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Characteristics of Aerobic, Solid Substrate Fermentation of Swine Waste-Corn Mixtures

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Summary. Aerobic fermentation of swine waste combined with corn produced differences in microbial and biochemical patterns dependent on use of fresh or stored excrement. Lactic acid fermentation and odor control resulted with either waste. Homofermentative lactic acid bacteria were present initially at 10^7 organisms/dry g with stored waste-corn cultures and total microflora amounted to 10^8 organisms/dry g. Fresh waste-corn fermentations initially yielded heterofermentative lactic acid bacteria at 10^7 organisms/dry g and total viable population was 10^9 organisms/dry g. These respective groups of lactic acid bacteria dominated from 12 through 144 h in cultures with either waste, and acid production (0.2 meq/dry g) decreased pH by 2 units to 4.5. The major acid component with stored waste-corn was lactic acid, whereas fresh waste-corn fermentation produced both lactic and homologous fatty acids from acetic through valeric acid. Coliform bacteria present initially at 10^5 organisms/dry g in stored waste-corn cultures were not detected after 36 h; coliform bacteria in fresh waste-corn fermentations persisted at 10^6 organisms/dry g. A silage-like fermentation product resulted which may have use in animal feed formulations.

Livestock raised in confined facilities provide problems in the management of their excrement (Loehr, 1968). Management may include recycling to use available nutrients (Anthony, 1970). Cattle feedlot waste liquid mixed with feedgrains were converted to an animal feed by a fermentation process (Rhodes and Orton, 1975) and changes in microbial patterns were described (Hrubant, 1975). Swine waste can be stabilized in an oxidation ditch prior to land application (Jones et al., 1970) but liquid from this aerobic culture process has been refed to pigs (Harmon et al., 1975) while nutritive value to swine of solid residue from such an oxidation ditch has also been measured (Harmon et al., 1972). This study chose a solid substrate form of aerobic fermentation for whole swine waste combined with corn and describes its microbial and biochemical characteristics.

Materials and Methods

Substrate for fermentation consisted of swine waste which had been washed into a concrete storage container covered by a slotted floor or from a feedlot with a concrete surface, both located in Central Illinois. Coarsely cracked corn was obtained from a local commercial elevator. Laboratory fermentations were done in Erlenmeyer flasks at 28°C. The flasks (0.3 and 2.0 l) were arranged in a horizontal position by snap-ring holders fastened to a circular wooden board held at an angle of 10° from the horizontal. The board was rotated by a 0.02 hp motor and reduction gears provided 0.6 rpm. This posture gave a slow tumbling action to the corn-waste mixture, and cotton plugs or gauze were generally not in contact with the material. Small flasks were charged with 25 ml waste diluted with water and combined with 50 g cracked corn to give preferred moisture levels of 40%; useful levels ranged from 34 to 43%. Large flasks were similarly prepared with 300 g diluted waste and 400 to 500 g corn. Fermentation equipment, though clean, was not sterilized and aseptic techniques were used only with procedures for microbiological analyses.

Microbiology. Certain groups of viable microorganisms were determined by blending 5.0-g samples with portions of water in a micro Waring Blendor for two 15-sec intervals, decanting liquid of each blend through glass wool and adjusting to a final volume of 50.0 ml. The initial dilution of 1:10 (v/v) was serially diluted. Four previously prepared media were used in triplicate petri plates for each of four to six dilutions. Volumes of 0.3 ml added to each plate were spread by sterile bent glass rods. The media used included Eugonagar for total counts, LBS agar for lactobacilli, eosin methylene blue (EMB) for coliform orgnanisms, Mycophil agar with dihydrostreptomycin sulfate (0.2 mg/ml) and penicillin G (330 units/ml) for yeasts. All these media were manufactured by BBL[1] (BBL, Division of Bioquest, Cockeysville, Md.). Lactobacilli MRS broth, manufactured by Difco, was also used (Difco Laboratories, Detroit, Michigan). Plates and tubes were incubated at 28°C except for those with EMB, which were held at 37°C. The latter were counted after incubating 1 day and the others after 3 days.

Chemical Analyses. Samples collected from fermentations were separated into 5.0-g aliquots for all analyses except for organic acids of fermentation which used 10 g. Distilled water was added to a 5.0-g sample, triturated, and allowed to stand 5 min before pH was determined by electrode. A constant dry weight was obtained on the same sample by drying overnight at 103°C; this information allowed all data to be calculated on a dry weight basis. Total acids produced by fermentation were determined on aqueous extracts of 5.0-g samples and were obtained by three 10-s blends in a micro Waring Blendor with filtration of liquid portions though glass wool. Filtrates were made up to 25.0 ml and titrated to pH 7.0 with 0.05 N NaOH. A similar aqueous extract was centrifuged (5,000 rpm, 15 min) and the supernatant was made up to 24.75 ml. It was added to a beaker together with 0.25 ml 10 N NaOH, magnetically

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

stirred, and analyzed for ammonia by a modification of the method described by Bremner and Tabatabai (1972), using an Orion ammonia electrode.

Odor was evaluated organoleptically by collective impressions of laboratory personnel. Fatty acids, which contribute to odor, were determined on samples stored at -15°C. Thawed samples were extracted with distilled water for 15 s in a Waring Blendor, filtered through glass wool, made up to 40 ml, and pH adjusted to 8 with sodium bicarbonate. The aqueous sample was extracted overnight with diethyl ether, followed by acidification of the aqueous portion, and further extraction with ether. The ether extract was concentrated on a steam bath to 0.2 ml, dried over sodium sulfate, and analyzed for fatty acids by gas-liquid chromatography (GLC) in an F&M model 700 chromatograph with dual columns (6 ft x 1/8 in, stainless steel) and packed with 10% SP-1200/1% H_3PO_4 on 80–100 mesh Chromosorb WAW (Supelco, Inc., Bellefonte, Pa.). The instrument was equipped with flame ionization detector and a disc integrator. The temperature program was 5°/min from 65 to 190°C. Standards were prepared in ether using 20% lactic acid and 2.0% of the following acids: acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, and caproic. Fatty acids gave retention times for comparison to fermentation acids and single symmetrical peaks except for lactic acid which provided two components.

Results

In order to establish the source of the inoculum which results in a lactic acid fermentaion, fresh swine waste was combined with cracked corn and incubated aerobically in Erlenmeyer flasks (300 cc) set in a horizontal attitude on a slowly rotating board (0.5 rpm, 28°C) for 24 h. Acid production dropped pH from 6.5 to 4.2 and fetid odor was replaced by one resembling silage. Sterilization of the corn component so that microorganisms were supplied by waste gave the same results. However, cultures with a sterilized waste component provided fermentation with little change in pH and an odor like wet corn.

Fermentations containing more than 15.6% waste on a dry weight basis (DWB) still retained an undesirable fetid odor at 45 h. The pH increased in a linear fashion with the percentage of waste component in culture flasks for a given fermentation time. The pH ranged from 4.31 at 8.2% waste to 4.53 at 18.9% waste.

A typical stored or fresh waste-cracked corn fermentation pattern is shown in Figure 1. After 36 h, the pH dropped 2 units in response to a ten-fold increase in titratable acid with little further change at 144 h. Original fetid odor of the stored waste component changed to one resembling a silage fermentation by 36 h.

As shown in Figure 1, the initial ammonia concentration at 0.8 mg/dry g with stored waste-corn cultures was reduced considerably in the first 12 h of fermentation and was associated with a rapid increase in lactic acid organisms (Fig. 2). Suspected uptake of ammonium ion by lactic acid bacteria was tested by determining difference in concentration of this ion between sterile and inoculated tubes of *Lactobacilli* broth (MRS, 0.28% NH_3-N content by ammonia electrode), at 24 and 72 h of growth. Inoculum came from 6-h isolates of lactic acid bacteria from another fermentation and consisted of three homofermentative and nine heterofermentative organisms (gas producers). No difference in ammonium ion level was detected.

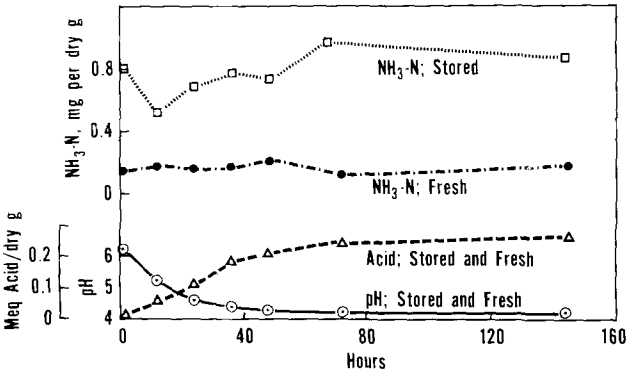

Fig. 1. Biochemical pattern of fermentations with fresh or stored swine waste combined with corn.

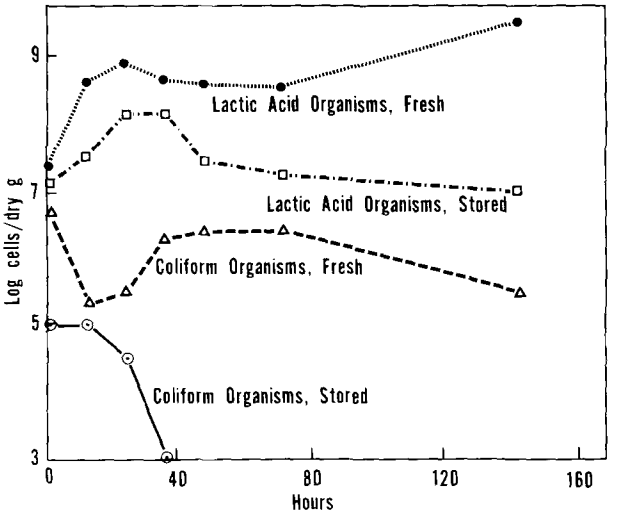

Fig. 2. Coliform and lactic acid organisms in fermentations of fresh or stored swine waste combined with corn

The population of lactic acid bacteria in cultures of fresh waste mixed with corn was 3 to 12 times greater after a 36-h incubation (Fig. 2). Lactic acid bacteria were present initially at 10^7 organisms/dry g in total populations of 10^9/dry g. By 12 h, lactic acid bacteria became the dominant group of microorganisms.

Coliform organisms in stored waste-corn cultures were initially present at 10^5 organisms/dry g. They were no longer detectable after 36 h. However, coliform organisms in cultures with fresh waste were present at levels of 10^6 to 10^7 organisms/dry g and diminished 20-fold in the first 12 h. Thereafter, they increased to initial levels. Total organisms and yeast counts (Fig. 3) show similar patterns of growth with fermentations containing either stored or fresh waste components. Total counts in fermentations employing fresh waste were ten-fold greater than those employing stored waste. Little change in total numbers of microorganisms was detected at 36 h over initial totals in either type of fermentation.

Acids resulting from fermentation of stored waste mixed with corn differed significantly from those of fresh waste plus corn. The major acid component from stored waste mixed with corn at 1 h was lactic acid (83.0%) with traces of butyric and valeric acids (Table 1). By 48 h, over 98% of the ether-soluble extract was lactic acid with the remainder being acetic acid.

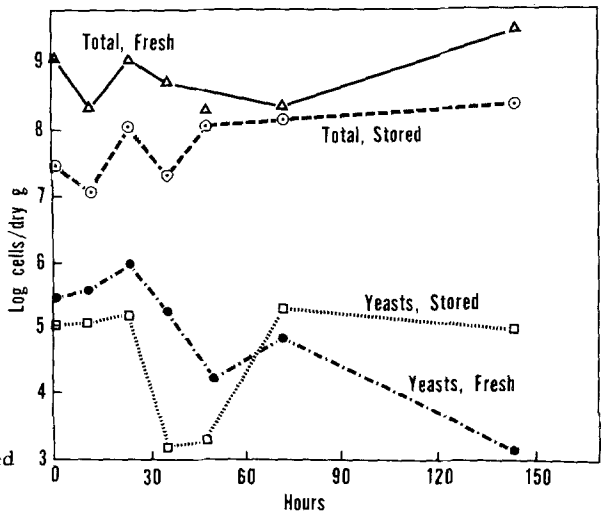

Fig. 3. Total microorganisms and yeast cells in fermentations of fresh or stored swine waste combined with corn

Table 1. Percent composition of fatty and lactic acids in fermentations of stored swine waste-corn

	Hours of fermentation				
Acid	1	12	24	36	48
Acetic	0	0.2	0	0.8	1.6
Butyric	0.5	0.4	0	0	0
Valeric	0.03	0	0	0	0
Lactic	83.0	86.4	89.6	94.6	98.9
Unknown	15.4	13.4	10.4	4.5	0

Table 2. Percent composition of fatty and lactic acids in fermentations of fresh swine waste-corn

	Hours of fermentation				
Acid	1	12	24	36	48
Acetic	13.2	10.0	29.7	24.9	12.4
Propionic	19.0	3.3	7.8	3.0	0.8
Isobutyric	1.9	0	0.2	0	0
Butyric	22.4	4.3	6.1	4.0	0
Isovaleric	7.7	0.7	0.8	0.3	0
Valeric	4.3	0.5	0.4	0.1	0
Lactic	14.2	70.2	54.1	60.3	87.4
Unknown	18.2	9.8	0.9	7.6	0

A contrasting pattern resulted from fermentation of fresh waste incorporated with corn (Table 2). Initially, all homologous fatty acids and certain isomers were detected in the series of acetic through valeric acids, including an average of 14.2% lactic acid. As fermentation proceeded through 36 h, acids at intermediate times showed decreases in percentage composition for homologous fatty acids from propionic through valeric and the isomers, isobutyric and isovaleric, while acetic and lactic acids increased to 24.9 and 60.3%, respectively (averages).

Discussion

Differences in biochemical and microbial patterns of fermentations were observed with stored vs. fresh waste combined with cracked corn, but silage-like fermentation products with lowered pH resulted from both types. Lactic acid fermentation produced acid with both types of waste and thereby diminished the disease potential of coliform organisms. Aerobic cultures of cracked corn tumbled with swine waste at 34 to 43% moisture levels and at temperatures of 27–33°C provided an acid fermentation with flexible operating parameters. Lactic acid bacteria are present initially in stored waste as 15% of the total microflora, but as less than 3% of all organisms in fresh excrement; however, this group of organisms completely dominated both types of culture by 12 h. Swine waste, not corn, supplied the microbial population that provided rapid acid fermentations with a silage-like odor. The surfaces of coarsely cracked corn appear to make convenient niches for growth of flora. Rate of fermentation with drop in pH and early reduction of fetid odor were modified by mixture ratios of waste and corn. More than 15.6% waste (DWB) in the corn-waste mixture retained a fetid odor at 45 h of fermentation, accompanied by diminished production of acid. Thus, waste fractions of cultures were chosen between 11 and 12% (DWB) and this level was associated with one part waste and two parts corn.

Acid production and pH in corn-waste culture with fresh or stored swine waste were comparable up to 36 h. Ammonium ion levels, however, were four-fold higher with stored waste-corn fermentation than with fresh waste-corn cultures and may reflect the higher deaminase levels associated with both microbial activity in stored materials of protein nature as well as their nonprotein N compounds (Looper and Stallcup, 1958). Significant differences in microbial pattern were found in fermentations of fresh or stored waste mixed with corn. Total viable flora and lactobacilli fractions were ten-fold higher in fermentations of fresh waste-corn compared to stored waste-corn, probably because of diminished availability of nutrients in old waste.

Coliform organisms, present at 0.5% or less of total flora with both types of waste, persisted with fresh waste at levels of 10^6 per dry g of fermentation product, but they were not detectable after 36 h in cultures with stored waste. Since both fermentations at 12 h contain low numbers of coliform organisms (10^5/dry g), produce comparable amounts of acids, and have similar pH levels, disappearance of these organisms is difficult to explain. However, the effect of pH can be significant as shown by McCasky and Anthony (1975) who tested 27 Salmonella cultures inoculated into cattle manure combined with Bermuda grass and ensiled for 3 days at pH levels of 4.0 to 4.5. At this pH level, no *Salmonella* were recovered while 25 of the 27 cultures were recovered at a pH of 6.0 to 6.5.

The acids responsible for reduced pH levels resulted from carbohydrate degradation by the dominant lactic acid bacteria group. Based on analysis of fermentation samples of ether-soluble acids, two types of lactic acid bacteria occurred dependent on use of either fresh or stored waste (Rogosa and Sharpe, 1959). Stored waste contained homofermentative lactic acid bacteria and, in cultures combined with cracked corn, produced almost all lactic acid. On the other hand, fresh waste-corn cultures produced 14.2 to 87.4% lactic acid along with other acids which result from growth of heterofermentative lactic acid bacteria.

Further information on the lactic acid bacteria group in animal waste was available from a comparable fermentation using cattle waste fractions combined with corn (Hrubant, 1975) which showed that the betabacterium, *Lactobacillus buchneri*-like, was present in greatest numbers for the first 24 h and is similar to our fermentation using fresh waste. His group three streptobacterium, *L. plantarum*-like, became the dominant lactic organism from 48 h on and compares to our homofermentative lactobacilli associated with stored waste-corn cultures.

Fresh swine waste combined with cracked corn in an aerobic tumbled vessel resulted in dominant growth by lactic organisms. Stored swine waste behaved similarly to give the same rapid acid fermentation and both types of waste plus corn provided a silage-like fermentation product with diminished disease potential which could be used in animal feed formulations.

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