Growth of Indigenous Organisms in Aerated Filtrate of Feedlot Waste

B. A. WEINER AND R. A. RHODES
Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

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Filtrates from feedlot waste were incubated under aerobic conditions to evaluate the availability of nutrients for cell production and to assess the capacity of indigenous flora to produce stabilized effluents. Incubation was carried out in 9-liter aerated jar fermentors. Three-fourths of the organic material and one-third of the nitrogen were taken up in 4 days; 90% utilization of organic material and nitrogen required almost a month. Acid was produced initially, but aerated liquid thereafter rapidly became alkaline. With pH controlled at 7.0, a comparable pattern of carbon utilization occurred, but nitrogen was incompletely used. The numerically dominant organisms in the waste inoculum were almost immediately displaced by an emergent population of a few types of organisms not originally evident. Maximal viable populations of $10^9$ to $3 \times 10^9$ cells/ml were obtained in aerated waste liquid within 48 h; subsequently, numbers declined quickly to initial levels. Numbers of fungi, yeasts, and streptomycetes slowly increased but never exceeded their initial concentration by more than tenfold.

Mechanized approaches to livestock handling produce 2 billion tons of animal waste annually. The major portion is contributed by cattle (5, 6), and the amount is expected to double by 1980 (1). Disposal of cattle waste usually involves land application (2, 7). Attempts to make useful material from the waste (10) include initial separation of solids and liquid. The liquid can be further fractionated to recover cellular protein and leave soluble nutrients as a possible substrate for growing microbes as a source of feed protein. Alternatively, the separated liquid is aerated in Pasveer ditches and lagoons to diminish the pollutants and stabilize the liquid. The spent liquid from these aerobic processes may be used for irrigation or mixed with surface waters if the nutrients have been sufficiently removed.

The liquid fraction of cattle waste contains soluble and finely divided material potentially usable by microorganisms. We have evaluated the ability of the indigenous flora to grow under aerobic conditions in this liquid and to utilize the nutrients present.

MATERIALS AND METHODS

The feedlot waste (FLW) came from an established operation of 5,000 to 10,000 head of cattle within 40 miles of Peoria, Ill. (9). Waste was collected from the paved areas of open pens having elevated areas of soil used by resting animals. The paved area was scraped clean almost daily. The feed was a high-energy type based on cracked corn.

Preparation of substrate. FLW with a solids content of about 30% was diluted with an equal volume of distilled water and mixed thoroughly to break up lumps. Diatomaceous earth was added at 15% (wt/vol), and the waste was then filtered by suction through Whatman no. 54 paper to provide a filtrate (FLWF). These steps gave FLWF both reproducible in composition and analogous to liquids resulting from waste processed to recover fiber and protein (10). This separation method was followed because screening gave liquids with particulate matter; reproducible samples of screened suspensions were difficult to obtain. FLWF was sterilized in 1-liter quantities at 15 lb/in² for 30 min.

Incubation conditions. Inocula were prepared by diluting fresh FLW 1:4 with sterile water. A 60-ml amount of the diluted waste was added to 3 liters of sterile FLWF in a 9-liter glass fermentor jar. Incubation was at 28 C. The stainless-steel head of the fermentor jar was equipped with conventional pipe openings, antifoam probe, and solenoid-controlled inlets for automatic addition of acid, base, or antifoam. A 1% silicone solution (General Electric 60) was added automatically in minute quantities to control foam.

Air sterilized through a glass-wool filter was supplied at the rate of 0.25 liters per liter per min through an Aloxite stone sparger. Liquid was agitated at 90 rpm with one set of blades placed at 3/8 the liquid depth. Another set was placed at the air-medium
interface to help alleviate foaming, which has been a serious problem in aeration of cattle waste (2).

Analyses. Immediately before sampling, sterile distilled water was added to replace evaporated liquid. A 40-ml sample was withdrawn for analysis; this volume was replaced with sterile FLWF at 50% original concentration. One milliliter of each sample was immediately diluted serially and spread on previously poured plates; triplicate plates were used for each dilution. Total counts were made on Eugen agar, gram-negative counts were made on eosin methylene blue, and fungal and yeast counts were made on Mycophil agar containing 0.2 mg of dihydrostreptomycin sulfate and 330 U of penicillin G per ml (all BBL, Division of Becton, Dickinson and Co., Cockeysville, Md.). Streptomycetes were counted on the salts-starch medium of Pridham (9) with 0.5 mg of cycloheximide added per ml to inhibit other growth.

The remainder of each sample was used for chemical analyses. Total solids were measured by drying 10 ml at 103 C for 24 h. Cell weight was determined by centrifuging a 10-ml portion at 3,000 x g for 20 min and drying the cell pellet for 24 h at 103 C. Titratable alkalinity (to pH 7.0), chemical oxygen demand (COD), nitrogen, and ammonia were determined on the supernatant after removal of cells. COD analyses were done according to Standard Methods (11). Kjeldahl-nitrogen was determined by an AOAC microprocedure (4). Ammonia was measured by a modified Folin-Farmer method (3); in this analysis, ammonia was sparged from a sample made alkaline with K2CO3 into 2% boric acid and titrated with standard HCl.

RESULTS

Growth patterns of microorganisms in FLWF under aerobic conditions are plotted in Fig. 1. Maximal growth occurred at 48 h, with gram-negative organisms representing one-third the total count of $3.5 \times 10^9$/ml. At 96 h, both total and gram-negative counts decreased sharply (82%) and then steadily diminished until numbers were nearly constant at about 20 days. Fungi and yeasts increased more slowly, and then they too decreased. The number of streptomycetes initially declined and thereafter slowly increased during later stages of the fermentation. The initial population was similar to that reported for FLW (9). Approximately $10^9$ coliforms/ml were detected on eosin methylene blue at 0 h, and none were detected thereafter. The yellow-pigmented corynebacterium, which constituted 30 to 50% of the total aerobic population of this FLW, was not detected after 48 h. Instead, 99% of the population consisted of rods, which formed small (1 to 3 mm), cream-colored, moist, entire colonies on Eugen agar.

Chemical changes associated with aerobic growth in FLWF are recorded in Table 1. In general, the extent of change correlated with viable counts. COD was reduced 63% in 48 h. An additional 11% reduction in COD occurred during the next 48 h, followed by a slow but steady decrease until more than 90% of the organic material was consumed in 28 days. The characteristic fetid odor of the waste was not detectable at 18 h.

Total solids were reduced at a constant rate of 5% per day for the first 10 days and at a decreasing rate until three-fifths the initial amount was gone by 21 days. No substantial change occurred thereafter.

Nitrogen concentration was reduced by one-third in the first 96 h and then at a lesser rate until more than 90% utilization was reached at 28 days. Ammonia constituted about half the initial nitrogen; the remainder was organic nitrogen. Urea was not detected. After an abrupt initial drop, ammonia increased to 82% of the total nitrogen by the 10th day and remained approximately at that proportion through the 17th day.

A basic pH was recorded for all but the first 48 h of fermentation, with alkalinity at 1.0 to 1.5 meq per 100 ml of culture supernatant. Onset of basic pH occurred when the viable population decreased sharply and ammonia concentration increased.

Results obtained when pH was controlled are plotted in Fig. 2. Maximal population (1.2 x $10^9$ cells/ml) occurred at 24 h, with a subsequent 90% decrease in viable organisms by 48 h accompanied by a 46% decrease in cell weight. COD decreased 45% in the first 24 h and 90% ultimately, but only a 30% reduction in nitrogen was achieved by the end of fermentations at pH 7.

DISCUSSION

Under aerobic conditions, microorganisms in FLW proliferate considerably on the soluble
TABLE 1. Changes in feedlot waste filtrates during aerobic submerged conditions

<table>
<thead>
<tr>
<th>Fermentation time (days)</th>
<th>pH</th>
<th>Titratable alkalinity (meq/100 ml)</th>
<th>Cell wt (mg/100 ml)</th>
<th>Total solids (mg/100 ml)</th>
<th>COD*a (mg of O₂/liter)</th>
<th>Kjeldahl N (mg of N/ml)</th>
<th>NH₃ (mg of N/ml)</th>
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<tr>
<td>0</td>
<td>6.47</td>
<td>1.77 (meq of acid/100 ml)</td>
<td>92</td>
<td>865</td>
<td>9,100</td>
<td>0.55</td>
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<td>2</td>
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<td>0.15</td>
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<td>4</td>
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<td>1.31</td>
<td>218</td>
<td>684</td>
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<td>10</td>
<td>8.73</td>
<td>1.35</td>
<td>131</td>
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<tr>
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<td>63</td>
<td>359</td>
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<td>1.04</td>
<td>104</td>
<td>427</td>
<td>700</td>
<td>0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*a COD, Chemical oxygen demand.

Fig. 2. Submerged aerobic incubation of feedlot waste at pH 7 by indigenous organisms. COD, Chemical oxygen demand.

nutrients of FLWF. Apparently abundant growth does not take place in the waste at the feedlot (9); neither are large populations found in lagoons receiving FLW (12) nor in aerobic treatment facilities (2). Most often, gram-negative organisms, coliforms, and enterococci diminish markedly (12; T. M. McCulla, J. R. Ellis, and W. R. Woods, Bacteriol. Proc., A27, 1969). In our study, a 1,000-fold increase in gram-negative organisms occurred in the first 48 h, but subsequently they decreased more sharply than did total numbers. Coliforms were not detected beyond inoculum time. The total numbers of organisms measured approximated initial levels by the 17th day.

After a 1,000-fold increase in viable count in the first 2 days, rapid decrease in viable count occurred during spontaneous aerobic growth in FLWF. Growth reduction was associated with alkaline pH, a condition reported by others (12; T. M. McCulla et al., Bacteriol. Proc., A27, 1969). Also, foaming is more pronounced at basic pH levels.

Control of pH was used in an attempt to extend viability. However, one-third less growth resulted even though uptake of organic matter was comparable to that in the uncontrolled system. Higher residual nitrogen levels in cultures at pH 7 represent retention of ammonia at neutrality during sparging. Undoubtedly, part of the apparent nitrogen utilization in uncontrolled alkaline fermentations was actually ammonia loss during aeration. The drop in viable count may have resulted from depletion of the limited supply of carbohydrate and other readily available nutrients.

Whole cattle waste has a COD of 80,000 to 130,000 mg of O₂ per kg and a biological oxygen demand (BOD)-to-COD ratio of 0.3:0.4 (6, 7, 12). Liquid from waste screened at 15% solids has a COD of 25,000 to 30,000 mg of O₂ per liter. The COD of liquids used in this study averaged about 10% of that of whole waste. The rapidly metabolized components measured as COD in this work probably encompass the materials in FLW which are measured in a standard 5-day BOD. The data indicate that the liquid fraction contains much of the readily degraded material in the waste because FLW organisms utilized 80% of the COD and 50% of the nitrogen in 2 to 4 days under aerobic conditions. This indicates why oxidation ditches for animal waste treatment are successful. Easily metabolizable components are made available to microorganisms through dilution (3 to 5% total solids, 20,000 COD in ditches), and aerobic conditions are established to ensure rapid metabolism.

Material remaining in FLW liquid after initial uptake is metabolized slowly. BOD analyses would not have been useful because liquids were largely stabilized. The COD after 4 weeks is 25% or more of that measured at 4 days. Fungi and streptomyces appear to be responsible for breakdown of the more resistant components during this period; although of negligible impor-
tance initially, these organisms persist in slowly increasing numbers as the rest of the population dies. Cell walls and other comparatively resistant material arising from death of organisms which initially grew on the soluble nutrients undoubtedly contribute to the supply of recalcitrant compounds. The decrease in cell mass after maximal growth indicates that cell fragments contribute to COD during prolonged incubation.

Soluble nutrients in FLW can serve as a substrate for growing cells of potential value as a protein source in feed. The drastic decrease in cell weight before maximal removal of COD would place such an operation at some disadvantage from the viewpoint of pollution control. However, rapid reduction in the number of gram-negative organisms, coupled with major reduction in total organic material, means that the resultant effluent could be used for irrigation. Although the flora of FLW can remove more than 90% of organic and nitrogenous material in 1 month under aerobic conditions, the effluent still would not be suitable for release to surface waters because of residual COD and nitrogen levels.

LITERATURE CITED