4—C_6 carboxylic acids and phenylacetic acid were malodorous and that phenols had a characteristic odor. However, an offensive odor was strengthened by mixing carboxylic acids and phenols and phenols added an adhensive nature to the total odor.

Strong aeration used in the second experiment is one of the most useful methods for deodorization. Strong malodor from liquid manure almost disappeared and the odor attributed to skatole remained when air was passed at a rate of 2 l/min for a week. A complete digestion under anaerobic conditions needs more than two months.[4] Gas-chromatographic analysis is facilitated by removing nonvolatile substances from sample. Therefore, a distillation method is very convenient, but steam distillation is not so good for this purpose. Distillation under reduced pressure, although it would be expected to be a useful method, was unsuccessful because of the violent frothing of the sample. In such a case flash-distillation under reduced pressure is very valuable and it was used successfully in this experiment. This method is suitable for isolating relatively volatile compounds, although it is time consuming. Continuous extraction with ethyl ether was used in order to avoid an accumulation of impurities from the large volume of solvent.

The gas chromatograms of each fraction were essentially identical. Each peak in the gas chromatograms was assigned by gas chromatography-mass spectrometry. The quantifications of fractions were carried out by gas chromatography and their results are shown in Table 2. The quantities of alkaline components did not change so much. The odor of anaerobically stored manure was attributed to the acidic components. The odor of indole and skatole from the anaerobically stored manure was felt a little, but it was fairly strong from aerobically digested manure. The aerobically digested manure did not exhibit the odor of phenols and carboxylic acids. This fact is compatible with the result of quantification. In particular, the concentrations of lower carboxylic acids decreased or were not detected after aeration. As the concentration of the valeric acid did not decrease significantly by bubbling nitrogen gas into the aqueous solution of the acid, its disappearance during aeration would be presumed to be caused by microbial action. The function of phenols with regard to odor diminished with decreasing carboxylic acids. As indole and skatole did not decrease so much and their threshold values for odor were very low, the odor of aerobically digested manure was dominated by them.

TABLE 2. QUANTIFICATION OF ODOROUS COMPONENTS IN ANAEROBICALLY AND AEROBICALLY DIGESTED LIQUID SWINE MANURE[a)]

Compound	Anaerobic wt/l	Aerobic wt/l
Aniline	76 μg	41 μg
o-Aminoacetophenone	248 μg	129 μg
Methylquinazoline	46 μg	29 μg
Dimethyl- or ethylquinazoline	44 μg	18 μg
Phenol	3.6 mg	0.79 mg
p-Cresol	29.2 mg	0.85 mg
p-Ethylphenol	3.8 mg	0.55 mg
Isovaleric acid	60 μg	—
Valeric acid	44 μg	—
2-Methylbutyric acid	12 μg	4 μg
4-Methylpentanoic acid	54 μg	—
Hexanoic acid	166 μg	6 μg
2-Methylpentanoic acid	18 μg	3 μg
4-Methylhexanoic acid	92 μg	9 μg
Heptanoic acid	166 μg	18 μg
Octanoic acid	166 μg	37 μg

a) These values are not corrected with recovery coefficients.

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