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Phenylacetic acid in an anaerobic swine manure digester

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SUMMARY

The presence of phenylacetic acid (PAA) in an anaerobic swine manure digester was determined by gas chromatography of the butyl ester and confirmed by mass spectroscopy. PAA concentration increased during start-up of a digester and with low carbon, high nitrogen loading. Unlike acetate, propionate and butyrate, the concentration of PAA varied little through the day in a stable digester loaded once per day. The laboratory scale digester was loaded at 4 g of swine manure solids/liter digester volume per day. The retention time and temperature were 15 days and 37°C. PAA is a microbial intermediate which is produced by one group of anaerobic bacteria and converted to methane by other members of the bacterial community in the digester. As such, it may be a useful indicator of the relative metabolic activity of the bacterial groups and thus of the overall stability of the anaerobic process.

INTRODUCTION

Anaerobic digesters play an important role in the stabilization of a variety of wastes and the production of methane. However, the variety of conditions leading to digester failure and the microbial ecology of the digester must be fully understood if we are to define the ranges and limits of operation. The most accepted indicator of digester condition is the level of volatile acids, especially propionate [6,17]. During operation of swine manure digesters we observed that the level of an unidentified substance changed with varying conditions. This paper identifies the unknown substance and describes some of the conditions affecting its concentration.

MATERIALS AND METHODS

Digester operation

The digester was a 14 liter (10 liter working volume) New Brunswick Model fermenter. The operating temperature was 37°C and with a hydraulic retention time of 15 days. Daily gas was determined by liquid displacement in a polypropylene tank containing an acidified salt solution.

Manure from swine on a corn-milo-based diet containing 14% protein was blended in a Waring blendor with a small volume of water for 3 min. The diluted, blended manure was pooled, mixed and added to 250-ml containers in which it was frozen until use. The blended manure contained

17% volatile solids. Unless stated otherwise, the digester was loaded at a rate of 4 g volatile solids/liter digester volume per day using manure defrosted overnight at 4°C.

The 10 l digester was started with the contents of a larger, stable swine manure digester (420 liter working volume) operated under the same conditions except that the manure was not blended [10]. The larger digester contents were sieved through a double layer of cheesecloth; sieved particles were blended for 2 min in a small volume of digester effluent and added to the 10 l digester. Subsequent digesters were started with the effluent from both the 10 and 420 l digesters.

PAA analysis is also presented from smaller digester runs completed earlier. Digester buffering capability was studied by adjusting the carbon/nitrogen ratio in the influent by the addition of glucose and urea [11]. The working volume was 1 liter, the retention time was 20 days, and the loading rate was 3 g volatile solids/liter digester volume per day.

Analysis

Volatile and non-volatile acids and gas composition were determined by gas chromatography as previously described [15]. Butyl esters of phenylacetic acid (PAA) were standardly quantitated with the non-volatile acids on a 3% JXR on 100/120 mesh Chromosorb W-HP column (Applied Science Laboratories, Inc.). The esters were also chromatographed on Chromosorb 101 (Applied Science Laboratories) to establish comigration with known compounds.

Confirmation of PAA as the *n*-butyl ester was performed by capillary-GLC mass spectrometry in the electron impact mode on a Kratos MS25 instrument with a DS55 data system interfaced with a Carlo Erba GC. A 15 m methylsilicone capillary column, 0.25 μm I.D., was used for the separation of the sample mixture with a starting temperature of 50°C increased by 10°C per min to 200°C. The electron energy was 70 eV and the ion source temperature was 250°C.

Total solids, volatile solids, pH and total alkalinity were determined by standard methods [2]; ammonia was determined by Orion probe. Chemical determinations were made on single samples taken at approximately weekly intervals. Biogas composition consisted of three samples.

RESULTS

An unknown compound, which has been found repeatedly in swine manure digesters, was identified as phenylacetic acid (PAA). PAA was identified by similarity of retention on two separate chromatography columns as compared to a standard compound and as the *n*-butyl ester by mass spectrometry (Fig. 1). The fragmentation pattern with the molecular ion m/z 192, the typical ions at m/z 136 ($M - 56$ rearrangement) and m/z 92, probably by loss of CO_2 from m/z 136, are in support of PAA. Ions at m/z 41 and 57 are related to the alkyl moiety. An intense m/z 91 in the spectrum most probably is related to the benzylium ion from free PAA with m/z 136 as M^+.

During the initial start-up period of a 10 l digester the concentration of PAA was found to increase in concentration (Fig. 2). The concentration remained high for approximately 7 weeks before decreasing to steady-state levels. The mean and standard deviation for samples following the period in Fig. 2 was 210 ± 32 mg/l (ten weekly samples). Other parameters did not change (Fig. 2 and Table 1). The values in Table 1 were for the same sample period as Fig. 2. 61% of the volatile solids were destroyed; 2.21 ± 0.085 l of biogas (mean ± S.D.) per liter of digester volume were produced. The bio-

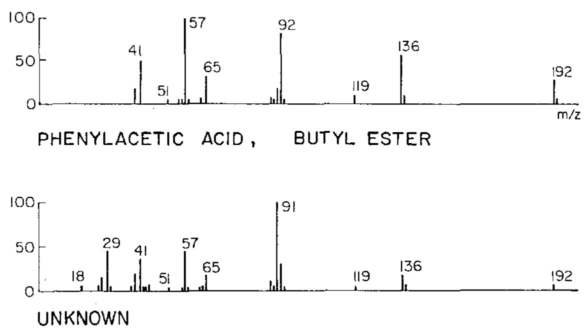

Fig. 1. Mass spectra of the butyl ester of phenylacetic acid and unknown compound from a swine manure anaerobic digester.

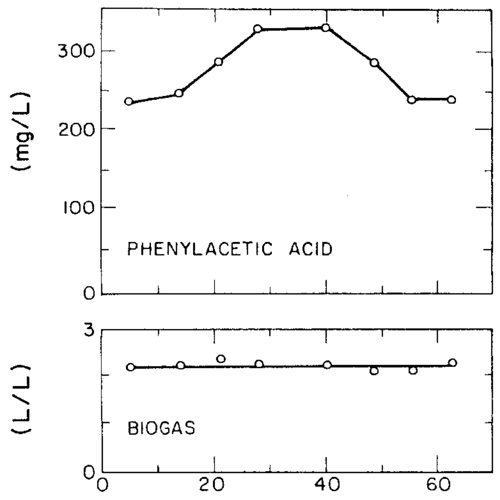

Fig. 2. Concentration of phenylacetic and biogas production in a swine manure anaerobic digester.

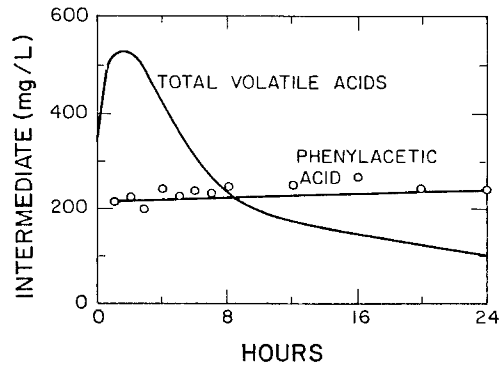

Fig. 3. Changes in the concentration of phenylacetic acid (O) and total volatile acids with time after loading.

gas was composed of 59.6 ± 1.9% methane and 38.8 ± 1.8% carbon dioxide; the values are for three samples taken at the end of the period in Fig. 1. Only trace amounts of hydrogen (55.6 ± 11.7 ppm) were detected. The pH in the digester was 7.45 ± 0.16.

Unlike volatile acids which have a distinct daily cycle in stable digesters loaded once per day, the concentration of PAA changed little through the day (Fig. 3). The concentration of volatile acids increased initially due to acids in the manure. That concentration increased, then decreased as the relative rates of production and utilization changed through the day.

During previous studies the carbon-nitrogen ratio in the influent of one liter digesters was adjusted by the addition of glucose and urea [11]. The concentration of PAA ranged from 25 to 345 mg/l (Table 2). The highest values were found at low carbon and high nitrogen combinations. The PAA levels tended to decrease as the carbon level (as added glucose) decreased. There was a positive correlation between PAA levels and ammonia (r = 0.62) and a negative correlation with carbon to nitrogen ratio (r = 0.78). PAA levels had very weak correlations with total acids (r = 0.05).

Table 1

Chemical parameters (mg/l) of a swine manure anaerobic digester effluent

Means ± S.D. are from nine samples taken at weekly intervals.

Total solids	39 100 ± 4500
Volatile solids	26 100 ± 3300
Alkalinity	12 200 ± 1700
Ammonia nitrogen	2160 ± 250
Total nitrogen	3000 ± 401
Acetic acid	108 ± 24
Propionic acid	12 ± 7
Butyric acid	trace
Lactic acid	35 ± 20
Succinic acid	59 ± 10

Table 2

Phenylacetic acid (mg/l) concentration in digesters loaded with different levels of carbon and nitrogen (g/l)

Carbon	Nitrogen			
	3.9	6.6	8.0	9.4
38	235	239	345	286
42	126	287	—	292
70	135	—	135	160
102			49	25

DISCUSSION

PAA concentration has been found to increase during initial start-up of a swine manure digester. The highest steady-state concentrations of PAA were found at low carbon and high nitrogen loading. The PAA levels decreased as the carbon loading increased.

While we have found that PAA levels change, the direct cuasative relationships need further study. During the past 4 years we have started and operated numerous digesters and have developed some ideas about these relationships. We feel that the level of PAA may possibly be related to the stress during start-up. An increase in PAA during start-up has been observed on a number of occasions; however, there were also runs where the level of PAA increased in only one weekly sample. Secondly, it appears that the stress does not have to be severe to cause an increase in PAA. In each case in which we have observed increases in PAA, the digesters were started with the contents of another digester and biogas production and the level of volatile acids were well within the range normally found in a stable digester. (The increase in PAA might be greater without the original inoculum or an inoculum from another source.) We have not observed a relationship between the level of PAA and that of volatile acids. The changes in concentration of PAA require more time than that of the volatile acids. Finally, we have observed changes in the steady-state and transient levels of PAA (up to 600 mg/l) under variable operational schemes.

Our observations are best explained by the concept of a continual production and removal of PAA. PAA is produced during the degradation of proteins, specifically derived from the aromatic amino acids tyrosine and phenylalanine [4,24]. *Bacteroides* and *Clostridium* are particularly known for their production of PAA [2,18,19,26]. Phenylalanine is degraded in a digester and is produced by several of the bacteria isolated from a swine manure digester (unpublished data; [14,15]). PAA is also potentially a breakdown product of ligno-cellulose.

PAA is degraded to methane as are other phenolic compounds; a wide variety of aromatic compounds has been found to be degraded under anaerobic conditions [3,5,8,9,12,13,23,25,30]. The degradation probably is the result of not just a simple food chain but microbial interactions [29]. *Syntrophus buswellii* is a benzoate catabolizer that has a requirement for low hydrogen concentrations [20]. Others have shown that the degradation of proteins etc. also results in low-molecular-weight substances that are converted to methane and involve interactions between organisms, including coupling to methanogenesis [21,22].

A build-up of PAA could possibly be toxic to digester bacteria. Plant phenolic acids have been shown to inhibit the cellulolytic activity of bacteria to varying degrees [7,16,27].

Anaerobic digestion is a microbial process in which complex molecules are degraded in multiple steps to methane [6,17,28]. The level of volatile acids is typically used as a relative measure of the degree of coupling of degradation of complex molecules to the terminal reactions [6,17]. However, several indicators, each an intermediate in the degradation of a different type of carbohydrate, lipid or protein, would present a more complete picture of the process. PAA is a microbial intermediate which could possibly be useful as an indicator of the metabolic conditions in the anaerobic process.

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