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Silage Fermentation of Swine Waste Combined with Corn

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Summary. Anaerobic fermentation of feedlot swine waste combined with corn was carried out in a newly designed laboratory silo. A lactic acid fermentation with rapid odor control resulted. Initial numbers of total and lactic acid bacteria (all per dry g) were 10^7. More than 75% of the initial total population were lactobacilli that increased 27-fold at 24 h and immediately entered a phase of decreasing viable numbers. The lactobacilli were largely the streptobacterium type. Lactobacilli were counted in both plates and roll tubes, and the counts in the roll tubes were as much as 100-fold greater; the difference was attributable to anaerobic lactobacilli. After 38 d, lactobacilli were no longer found in plates, but obligately anaerobic lactobacilli persisted at 10^3 through 90 d. Fecal coliform bacteria, initially present at 10^6, were not detected after 24 h. Yeast cells, starting at 10^6, decreased 100-fold at 3 d, and clostridia never exceeded 82 cells. Both groups were of minor importance in this fermentation. Virtually all the acid produced was lactic, measuring 3.83 (% dry matter) at 2 d and rising to a maximum of 12.45 at 69 d. In response, the starting pH of 6.80 decreased to 4.23 and then remained near 4 thereafter. Fumaric and succinic acid levels never exceeded 0.2 (% dry matter). Of the volatile fatty acids measured, acetic was maximum at 0.45 (% dry matter), but n-butyric and propionic were never more than 0.06. Fermenting a swine waste-corn mixture in a laboratory silo conferred preserved properties and diminished disease potential on a moist silage that can serve as a major component in an animal ration.

Animal waste from confined livestock production facilities continues to cause management problems, and additional methods of handling other than land application would be useful. The available nutrients in waste can be utilized (Smith and Wheeler 1979; Anthony 1974; Diggs et al. 1965) by refeeding as part of an animal ration. This procedure represents a disease potential (Bhattachara and Taylor 1975; Fontenot and Webb 1975) that can be decreased by fermentation processes (McCaskey and Anthony 1979; Creger et al. 1973; Caswell et al. 1974). The processing of swine waste-corn mixtures in an aerobic, motor-stirred system has yielded products with reduced numbers of fecal coliform bacteria (Weiner 1979). The present study measured the changes in the biochemical composition and microbial population, particularly the fecal coliform and lactobacillus community that occurred in swine waste-corn mixtures ensiled by a static, energy-saving, anaerobic process.

Materials and Methods

Silage Process

Fresh swine waste was obtained from a feed lot with open front sheds and concrete surfaces located in Central Illinois. Cracked corn came from a local elevator. Waste (6.5 kg) was mixed with distilled water (3.9 kg) and cracked corn (13.0 kg) to give a mixture with a moisture content of 38%. A newly designed laboratory silo was filled from the top with intermittent tamping with a wooden stave. The silo was closed as described below and placed in an incubator room at 30 °C.

Construction of the Laboratory Silo

A 14.2 x 109 cm cylinder made of 0.64 cm thick transparent plastic (Leucite) provided a capacity of 17.3 l for silage (Fig. 1). Leucite lids, 2.5 cm thick, were fitted into the cylinder to a 1.0 cm depth by step construction and pressed against rubber "0"

1 The mention of firm names or trade products does not imply that they are endorsed by the U.S. Department of Agriculture over other firms or similar products not mentioned

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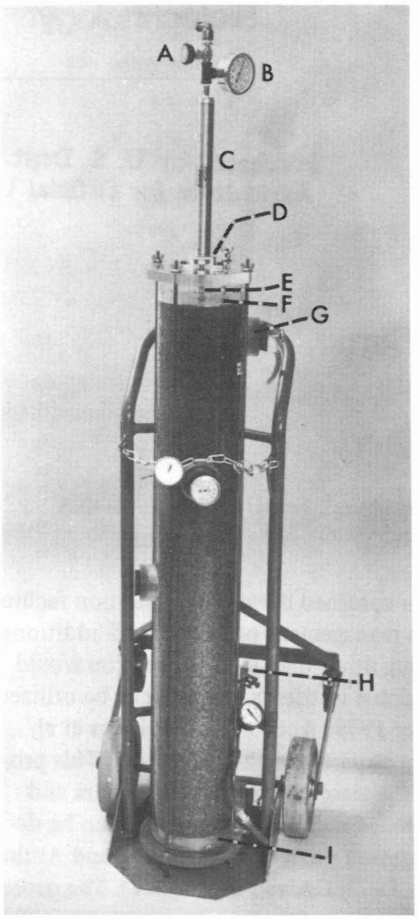

Fig. 1. Laboratory silo filled with silage and silage surface under a pressure of 14.2 kg/cm^2. Important silo components: *A* Hoke valve; *B* – gas gauge to show current pressure of air cylinder; *C* – air cylinder; *D* – platform, bolted to lid of silo to hold air cylinder, *E* – piston from air cylinder, *F* – plate pressed down on silage surface by piston, *G* – top sampling port with Bunsen valve, *H* – hose outlet for release of silage liquid, *I* – sieve disc to separate out silage liquid

rings to ensure air-tightness. The lids were held in place by tightening thumb screws on each of the four 0.64-cm threaded steel rods that were passed through holes in the lids. The vessel was fitted with 5 short plastic tubes glued in place with ethylene chloride, each to accept No. 10 rubber stoppers. The tubes were helically placed about the cylinder to serve as anaerobic sampling ports; the vessel was laid horizontally and flushed continuously with oxygen-free carbon dioxide. Exit of silage liquid from the bottom was through a perforated plastic disc resting on the bottom lid. The disc was hollowed on its lower surface, as was the upper portion of the bottom lid, to form a lens-shaped cavity. A center hole in the bottom lid connected to a horizontal channel drilled through a glued-on solid rectangle of plastic. The horizontal channel terminated in a tube shape, and an attached rubber tubing with clamp acted as a valve for release of silage liquid. Continuous silage compaction to simulate cylindrical farm silos (El Hag et al. 1982; Otis et al. 1959; Otis 1950) was achieved by a pneumatically driven piston-cylinder system attached to a platform held on the silo lid. The stainless-steel piston passed through a gland in the lid fitted with a rubber "0" ring, and the piston fastened into a stainless-steel plate that pressed downward on the silage surface. Compressed nitrogen was passed through a Hoke valve and a gas pressure gauge to the air cylinder to produce a pressure of 14.1 kg/cm^2 (200 psi). The rubber stopper in the top sampling port was drilled to accommodate a Bunsen valve, which consisted of a rubber tubing with a sealed end and a small slit through which internal gas pressure would be released but entrance of outer air would be prevented. Handling and mobility for the silo were facilitated by a compressed gas cylinder cart.

Microbiology

The roll tube technique for counting and culturing anaerobic bacteria (Hungate 1969) required flowing supplies of carbon dioxide, hydrogen, and nitrogen that were free of oxygen. Oxygen-free gases, made by passage through a column of hot (350 °C) copper wire or copper filings (Moore 1966; Bryant 1972), were used in culture tubes or flasks to prevent entrance of air and to prepare prereduced anaerobically sterilized bacterial growth medium (Holdeman et al. 1977). Selected groups of anaerobic microorganisms were determined on 5 g of material taken from a sampling port as the silo lay in a horizontal position. Two sterile gas cannulae, bent at right angles and attached to sterile cotton filters, allowed CO_2 to flow into both the sample port and a tared sterile jar to effect anaerobic and aspetic transfer of sample. The sample and sterile mineral solution were transferred to a micro Waring Blendor under a flow of CO_2. After blending for three 15-sec intervals, the top liquid was serially diluted in mineral salts solution and then added to four previously prepared and freshly melted agar media in duplicate roll tubes. The tubes were horizontally placed on a motor-driven tube spinner and positioned under a flow of cold water to congeal the inoculated agar medium into a thin smooth layer over the entire inner wall of the roll tube (Holdeman et al. 1977).

Total numbers of bacteria were counted in a modification of Medium 10 (Caldwell and Bryant 1966; Holdeman et al. 1977) containing the following materials in 93 ml: glucose and cellobiose, 0.025 g each; soluble starch and yeast extract, 0.05 g each, trypticase, 0.2 g; hemin solution, 1.0 ml (0.028 g KOH, 25 ml ethanol and 100 mg hemin dissolved and made up to 100 ml with water); mineral solution 1, 3.8 ml (0.6% K_2HPO_4); mineral solution 2, 3.8 ml (KH_2PO_4, 0.6 g; $(NH_4)_2SO_4$, 0.6 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NaCl, 1.2 g; $CaCl_2 \cdot 2H_2O$, 0.16 g; make up to 100 ml with distilled water); volatile fatty acid solution, 0.31 ml (glacial acetic acid, 17 ml; propionic acid, 6 ml; n-butyric acid, 4 ml; n-valeric acid, 1 ml; iso-valeric acid, 1 ml; DL-α-methyl butyric acid, 1 ml; resazurin, 0.1%, 0.1 ml). The pH is adjusted to 6.5 and 2 g agar is added. At this point in the preparation of modified Medium 10 and all subsequent anaerobic media, the medium must be pre-reduced. This is done by boiling briefly in a round-bottomed flask under a flow of CO_2. A rubber stopper is wired in place and the flask is autoclaved for 15 min at 121 °C. The procedure that pre-reduces and anaerobically sterilizes the medium is designated PRAS. After sterilization and cooling to 47 °C, PRAS solutions of 8% Na_2CO_3 (5 ml) and 2.5% cysteine · HCl · H_2O (2 ml) are added. Transfer 9 ml medium per roll tube and tightly insert rubber stoppers.

Serial dilutions for counting in roll tubes were done with mineral solution prepared as follows: to 92 ml of water add mineral solution 1, 3.8 ml, mineral solution 2, 3.8 ml, and resazurin, 0.1% ml, then prereduce and anaerobically sterilize at 121 °C for 15 min. Cool and add PRAS solutions of 5 ml 8% Na_2CO_3 and 2 ml of 2.5% cysteine HCl · H_2O. Mineral solution for serial dilution in plate counts was prepared by mixing 3.8 ml

each from mineral solution 1 and mineral solution 2, diluting to 100 ml with distilled water, and sterilizing 15 min at 121 °C.

Lactic acid bacteria were counted in Rogosa SL Agar (RSL) (Difco), modified for use in anaerobic roll tubes. To 92 ml of water were added Rogosa SL Agar, 7 g; agar, 0.5 g; resazurin, 0.1%, 0.1 ml; and glacial acetic acid, 0.13 ml. Heat to boiling under CO_2 and add 5 ml 8% Na_2CO_3, 2 ml 2.5% cysteine · HCl · H_2O, and concentrated HCl to bring pH to 5.4. This mixture may not be autoclaved and was dispensed into roll tubes under flowing CO_2 in volumes of 9 ml.

Types of lactobacilli were differentiated on modified Lactobacilli MRS Broth medium (Difco) (Nakamura and Crowell 1978): proteose peptone #3, 10 g; yeast extract, 5 g; Tween 80, 1 g; glucose, 10 g; ammonium citrate, 2 g; sodium acetate, 5 g; $MgSO_4$ · $7H_2O$, 0.1 g; $MnSO_4$ · H_2O, 0.05 g; Na_2HPO_4 · H_2O, 2 g; resazurin, 0.1%, 0.1 ml. The medium was diluted to 930 ml, prereduced, and anaerobically sterilized at 121 °C for 20 min. After cooling PRAS solutions were added of 50 ml 8% Na_2CO_3, 20 ml 2.5% cysteine HCl · H_2O, and concentrated HCl to bring pH to 6.5. For selected sampling times, one to two Lactobacilli roll tubes, with either the countable dilution or the next higher dilution, were picked in entirety (20 to 70 isolates per sample time) with the aid of a dissection microscope and flowing CO_2 and transferred to MRS slants (2% agar). After 48 h of anaerobic incubation at 30 °C, growth from an isolated colony was transferred to two RMS broth tubes, one of which was preincubated at 15 °C and after inoculation was held at that temperature for 6 d. The second MRS broth tube was preincubated at 45 °C, inoculated, and then kept at this temperature for 6 d. Finally, production of gas from glucose was tested by inoculating from the same colony into butt tubes of MRS agar, which was then overlain with a PRAS agar seal that contained water, agar, 8% Na_2CO_3, 2.5% cysteine HCl · H_2O, and 0.1% resazurin (93:2:5:2:0.1) and incubated for 6 d at 30 °C (Gibson and Abdel-Malek 1945). Yeast cells were counted in Mycophil Agar (BBL), which was prepared as follows in 90 ml of water: Mycophil Agar, 3.6 g; agar, 0.4 g; 0.1% resazurin, 0.1 ml. After prereduction and anaerobic sterilization at 118 °C for 15 min, the medium was cooled and PRAS solutions were added of 5 ml 8% Na_2CO_3, 2 ml 2.5% cysteine HCl · H_2O, 0.75 ml 25% lactic acid, 1 ml Penicillin G (33,000 units), and 1 ml dihydrostreptomycin sulfate (20 mg). Clostridia were enumerated in modified Clostrisel Agar (BBL) and prepared in 94 ml of water as follows: Clostrisel Agar, 4.6 g; agar, 0.6 g; 0.1% resazurin, 0.1 ml. Pre-reduced and anaerobically sterilized at 118 °C for 15 min, the preparation was cooled and PRAS solutions were added of 5 ml 8% Na_2CO_3 and 1 ml 2.5% cysteine HCl · H_2O and adjusted to pH to 7.0. Total population, lactobacilli, yeast cells, and clostridia were incubated in roll tubes 6 d at 30 °C and then counted. Aerobic and facultatively anaerobic bacteria were enumerated at the same time by blending another 5-g sample from the silo with distilled water, filtering through glass wool mats, and adjusting to a final volume of 50.0 ml. Serial dilutions were made with mineral solution for plate counts. Three previously prepared media were used in triplicate Petri plates for each of four to six dilutions. Volumes of 0.3 ml of dilution were added to each plate and spread with sterile bent glass rods. The media used included Rogosa SL Agar (Difco) for lactobacilli and mFC Agar (Difco) for fecal coliform bacteria (Geldreich et al. 1964). The inoculated mFC Agar plates were enclosed in Zip-Lok bags in an inverted position (agar face down); the bags were then placed in wire baskets held below the surface of water maintained at 44.5 °C and incubated 24 h. In duplicate test tubes (containing Durham tubes), lactose broth (10 ml) was inoculated with 1.0-g quantities of fermented sample and incubated for 24 h at 37 °C to test for the presence of coliform bacteria. Microbial growth media were supplied by BBL (BBL, Division of Bioquest, Cockeysville, Maryland) and Difco (Difco Laboratories, Detroit, Michigan).

Chemical Analyses

Distilled water was added to a 5.0-g sample collected from the silo, which then was triturated and allowed to equilibrate 5 min before pH was measured by electrode. A constant dry weight was taken on the same sample by drying overnight at 103 °C. Organic acids produced by fermentation were determined on aqueous extracts of 5.0-g samples that were obtained with three 15-sec blends in a micro Waring Blendor and subsequent filtration of the mixture through a glass wool mat. Filtrates were made up to 50.0 ml and centrifuged at 750 × *g* for 10 min. The supernatant was stored at −20 °C until volatile fatty acids and nonvolatile acids were analyzed by gas chromatography according to Holdeman (Holdeman et al. 1977) but modified by the use of internal standards (McNair and Bonelli 1968). Volatile fatty acids were determined on 0.9-ml portions of the aqueous extract. Also added to the aqueous extract was an internal standard of 0.10 ml benzoic acid (1.0 meq/100 ml). Nonvolatile acids of fermentation were also determined as methyl esters of 1.0 ml of the aqueous extract which was cooled in ice-water and 1.0 ml propionic acid (1.0 meq/100 ml) was added as an internal standard. Standards were prepared using acids at 1.0 to 6.0 meq/100 ml and extracting with either ether or chloroform and injecting 2.0 μl. Standard acids gave retention times for comparison to fermentation acids. Gas chromatography was done in a Packard Model 428 equipped with a flame ionization detector and employed a coiled glass column (6 ft × 1/8 in.) packed with 10% SP 1200/1% H_3PO_4 on 80/100 mesh Chromosorb W AW (Supelco, Inc., Bellefonte, Pennsylvania). Area was determined by a computer program (Butterfield et al. 1978). The temperature program was 8°/min from 60 to 150 °C for methylated acids and 60 to 180 °C (held for 6 min) for the volatile fatty acids. Temperatures for the detector block and injection port were 200 and 150 °C, respectively. A nitrogen gas carrier flow rate of 20 cc/min was used.

Redox potentiometer potential was measured with a combination redox electrode Model 96–98 (Orion Research Incorporated, Cambridge, Massachusetts). Samples were anaerobically transferred to a beaker sealed with a plastic film (Parafilm, American Can Company, Greenwich, Connecticut) and continuously supplied with flowing CO_2. The electrode was inserted through a tightly fitted hole in the film and equilibrated 15 min before the potential was read on a digital pH meter (Model 701, Orion Research, Inc., Cambridge, MA).

Results

The total number of anaerobic microorganisms (all data per dry g), which was 10^7 at the start of fermentation, increased 10-fold in 48 h (Fig. 2). This value decreased to 10^4 at 27 d and remained at this level thereafter. Lactobacilli, also with an initial count of 10^7 (plates), increased 20-fold in 9 h and, like the total anaerobic population, decreased to 10^4 at 27 d. After 38 d, lactobacilli could not be detected in aerobic plates. In contrast, anaerobic lactobacilli numbered 10^7 at the start of fermentation and increased 27-fold in 24 h. The count of these organisms decreased to 10^3 at 38 d but exceeded corresponding

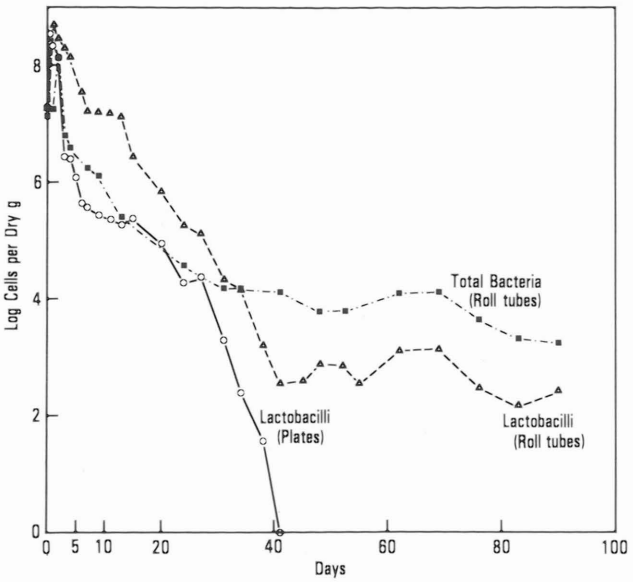

Fig. 2. Growth pattern of total bacteria and lactobacilli in swine waste-corn and silage in a comparison of aerobic and anaerobic counts

Table 1. Percent distribution of lactobacilli during silage fermentation of swine waste combined with corn

Days	Thermobacteria	Streptobacteria	Betabacteria
0	2.8	83.3	13.9
0.4	0	84.6	15.4
1	0	93.6	6.4
2	5.9	92.2	1.9
3	13.5	59.6	26.9
4	24.0	64.0	12.0
5	29.6	63.6	6.8
6	2.2	95.6	2.2
13	100.0	0	0
31	94.0	6.0	0

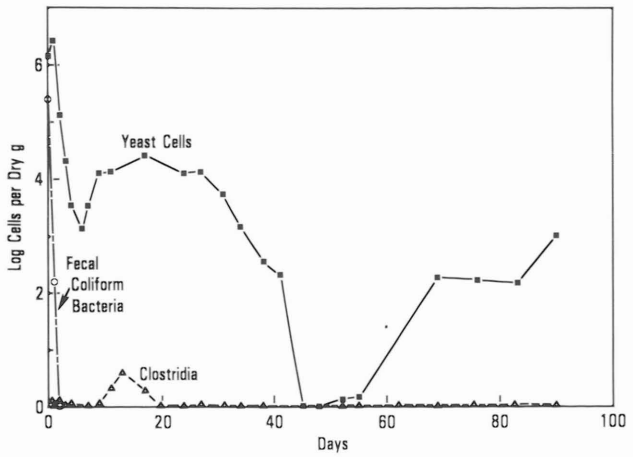

Fig. 3. Growth patterns in swine waste-corn silage of fecal coliform bacteria, yeast cells, and clostridia

counts of lactobacilli by as much as 100-fold and persisted at this level to 90 d. Persisting lactobacilli were gram positive. More than 75% of the total number of microorganisms at the start of fermentation were lactic acid bacteria, and all three types of lactobacilli were present (Rogosa 1974). Initially, the fractions of the homofermentative streptobacteria and thermobacteria were 83.3 and 2.8%, respectively, and that of the heterofermentative betabacteria was 13.9% (Table 1). Streptobacteria persisted as the predominant type of lactobacillus through 6 d, whereas betabacteria declined to 2.2%. At 13 and 31 d, thermobacteria became the dominant type of lactobacillus.

Swine waste combined with corn in the laboratory silo initially contained 5.41×10^5 fecal coliform bacteria (Fig. 3). These organisms decreased to 2.04×10^2 at 24 h and were not detected again through 90 d. Coliform bacteria were not present at 3, 20 or 24 d according to presumptive tests in lactose broth with Durham tubes in place. Yeast cells at starting numbers of 10^6 decreased 1,000-fold at 7 d, stabilized at 10^4 until 27 d, and dropped to less than 10^3 thereafter. Clostridia were not always detected during the fermentation but when found never exceeded 82 cells. Redox potentials measured −66 mV at 9 h, dropped to −384 mV at 17 d, rose to 30 mV at 34 d, and persisted at this level to 90 d.

Lactic acid, present at the start of fermentation (% dry matter) at 0.5, increased to 3.83 at 2 d, 7.70 at 4 d, and attained the highest level of 12.45 at 69 d (Table 2). Fumaric and succinic acids were other nonvolatile acids found through most of the fermentation at levels of 0.01 and 0.06, respectively, but acetic acid ranged from 0.3 to 0.5 after 20 d. Valeric, iso-caproic, and caproic acids were also detected in the zero sample at less than 0.1 concentration. Acid production was reflected by the decrease of initial pH values of 6.80, to 4.50 at 24 h, to 4.2 at 2 d, and to near 4 at 20 d and thereafter. Dry matter loss during fermentation was 0.8%.

Discussion

A swine waste-cracked corn mixture placed in an anaerobic environment resulted in a lactic acid fermentation characterized by a disappearance of fetid odor by 24 h. More than 99% of the total number of microorganisms during fermentation were lactobacilli; this preponderance continued for 34 d, during which levels peaked at 24 h and then decreased rapidly to 10^7 at 7 d. This phase of proliferation followed by decreasing viable counts is common in silage fermentations (Gibson and Stirling 1969). Lactobacilli were counted at the same time in both plates and roll tubes, and the population in roll tubes was as much as 100-fold greater than that in plates, the difference being attributable to obligately anaerobic lactobacilli. Streptobacteria are homofermentative organisms, and

Table 2. Organic acid content and pH of silage fermentation of swine waste combined with corn

Fermentation days	Percent of dry matter						
	Lactic	Fumaric	Succinic	Acetic	Propionic	Butyric	pH
0	0.5	0	0	0.01	0.01	0.02	6.80
2	3.83	0	0	0.01	0.02	0.03	4.23
3	7.79	0.04	0.06	0.02	0.02	0.05	4.22
4	7.72	0.04	0.06	0.02	0.03	–	4.16
20	8.22	0	0.11	0.33	0.04	0.06	4.06
24	9.23	0.02	0.21	0.31	0.04	0.06	4.05
31	9.56	0.04	0.21	0.36	0.04	0.06	4.02
45	10.74	0.04	–	0.33	0.04	0.06	4.03
48	10.61	0.04	0.20	0.37	0.04	0.06	4.09
52	10.81	0.04	0.21	0.35	0.04	0.06	4.10
62	10.34	0.04	0.20	0.36	0.04	0.06	3.96
69	12.45	0.04	0.23	0.44	0.03	0.06	3.97
123	11.55	0.05	0.24	0.45	–	0.06	4.03

Additional acids found at zero days were valeric, 0.01, isocaproic, 0.07, and caprioic, 0.01, and at 4 days, valeric, 0.01

lactic acid, the major product of glucose metabolism, was rapidly produced to lower the pH to 4.2 in 2 d – a lower level than would be expected if heterofermentative betabacteria were the dominant group of lactobacilli. Studies with pure cultures of lactobacilli showed greater reduction in pH with streptobacteria and thermobacteria than with betabacteria (Rogosa et al. 1953). As was found in aerobic cattle waste-corn fermentations (Hrubant 1975), the production of acid and pH levels near 4 probably were responsible for the disappearance of the fecal coliform bacteria. Weiner (1977) suggested that coliform-inhibiting substances other than organic acid were formed during aerobic lactic acid fermentation of swine waste-corn mixtures. Yeast cells, never exceeding 4.32×10^6, were not an important group of organisms in this fermentation. Yeast cell proliferation would be promoted by increased oxygen levels, which has been shown to be a deterrent to the production of lactic acid (Takahashi 1970). The effect of oxygen in a grass silage fermentation was to prevent a drop in pH with time (Ruxton and McDonald 1974). A good measure of the degree of anaerobiosis of this swine waste-corn fermentation at 17 d was the minimum measured redox potential of –384 mV; this value reflected both the air tightness of the laboratory silo and the sampling protocol when done under a flow of oxygen-free carbon dioxide. Clostridia were even less important than yeast cells in this fermentation, as shown by low numbers of cells. If present in large numbers, clostridia will utilize lactic acid and residual sugars and carry out putrefactive processes that will spoil silage (Whittenbury 1968; Gibson 1965). Since clostridia have been shown to be depressed by low moisture levels and low pH (Gibson 1965), the 38% moisture level and rapid pH decrease to near 4 effectively inhibited this group of organisms in the present fermentation.

Raw swine waste combined with corn and ensiled under anaerobic conditions undergoes a lactic acid fermentation to yield a product which is well preserved, has diminished threat of transmitting disease, and rapidly develops a silage-like odor. The product can be used as a major component in an animal ration and as a help in the management of animal waste.

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