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Volatile fatty acids in anaerobically stored piggery wastes

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Summary

Volatile fatty acids (VFA) were estimated in 30 samples of farm slurries. The total concentration of VFA in these samples ranged from 4.0 to 27.6 g/litre (average 11.8 g/litre with standard deviation of 5.6 g/litre). From laboratory experiments it was derived that VFA are produced from faeces whilst only nonsignificant amounts originate from urine. During 70 days incubation of a mixture of freshly voided faeces and urine, 43 % of the protein and 24 % of the plant-fibre residues were degraded. The resulting products were predominately VFA and carbon dioxide.

Introduction

During the storage of mixed wastes from piggeries in pits under slatted floors, anaerobic microbial decomposition of the wastes takes place. Under the prevailing conditions, numerous organic compounds of low molecular weight are accumulated. Among these, the volatile fatty acids (VFA) are quantitatively the most important group. The VFA in piggery wastes have received some attention in literature. They have been demonstrated to contribute to the bad smell of the wastes (Schaefer et al., 1974). Removal of VFA from the wastes by methane fermentation has been studied (Hobson & Shaw, 1974; van Velsen, 1977). In addition attempts have been made to utilize the VFA of the wastes as substrates for microorganisms, with the object to produce single cell protein (Henry et al., 1976; Ensign, 1977; McGill & Jackson, 1977). The processes leading to the accumulation of VFA in the wastes have received little attention. In the work reported here, information is given about concentrations of VFA in piggery wastes and about their precursors.

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Materials and methods

Samples
Faeces and urine were collected separately from pigs held in metabolism cages. Samples of slurries from farms with storage of wastes under slatted floors were composed from grab samples. The sampling procedures have previously been described in more detail (Spoelstra, 1977).

Experiments

Urea in the urine used in the experiments was hydrolysed by incubation with urease and the liberated ammonia neutralized with 2 *N* HCl previously to mixing the urine with faeces (Spoelstra, 1977). Incubation mixtures were seeded with a drop of farm slurry.

Experiment 1. A mixture was prepared of freshly voided faeces and urine (mixing ratio 1 : 1 w/w) and diluted with water to a dry matter content of 6 %. This suspension and a mixture of faeces with water but without urine were incubated at 15 °C and 25 °C in closed 1-litre flasks. The pH value of the suspension was maintained at 7.0. Samples to be analysed for VFA were taken periodically.

Experiment 2. In another experiment 8 litres of a mixture of faeces and urine (mixing ratio 1 : 1.1 w/w, dry matter content 9 %) was stored anaerobically for 150 days at about 18 °C. The initial pH value was brought to 7.0 and was not adjusted during the experiment. Samples were taken and analysed for VFA, dry matter, ash, pH, NH_4^+-N, Kjeldahl-N and fibre content. In addition gas production and the composition of gas formed were analysed.

Estimation of VFA
Waste samples were centrifuged at 24 000 *g* for 20 minutes. The supernatant was subjected to gas chromatographic analysis without further treatment. It was introduced on to a glass column (1 m × 4 mm) packed with 20 % Tween 80 on Chromosorb W-AW (80-100 mesh). The column was installed in a Packard model 409 gas chromatograph equipped with a flame ionization detector. The temperature setting of the column oven was 115 °C and that of the injector and the detector 170 °C. As carrier gas nitrogen (80 ml/min) saturated with formic acid was applied (Fohr, 1974). Formic acid it not detected by this system. Isovaleric and α-methylbutyric acids are not separated by the column used. Both isomers are probably present in the wastes being decomposition products of amino acids. The concentration of isovaleric acid plus α-methylbutyric acid will be referred to as branched valeric (b-valeric) acids.

Gases
Gas production was measured by replacement of acidified water by the gas formed. The gas composition was determined in samples drawn from the head space of the container in which the slurry was stored. The samples were analysed gas chromatographically as described by van Kessel (1978).

Nitrogenous compounds
Protein content was calculated as the difference between total Kjehldahl-N and NH_4^+-N multiplied by 6.25. NH_4^+-N was determined by distillation of NH_3 from a sample to which borate buffer (pH 8.6) had been added. The ammonia was collected in a solution of boric acid which was subsequently titrated with HCl.

Fibre content
Samples were washed with an equal volume of a 0.05 *M* EDTA solution (pH 7.0) and centrifuged for 30 min at 39 000 *g*. Washing with 0.5 *M* EDTA was repeated twice and the residues were subsequently subjected to three washings with each of the solvents 5 % n-butanol in water, 96 % ethanol, and acetone. The acetone-washed residues were dried and weighed.

Results

VFA in farm slurries
Concentrations of VFA in samples of farm slurries are presented in Table 1. The VFA contents have been expressed on a wet weight basis to avoid the introduction of the large errors in the estimation of the dry matter contents due to sampling difficulties. The samples from piggery number 1 were taken at weekly intervals from December 1976 to April 1977. The temperature of the waste during this period fluctuated between 10 and 15 °C. In this farm, pigs have free access to water which is supplied by drinking nipples. A considerable amount of the drinking water is spoiled and consequently the wastes are diluted. This is in contrast with piggery 3. Here liquid feeding is practised and very high waste concentrations are reached. The concentrations of VFA in samples from this piggery were also found to be high.

About 30 samples of slurry from 6 farms were analysed for VFA. The total

Table 1. Concentrations (g/kg wet weight) of VFA in slurry samples from 6 piggeries.

Piggery number	1	2	3	4	5	6
Number of samples	19	3	3	1	1	1
Sampling period	Dec. '76–Apr. '77	July '75	Febr.–July '77	Jan. '77	Nov. '75	July '77
Dry matter	45.4 ± 18.5 [1]	63 ± 24	123 ± 33	26	14	71
pH	7.19 ± 0.17	N.D. [2]	7.6 ± 0.14	7.5	7.4	7.2
Formic acid	N.D.	N.D.	N.D.	N.D.	N.D.	0.07
Acetic acid	6.0 ± 2.5	8.1 ± 1.8	12.3 ± 3.9	4.7	6.9	8.1
Propionic acid	2.8 ± 1.3	3.4 ± 1.0	4.2 ± 0.8	1.27	1.24	2.85
Isobutyric acid	0.33 ± 0.12	0.56 ± 0.17	0.90 ± 0.49	0.32	0.27	0.69
Butyric acid	0.71 ± 0.29	1.26 ± 0.67	1.72 ± 1.24	0.79	0.30	0.60
b-Valeric acids	0.36 ± 0.13	0.92 ± 0.29	1.59 ± 0.31	0.47	0.20	1.17
Valeric acid	0.20 ± 0.05	0.24 ± 0.06	0.27 ± 0.07	0.37	0.04	0.21

[1] Mean and standard deviation of the mean.
[2] Not determined.

amounts ranged from 4.0 to 27.6 g/litre (mean and standard deviation of the mean were 11.8 ± 5.6 g/litre). Generally, the lower VFA concentrations were found in the more diluted wastes. In the samples tested, acetic and propionic acid represented 53.5 ± 12.2 % and 24.4 ± 8.4 % (mean and standard deviation of the mean), respectively, of the total amount of VFA present in the wastes. These figures amounted to 3.5 ± 1.5, 6.8 ± 5.9, 4.2 ± 2.3 and 2.4 ± 2.9 for isobutyric, butyric, b-valeric and valeric acids, respectively. Formic acid was determined in one sample only (piggery 6). The concentration found corresponds with 0.6 % of the total amount of VFA in this sample.

Experiment 1

No important differences in the accumulation of VFA were observed between mixtures with and without urine added to the faeces. In the presence of urine, VFA tended to be slightly higher at the end of the incubation period. The mixtures incubated at 25 °C showed a much larger increase in the content of VFA than those incubated at 15 °C (Fig. 1).

Experiment 2

Data on the production of VFA during an incubation period of 150 days of a

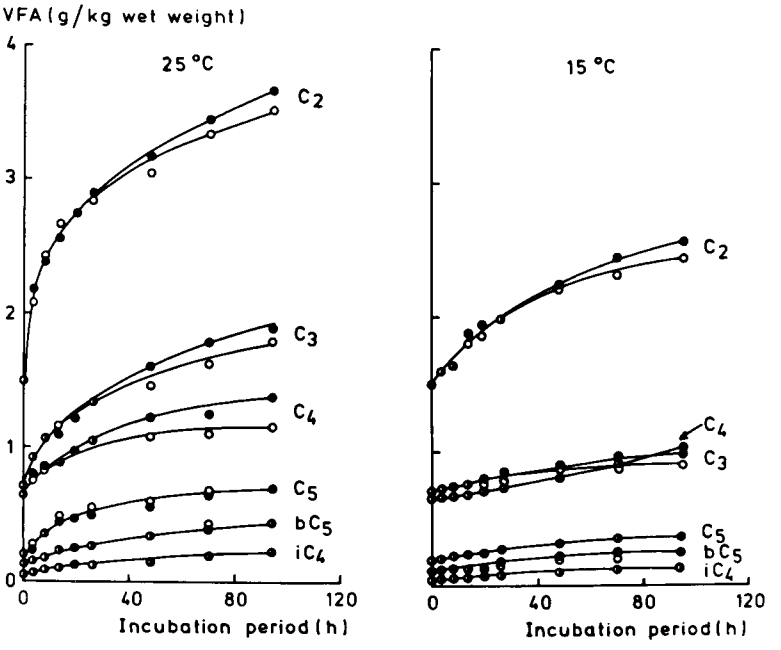

Fig. 1. Production of VFA in mixtures of faeces plus water (o———o) and faeces plus urine (•———•) incubated at 25 and 15 °C, respectively.
C_2 = acetic acid; C_3 = propionic acid; iC_4 = isobutyric acid; C_4 = butyric acid; bC_5 = branched valeric acids; C_5 = valeric acid.

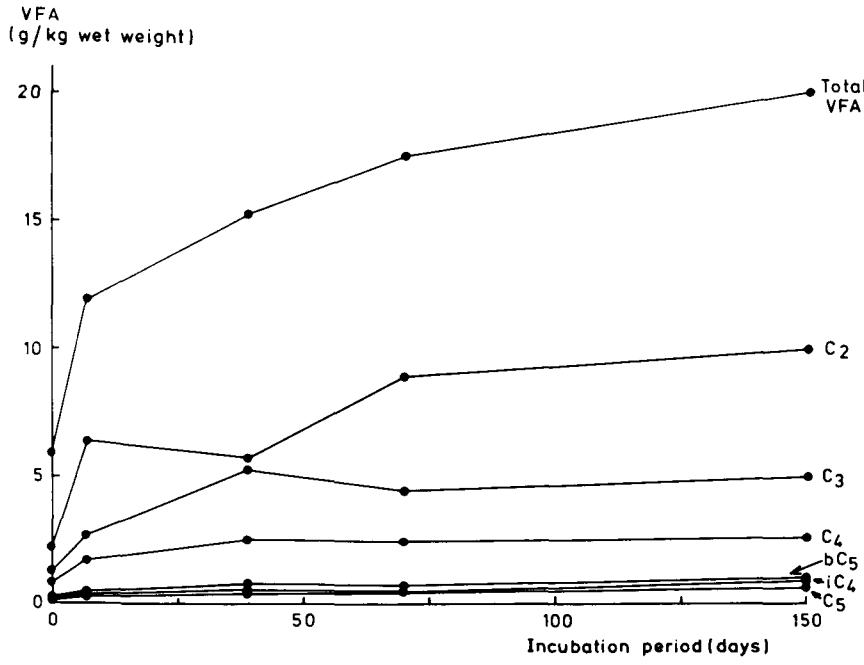

Fig. 2. Production of VFA in a mixture of faeces and urine incubated at 18-20 °C. C_2, C_3, i C_4, C_4, bC_5 and C_5: see Fig. 1.

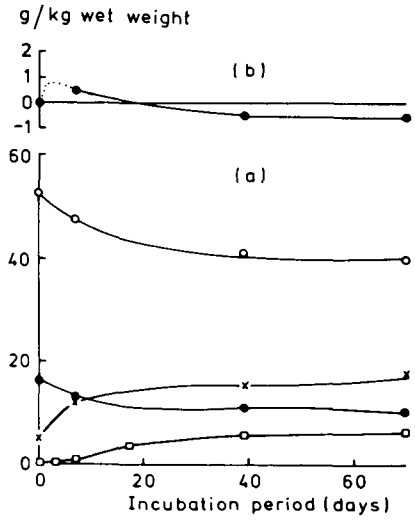

Fig. 3. (a) Decrease of fibre (o———o) and protein (•———•) content, increase of VFA (×———×) and gas production (□———□) in an anaerobically stored mixture of faeces and urine.
(b) Mass balance calculated from the data in Fig. 3a. Note different scale.

mixture of faeces and urine are presented in Fig. 2. The total amount of VFA increased from 5 g/litre to 20 g/litre. Acetic, propionic and butyric acids were in this order the most important acids. The other acids accumulated from about 0.1 to 0.8 g/litre. The pH value declined during the storage period from 7.0 to 6.4. The NH_4^+-N concentration increased from 2.63 to 4.11 g/litre during the same period.

The results on decomposition of fibre and protein and the production of gas and VFA during the first 70 days of the experiment are given in Fig. 3. Gas was produced during the first 40 days of the experiment, afterwards no gas production was measurable. The average composition of the gas produced was 80 % CO_2 and 20 % methane. Hydrogen was always present in low amounts (about 0.02 %). During the 70 days period about 24 % of the fibre and 43 % of the protein was degraded.

From the available information a mass balance was calculated which is presented in Fig. 3b. An attempt was made to differentiate the fibre fraction in cellulose, hemicellulose and lignin. The analytical results were, however, not accurate enough to allow conclusions about the extent to which the cellulose and hemicellulose fractions were degraded.

The percentage of fibre that was hydrolysed after incubation for 30 min with 72 % H_2SO_4 at ambient temperature and subsequently 6 h at 100 °C decreased during the 70 days incubation period from 78 to 67.

Discussion

The amounts of VFA in farm slurries (Table 1) show large variations which must mainly be attributed to the different amounts of water entering the pits. However, variations in microbial activity due to storage conditions (temperature) also influence the content of VFA. Generally, the mutual ratios of the VFA were found to be rather constant. Acetic acid, propionic acid and butyric acid showed in this order the highest concentrations. The remaining acids are ususally present in concentrations of about 5-10 % of the acetic acid concentration. Formic acid is probably present in even lower concentrations. These results are consistent with those of Cooper & Cornforth (1978).

From Fig. 1 it is concluded that VFA are mainly produced from constituents of the faeces. No significant increase of the VFA content was obtained when urine was added to the faeces. The production of VFA proceeds more rapidly at higher storage temperatures.

The results presented in Fig. 3 suggest that the main overall processes which take place in anaerobically stored piggery wastes are (1) degradation of plant fibre residues to VFA, and (2) degradation of protein to VFA and ammonia. The decomposition of fibre and protein is accompanied with the formation of CO_2. Besides some methane is formed in the slurry. Production of CO_2 and CH_4 in piggery waste has also been observed by Stevens & Cornforth (1974).

As is shown in Fig. 3b the production of gas and VFA does not balance completely with the degradation of fibre and protein. After an incubation period of 7 days more VFA and gas is formed than polymers are degraded. This is possibly

the result of the formation of VFA and gas from easily degradable compounds that have not been included in the analysis. A further factor which may be responsible for this surplus is the incorporation of water during hydrolysis of polymers like various soluble carbonaceous components and possibly fat. At the end of the experiment about 0.5 g more fibre and protein were degraded than gas and VFA formed. This may be explained by the formation of soluble products other than VFA, such as alcohols, phenols (Spoelstra, 1977) and phenolic acids.

Plant fibre consists of cellulose, hemicellulose and lignin which is probably not degraded under anaerobic conditions. Cellulose and hemicellulose are first decomposed to oligomers or monomers, which subsequently are converted to mainly acetic, propionic and butyric acids. Most amino acids – the monomers of protein – are also decomposed to one or more of these VFA. However, valine, leucine and isoleucine are degraded to isobutyric, isovaleric and α-methylbutyric acids, respectively (Allison, 1978). Acetic acid may also be synthesized from H_2 and CO_2 as has been demonstrated to occur in the intestine of some rodents (Prins & Lankhorst, 1977). The VFA content of the wastes may be lowered by methane fermentation, in which formic or acetic acid may be used as substrate. Methane fermentation in stored farm wastes is usually not a quantitatively important process. This has also been observed for slurry of cattle and poultry (Ensign, 1977).

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