Solid Substrate Fermentation of Feedlot Waste Combined with Feed Grains

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ANIMAL production based on confinement of animals in large groups means that animal wastes also are confined. This waste can be considered a raw material whose on-site concentration allows continuous collection and processing. For example, animal wastes contain sufficient nitrogen in the form of protein (20 percent of total N) and in forms readily convertible by microorganisms to protein (urea and ammonia nitrogen constitute about 30 percent of total N) to be potentially useful as a nutrient source for feeds.

Animal wastes have been fed to the same or different species, generally as a nitrogen source. These studies have been discussed by Anthony (1971a) and extensively reviewed in detail by Smith (1972, 1973). Supplementation of conventional feeds with animal waste need not depress consumption, digestibility, or gain, although data on true nutrient utilization are yet sparse.

In contrast to direct refeeding, feedlot manure has been ensiled with roughage in the ratio 57 manure:43 hay and termed Wastelage (Anthony 1969, 1971b). The ensiled mixture fed at 40 percent of a corn ration gave satisfactory gains, although feed:gain ratios were somewhat higher than with control rations. An anaerobic lactic fermentation of whole manure neutralized with anhydrous ammonia has been reported but details are lacking (Moore and Anthony 1970). Acid treatment of the cellulosic fraction of manure to provide a substrate for yeast production has been proposed (Singh and Anthony 1968).

Nitrogen is not evenly distributed among the major components of animal wastes. A large proportion (70 percent of total N) is recovered in solution or fine particles separated by screening from the essentially fibrous portion (Jones et al. 1972, Sloneker et al. 1973). Separation of the nitrogenous fraction from the bulk of fibrous material in cattle waste probably is essential to fullest utilization of the waste. The dissimilar composition of these two fractions indicates that they should be processed separately.

The nitrogen-rich liquid fraction of cattle waste has the best immediate potential as a raw material for protein production by microorganisms. Certain characteristics of this fraction are important in considering such a fermentation process: (a) Much of the protein is present in bacterial cells; this protein should not be broken down and new protein synthesized. (b) A large proportion of the microbial cells are alive and can be induced to grow and convert inorganic nitrogen to cellular protein. (c) The microbial growth necessary for protein synthesis is limited by the relatively small amount of readily metabolizable carbohydrate present in the waste liquid. (d) Microbial protein production by conventional liquid fermentation systems would be difficult to control; this is particularly true in light of the large population of different organisms present.

We have devised a solid-substrate fermentation system which processes the waste liquid in mixture with feed grains. Microbial growth is enhanced selectively in a restrictive environment which spontaneously promotes the growth of certain indigenous organisms and restricts that of others. The objective of this study was to provide a basis for microbial protein production for animal feeds.

MATERIALS AND METHODS

Materials

Fresh manure (1-24 hr accumulation) was collected by hand shovel from paved areas of an outdoor commercial beef cattle feedlot. The animals were fed a low roughage high energy ration based on cracked corn. The circumstances surrounding this feedlot were described earlier (Rhodes...
Preparation of Feedlot Waste Liquid (FLWL)

Raw waste was mixed with a calculated amount of water and stirred to a homogenous slurry of desired solids content (w/w). Initial studies were done with waste diluted to different solids content; most separations were done at 15 percent solids. In laboratory studies, the diluted waste liquid was expressed from diluted waste by hand squeezing through two layers of cheesecloth. Larger volumes were processed with the reciprocating screen shown in Fig. 1. A copper screen of 30 mesh (0.33 mm wire, 0.59 mm openings) was fastened over a rectangular wooden frame; an open three-sided wood frame was fastened on top of the screen frame to contain the slurry. The screen assembly was held tightly over a stainless steel tray which has separated openings at the lower end to discharge liquid and solids. The entire tray-screen assembly was held at 11 deg from horizontal and moves with a reciprocating motion through a 2-cm displacement at ca. 300 strokes per min when loaded. The screen was driven through a gear box and belt by a 1/4 hp electric motor. Waste ladled onto the high end of the screen traversed the length of the screen in about 1 min under impetus of the screen motion. Liquid which separated from the waste through the screen drained from the receiving tray into a receiving vessel; fibrous solids migrated off the open lower end of the screen into a separate container. The liquid was stored at 4 C in plastic containers until used. Fibrous solids were discarded.

Fermentation Process

The liquid fraction (FLWL) was mixed with coarsely cracked feed grains so that the resultant grain-FLWL mixture had a moisture content of 35-42 percent. Grain: FLWL mixtures at ratios of 2:1 to 2:1.2 (w/w) have this moisture level. At these ratios, the viscous FLWL fully adhered to the grain and coated it so that no free liquid existed within 10 min after mixing. Grain-FLWL mixtures were incubated in nearly horizontal containers rotating slowly.

Flask Studies

Erlenmeyer flasks were used in laboratory studies. Quantities of grain-FLWL mixtures which approximated 25 percent of flask volume afforded proper mixing of the grain during fermentation (75 g in 300-ml flasks, 500 g in 2000-ml flasks). Flasks were held by metal spring clips perpendicularly to a board rotating at 0.6 rpm at 9 deg from vertical. This configuration, together with the 40 percent moisture content and quantity of solids in flasks, allowed a continuous tumbling of grain-FLWL so that particles remained separated. Flasks were plugged with cotton or the opening was covered with gauze. Incubation was at 28 C.

Large-Scale Fermentations

A standard cement mixer with a 130-l bowl (70-l capacity) was used for volume fermentations. The mixer was belt-driven through a reduction gear on a 1/4-hp electric motor so that the chamber rotated at 0.5 rpm. The interior of the mixer bowl (including mixing baffles) was sand blasted and painted with a two-component epoxy paint before use to eliminate rust formation from the acid fermentation. The mixer was charged with 23 kg of grain and 11-13 kg of FLWL. The bowl was held at 40 deg from horizontal. The mixer operated at ambient temperatures (30-35 C).

Fermentations done in the cement mixer were terminated after 36 hr and the fermented product was dried in situ by blowing 60 C air into the opening of the bowl while it continued to rotate. The fermented grain dried to a moisture content of 12 percent or less in 12-14 hr. Dried product was dumped freely and was bagged and held for animal tests.

Analytical Procedures

Analytical results were calculated to dry weight of fermented product. Moisture was determined by drying a weighed sample at 100 C for 24 hr. Ash was measured by standard AOAC method. Total nitrogen was determined by micro-Kjeldahl. pH of fermented product was measured on a 5-g sample triturated in distilled water for 10 min.

Microbial counts were done on material prepared by blending a 5-g sample (wet wt) for 30 sec in 20 ml of cold 0.1 M phosphate buffer at pH 7 and then filtering and rinsing to volume through a loose fiberglass plug in a funnel. The turbid filtrate then was serially diluted in sterile distilled water. Counts were made by spread plating 0.3 ml of appropriate dilutions in triplicate. Eugon agar was used for total counts and EMB for coliforms (both BBL, Bioquest Division of Becton, Dickinson Co.).* Eugon plates were counted after 48 hr incubation at 28 C and coliform counts were made after 24 hr at 37 C. Ammonia and total acid determinations were performed on comparable filtrates prepared with distilled water. Total acid was measured by titration with 0.05 N NaOH to pH 7. Ammonia was measured with an ion-specific electrode (Orion Company, Cambridge, Massachusetts) on the supernatant of a blended sample centrifuged at 10,000 rpm for 1 hr under refrigeration.

Amino acids were measured with a Beckman Model 120B automatic analyzer on dried product ground in a Wiley Mill to pass a 20-mesh screen. Samples for fatty acid identification were frozen until analysis. One hundred-gram samples were triturated with 200 ml distilled water for 10 min and then were filtered through glass wool with the first 100 ml collected. These water extracts were centrifuged twice at 15,000 rpm for 30 min under refrigeration; pellets were discarded. The resultant clear supernate was made alkaline with NaHCO₃ and extracted for 24 hr with ether in a liquid-liquid extractor to remove nonacidic components (discarded). The alkaline supernatants then were made acid with concentrated HCl and extracted with ether for an additional 24 hr. The ether phase from the acidic extraction was concentrated to a small volume and used for gas chromatography.

Fatty acids were determined by GLC on two different polar columns; dual flame ionization detectors were used and disc integrators approximated quantity of component peaks. A standard mixture consisting of acetic, propionic, iso- and n-butyric, iso- and n-valeric, iso- and n-caproic, and L-acids at 2-5 uM/ml in anhydrous ether was used to establish retention times.

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.
total nitrogen (Kjeldahl) increases 12 percent. Thus, there is a preferential increase of protein at the expense of nonprotein nitrogen. Acceptability of the fermented product for hogs was tested by B.G. Harmon of the Animal Science Department at the University of Illinois. Fermentation product was substituted for corn in a standard diet (corn, soybean meal, minerals, vitamins) and offered in a cafeteria test with the standard diet. The trial was done for 10 days with consumption measured every other day. Three of the four pens showed a preference for the regular diet but the fermented product was not rejected.

Fermented product collected from flask fermentations done over several months was blended and offered to white Swiss mice in comparison to unfermented corn and a commercial pelleted diet. The fermented product and the unfermented corn were coarsely ground, cooked briefly in minimal amount of water to partially gelatinize the starch, and then formed into pellets. Each of the three diets was fed ad libitum for 2-1/2 months to six mice separated in cages of three segregated by sex. Mice were weighed every 3 or 4 days; weight data are shown in Table 4. The fermented product exhibited no overt toxicity to mice and yielded equal growth rates compared to corn. The product, as well as unfermented corn, does not appear to be nutritionally complete for mice.

**DISCUSSION**

Much of the proteinaceous component of FLW is contained in microbial cells or other discrete particulates. A large number of the cells are alive. Most of the microorganisms and much of the potentially useful nutrients in FLW are separated together as a liquid from the fiber portion by screening. Addition of the resultant viscous liquid to cracked grain provides inoculum and nutrients and simultaneously establishes a selective environment for microbial growth. The grain absorbs moisture and provides carbohydrate needed for microbial growth. The waste liquid is nitrogen-rich.

Although lactic acid bacteria constitute less than 1 percent of the total microbial population in cattle waste, conditions selectively permit their growth with exclusion of other organisms from the waste. Rapid acid production by these bacteria further limits competitive growth. As a result, the fetid odor of the waste is replaced within 6-8 hr by a typical silage odor of acetic and lactic acids. Growth of lactic acid bacteria does not greatly increase the protein content of grain but it does establish a selective environment for growth of yeasts which have the capability of utilizing nonprotein nitrogen. Regardless, most of the valued nutrients in the waste are combined with grain in a palatable form.

The process is simple and is adaptable to both small and large animal production units. It depends upon the intrinsic flora of the waste. The solid-substrate configuration for the fermentation with particulate substrate and a controlled moisture content and degree of aeration provides a simple, self-stabilized process without the need for complex control systems commonly required in liquid fermentations. The fermentation pattern described typically results if fresh manure is used. Cattle waste from a second feedlot has been used successfully in laboratory trials. Weathered waste or that excessively leached by rain do not yield good fermentations. Fermentations done with combinations of sterile corn and sterilized waste liquids showed that it is the waste flora and not the flora of the grain which produces the rapid acid fermentation. Cracked corn has the capacity to reduce odor to some extent by absorption without fermentation.

Preliminary studies with scanning electron microscopy indicate that microorganisms grow on the exposed, porous starch areas of corn but not on the smooth hull. The waste liquid appears to be preferentially absorbed in the starch areas. The moisture level during fermentation is rather critical. Less than 35 percent moisture limits growth and acid production by lactics so that pH values of only 5.0-5.5 are obtained. At such low moisture levels yeasts grow and the pH increases rapidly. Grain-FLWL mixtures with moisture contents over 42 percent tend to be gummy and aggregate undesirable. The moisture content of fermenting grain-FLWL remains remarkably constant for extended incubation periods in spite of open containers and exposure by continuous tumbling of particles. There is a tendency for a slight increase in moisture with time, probably as a result of moisture incorporation during starch hydrolysis. This causes some particle aggregation after 3-4 days. The fermentation has been done in flasks with cracked milo and with cracked wheat. Essentially, the same fermentation occurred but the structure of milo makes it difficult to expose the starch without shattering the grain into fine particles. The different moisture absorption characteristics of these grains may require different grain-liquid proportions for optimum fermentation. Preliminary examination indicates yeast growth occurs earlier than with corn, probably because moisture is limited.

The temperature of fermentation does not seem to be a problem. Excessive temperatures within the slowly turning mass have not been encountered in spite of high ambient temperatures. Tests with both bacteria and yeasts isolated from the fermentation show that they will grow at the temperatures encountered. Although we have done no work on minimum temperature requirements, it is likely that 25 C or above will be required for rapid fermentations; even though most isolates grow slowly at 20 C or below.

Fermentations also have been done with unfractionated raw waste; results are similar. However, processing whole waste would in effect dilute the nutrient content of the product by recycling unaltered fiber from the waste. Hog waste, which contains relatively less fiber than does cattle waste, is being fermented without fractionation in other work underway.

Two lines of future work on this fermentation are planned. First, to

### TABLE 4. GROWTH OF MICE ON FERMENTED CORN-FLWL PRODUCT

<table>
<thead>
<tr>
<th>Diet</th>
<th>Days on diet, avg. weight in grams*</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Unfermented corn</td>
<td>14.7</td>
</tr>
<tr>
<td>Fermented corn</td>
<td>14.8</td>
</tr>
<tr>
<td>Fermented corn-FLWL</td>
<td>12.5-16.3</td>
</tr>
<tr>
<td>Commercial feed</td>
<td>13.0</td>
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<tr>
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<td>(10.7-15.5)</td>
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*Average weight of six white Swiss mice per diet. Parenthetical values are the range in weight within the group.
achieve complete recycling of organic nitrogen, the raw waste needs to be fractionated at a higher solids content to yield a liquid of about twice the solids content presently obtained. This liquid would be used at approximately a 1:1 ratio with cracked grain to obtain a mixture of 40 percent moisture and still retain the desired solid-substrate configuration which makes the process convenient. Second, a greater net protein content in the product should be attainable by adding inorganic nitrogen for protein synthesis by yeasts whose growth would be encouraged early in the fermentation; addition to the grain-FLWL mixture of material from a previous fermentation could provide a massive inoculation of yeasts for this purpose.

The rapid decrease in pH destroys many enteric and other bacteria. Sometimes, reduced numbers survive. Regardless, the strongly acid environment does decrease potential health hazard from refeeding waste. The nutritional value of the product is to be assessed in growth trials with pigs. Dried product will be used in these trials because this is the only feasible way to store sufficient material for feeding. In practice, the fermented product probably would be fed in the moist state with the probability that nutrients would be more available.

SUMMARY

A new process is being developed for recycling animal wastes based on a solid-substrate fermentation with cracked grains. To date, the work has been done mainly with corn and the liquid fraction of cattle feedlot waste. This liquid, separated by an oscillating 30-mesh screen, contains 10 percent solids in the form of microbial cells and either fine or soluble waste components; it has a nitrogen content of 3-4 mg/ml. When added to cracked corn at a ratio of 1:2, the thick liquid adheres to grain surfaces. Grain-waste mixtures at about 40 percent moisture, incubated aerobically by tumbling in a slowly revolving (0.5 rpm) vessel, such as a small cement mixer, rapidly undergo an acid fermentation caused by the selective growth of lactic acid bacteria originally present in the waste; other waste organisms die. The number of bacterial cells increases 100-fold with 24 hr; yeasts emerge after extended incubation. The fetid waste odor quickly disappears as 0.1 meq of acid per wet gram of fermented solids is generated while nitrogen is conserved. The acids formed are primarily lactic and acetic, with lesser amounts of propionic and butyric.

Exploratory tests with milo and wheat, as well as with wastes from hogs and cattle on different rations, indicate that the new process may be widely applicable. Diluted whole waste may be used when inclusion of fiber in the fermented feed product is of no concern. The solid-state system provides an easily established selective environment for microbial growth. Specific organisms and added inorganic nitrogen may be used for a continuous operation to generate higher protein content in grain-based rations.

References