Bacterial Fermentation of Cheese Whey for Production of a Ruminant Feed Supplement Rich in Crude Protein

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A simple and efficient process for the production of a ruminant feed supplement, rich in crude protein (defined as total N \( \times 6.25 \)), by bacterial fermentation of cheese whey has been developed. The lactose in unpasteurized whey is fermented to lactic acid by Lactobacillus bulgaricus at a temperature of 43°C and pH 5.5. The lactic acid produced is continually neutralized with ammonia to form ammonium lactate. The fermented product is concentrated by evaporation to a solids content of about 70% and adjusted to pH 6.8 with additional ammonia. The concentrated product contains about 55% crude protein. Approximately 6 to 8% of the crude protein is derived from bacterial cells, 17% from whey proteins, and 75 to 77% from ammonium lactate. The efficiency of conversion of lactose to lactic acid usually exceeds 95%. The fermentation time is greatly reduced upon the addition of 0.2% yeast extract or 0.1% corn steep liquor as a source of growth factors. Whey containing lactose at concentrations up to 7% can be fermented efficiently, but at higher concentrations lactose is fermented incompletely. The process has been scaled up to a pilot plant level, and 40 tons of concentrated product were produced for animal feeding trials, without ever encountering putrefactive spoilage.

Whey is a lactose-rich, watery by-product of the cheese manufacturing process (42). Nearly, \( 1.36 \times 10^8 \) kg of whey is produced annually in the United States (20). Out of this, only 56% of the whey solids are currently processed into animal and/or human food products (20).

Several problems are encountered in disposing or reutilizing whey. It is uneconomical to transport whey because of its high water content. It is readily subject to bacterial and fungal spoilage and cannot be stored for any length of time unless special precautions are taken. Drying whey requires a large capital investment, is energy intensive, and is not economically profitable (6, 28). Disposal of whey within federal environmental standards is expensive because of its high biological oxygen demand. Besides, whey contains large amounts of potentially recyclable nutrients. It is estimated that discarded whey contains about \( 3.6 \times 10^8 \) kg of lactose and \( 6.9 \times 10^8 \) kg of protein. Thus, there is an obvious need for finding a solution to the whey disposal problem and for recycling the nutrients in whey as feed and/or food material.

A number of processes have previously been described for the batch bacterial fermentation of lactose in cheese whey and production of lactic acid (36, 43; H. C. Jansen, Dutch Patent 57848, 1945; A. H. Johnson, S. M. Weisberg, J. J. Johnson, and M. E. Parker, U.S. Patent 2071346, 1937; S. M. Weisberg, F. L. Chappell, E. Stringer, S. Stevens, and H. A. Trebler, U.S. Patent 2071368, 1937) or microbial protein (4, 6). A continuous fermentation process for lactic acid production from cheese whey was also described (44). Further, the production of ammonium lactate, as a source of crude protein (defined as total N \( \times 6.25 \)) for cattle, by fermentation of cheese whey or other materials rich in carbohydrates has been described (2; E. J. Czar- netzky, U.S. Patent 2,094,437, 1959; Jansen, Dutch Patent 57848, 1945; L. H. Perquin, Dutch Patent 58545, 1946). Several investigators have shown that ammonium salts of short-chain organic acids, including ammonium lactate, produced either synthetically or by fermentation were superior to urea and equivalent to soybean meal as nitrogen supplements to ruminants (1, 5, 17, 22–24, 39).

The previous methods for the production of lactic acid, salts of lactic acid, or SCP (single cell protein), by fermentation of cheese whey, suffered from one or more of the following disadvantages: (i) there was a long lag period prior to active lactic acid fermentation and/or unus-

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ually long fermentation times that required greater fermentor capacity and increased operational costs (4, 36; Czarnezky, U.S. Patent 2,094,437, 1959); (ii) manual procedures used for the addition of ammonia or calcium ion to neutralize the lactic acid produced during the fermentation were tedious and contributed to increased product variability (18, 36; Czarnezky, U.S. Patent 2,094,437, 1959; Jansen, Dutch Patent 57848, 1948; Perquin, Dutch Patent 58545, 1946); (iii) large levels of inocula (10 to 20% by volume) were required to obtain vigorous fermentation and, hence, were less economical (2, 4, 6, 36, 43; Czarnezky, U.S. Patent 2,094,437, 1959; Jansen, Dutch Patent 57848, 1945); (iv) putrefactive spoilage of the fermentations was encountered (2); (v) the crude protein content of the product was relatively low (4, 6; Czarnezky, U.S. Patent 2,094,437, 1959; Perquin, Dutch Patent 58545, 1946); (vi) relatively high costs were involved in the pasteurization or sterilization of whey prior to fermentations (2, 6, 36; Czarnezky, U.S. Patent 2,094,437, 1959); (vii) there were high aeration costs during fermentation (4, 6, 32); and (viii) there were palatability problems for the animals with the product that resulted in decreased dry matter intake (21).

The objectives of this study were twofold: (i) to develop a simple, efficient, and nonaseptic fermentation process for the production of a nitrogenous feed supplement, for ruminant animals, from cheese whey and (ii) to investigate the feasibility of producing such a product on a pilot plant scale.

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MATERIALS AND METHODS

Bacterial culture. After a careful examination of a large number of Lactobacillus strains representing several different species, L. bulgaricus strain 2217 (supplied by Robert Sellars, Chris Hansen Laboratories, Milwaukee, Wis.) was used in this study because of its high rate of acid production in the pH range 5.0 to 6.0, greater acid tolerance, and short generation time. This organism was routinely maintained in sterile skim milk medium (SSMM), 10% (wt/vol) skim milk powder in distilled water, and autoclaved at 121°C for 15 min. About 0.1 ml of the culture was transferred once every 4 weeks to 10 ml of SSMM in a screw-cap tube. Inoculated tubes were incubated at 44°C for 24 h and stored at 4°C until further use.

Bench-top fermentor. The inoculum for a 14-liter bench-top fermentor (model Ma 140 F1, Fermentation Design, Allentown, Pa.) was prepared by transferring 0.1 ml of a 24-h culture of SSMM-grown L. bulgaricus to 10 ml of the same medium, in a screw-cap tube, and incubating for 12 h at 44°C. Coagulation of SSMM occurred in 8 to 10 h as a result of bacterial growth and lactic acid production. About 0.1 ml of this culture was added to another tube of SSMM and incubated as above. The contents of the second tube were transferred to 500 ml of SSMM in a 1-liter foam-plugged Erlenmeyer flask. After incubation, the contents of the flask were used to inoculate 9.5 liters of whey in the fermentor.

All fermentations were conducted as follows. Nonsterile, reconstituted whey (spray-dried, cottage cheese whey powder [Michigan Milk Producers, Ovid, Mich.], reconstituted with distilled water to give a lactose concentration of approximately 5.0% [wt/vol] was added to the fermentor. Unless specified otherwise, sterile corn steep liquor (CSL; Corn Products Corp. International, Argo, Ill.) was added to the whey (0.1% final concentration) as a source of supplemental growth factors for L. bulgaricus. The medium was continuously agitated at 150 rpm. The temperature was adjusted and maintained at 43 ± 0.5°C. The pH was adjusted and maintained at 5.5 ± 0.1 by an automatic pH recording controller (model PHRT, Fermentation Design, or model pH 22, New Brunswick Scientific Co., New Brunswick, N.J.). Either anhydrous (99.9%; Matheson Gas Products, Chicago, Ill.) or aqueous (27% in water; Carrier Stephens Co., Holt, Mich.) ammonia was used as the neutralizing agent. Inoculum was added at the 5% level unless otherwise specified. No aseptic precautions were taken for adding the inoculum, collection of samples, addition of growth factors, or other manipulations. Fermentation was terminated at the end of 16 to 24 h. Samples (~40 ml) were collected in screw-cap glass sampling bottles just before adding the inoculum, at selected intervals during the fermentation, and at the end of fermentation and stored at −18°C until analyzed. All samples were analyzed for lactose, lactic acid, total nitrogen, and ammonia nitrogen.

Pilot plant fermentor. All pilot plant scale fermentations were conducted in a 2,270-liter stainless-steel fermentor (model FS-3, Bernard and Leas, Cedar Rapids, Iowa) operated at a 1,940-liter capacity. The temperature in the fermentor was controlled with an ACCO-Bristol temperature controller (model 624-II, ACCO-Bristol Div., Waterbury, Conn.), and the pH was continually measured and controlled at a set point by an electrode (model 764-31, Ingold Electrodes Inc., Lexington, Mass.) fitted into the side of the fermentor and connected to an automatic pH recording controller described above.

All fermentations using a 2,270-liter fermentor were conducted as follows. About 1,890 liters of fresh cottage cheese whey or sweet whey was added to the fermentor, agitation was provided, and 20 liters of CSL was added. The temperature and pH were adjusted to and maintained at 43 ± 0.5°C and 5.5, respectively, as described above. The inoculum was prepared by transferring 10.0 ml of a 12-h-old culture of L. bulgaricus, grown in SSMM, to 2 liters of the same medium in a 3-liter foam-plugged Florence flask. After incubation for 12 h, the contents of the flask were transferred to 45 liters of commercially available pasteurized skim milk, and after 12 h of incubation the latter served as the inoculum. Immediately after adding the inoculum, a sample was
collected. Fermentation was terminated after 24 h. another sample was collected, and the fermented ammoniated whey (FAW) was concentrated 10-fold by using a 378-liter, single-effect, rising film evaporator (Rogers Co., Detroit, Mich.). The pH of the concentrated product was adjusted to 6.8 with additional ammonia to further increase the crude protein level in the product. This final product, referred to as fermented, ammoniated, condensed whey (FACW), was stored in 3,785-liter steel tanks until further use.

Analytical procedures. Total nitrogen was determined by the micro-Kjeldahl procedure (27). Lactose was determined by the procedure of Dubois et al. (16) as modified by Montgomery (33). Ammonia nitrogen was determined by Nesslerization (34) and by Conway microdiffusion analysis (11). Lactic acid was quantitatively determined by the procedure of Barker and Summerson (3) as modified by Davidson (15). Metabolic end products produced during the fermentation were qualitatively analyzed on a gas chromatograph (Dohrmann Anaerobic Bacteriology Analyzer, Clinical Analysis Products Co., Sunnyvale, Calif.), using the procedures described by Holdeman and Moore (25). Optical rotation of the lactic acid produced during the fermentation was determined by the procedure of Cato and Moore (10). The percentage of solids in FACW was determined by the procedure of Hood et al. (26) or by air drying in a forced-air oven at 60°C for 48 h.

RESULTS

Rate study. Decreases in lactose concentration and increases in concentrations of lactic acid and ammonia during the fermentation are shown in Fig. 1. Lactose was rapidly metabolized and in many cases fermentation was essentially complete within 14 to 16 h. About 95 to 98% of the lactose was metabolized during the fermentation. As the lactose concentration decreased, there was a proportional rise in the concentration of lactic acid. Lactic acid was the only major acid produced during the fermentation, suggesting that the lactose was metabolized by a homolactic acid type of fermentation.

For each gram of lactose fermented, about 0.189 g of NH₃ was required to neutralize the lactic acid produced, and the ammonium ion concentration curve essentially paralleled the lactic acid concentration curve. Therefore, the progress of the fermentation could be monitored equally efficiently by measuring either the decrease in concentration of lactose or the increase in concentration of lactic acid or ammonium ion.

Effect of pH. As shown in Fig. 2, the rate of utilization of lactose appeared to be better at pH 5.5 than at 5.0, 5.7, 5.9, and 6.1, especially in the first 16 h of fermentation. However, note that at the end of 24 h the residual lactose concentration is the same regardless of whether the fermentation was conducted at pH 5.5 or 5.0. At pH 7.0 there was little utilization of lactose, even at the end of 24 h.

Inoculum size. As shown in Fig. 3, rates of lactose utilization and ammonia uptake were similar with 10 and 20% inocula. With a 1% inoculum the rate of fermentation was considerably lower, especially in the first 12 h of

Fig. 1. Lactose utilization (○), lactic acid production (●), and ammonia uptake (△) during the fermentation of whey by L. bulgaricus at 43°C and pH 5.5.

Fig. 2. Effect of pH on the rate of utilization of lactose. Symbols: ○, pH 5.0; ●, pH 5.5; △, pH 5.7; □, pH 5.3; ■, pH 6.1; △, pH 7.0.

Fig. 3. Effect of inoculum level on lactose utilization and ammonia uptake. Inoculum levels: ■, 1%; ●, 10%; ○, 20%.
fermentation. However, note that at the end of 24 h the residual lactose concentration and the ammonia concentration were comparable with all three levels of inocula. We have obtained additional data which showed that 2.5 and 5% inocula gave as fast a rate of fermentation as those obtained with 10 and 20% inocula. No putrefactive spoilage was encountered in any of the fermentations.

Effect of addition of growth factors. Lactobacilli are nutritionally fastidious organisms and are known to require a number of growth factors (7, 32, 38). The effect of adding different sources of growth factors on whey fermentation, as measured by the rate of lactose utilization, was investigated (Fig. 4). Higher rates of fermentation were obtained when 0.1% CSL or 0.2% yeast extract (Difco) was added. At about 10 h after the initiation of the fermentation, control whey medium (without any growth factors added) contained 3.4% lactose, whereas the same medium supplemented with 0.1% CSL or 0.2% yeast extract contained only 1.24 and 1.7% lactose, respectively. Supplementation with 0.1% yeast extract also resulted in an appreciably higher rate of fermentation. Trypsinase (BBL), Casamino Acids (Difco), or malt extract (Difco), when added to whey at a 0.1% level (data not shown), had no stimulatory effect on fermentation, especially in the first 16 h of fermentation. At the end of 24 h, however, the differences in residual lactose concentrations were less marked.

Considering the stimulatory effect of CSL on fermentation and the fact that it is less expensive than yeast extract, the effect of different levels of CSL on fermentation was determined. As shown in Fig. 5, supplementation with 0.1% CSL appears to give a higher initial rate of fermentation, whereas with 0.4, 0.6, and 1% levels of CSL, the rate of utilization of lactose was somewhat lower. This suggested that higher levels of CSL contained a compound(s) at concentrations high enough to inhibit fermentation. The effect of CSL at levels lower than 0.1% has not been tested. Interestingly, the residual lactose levels at the end of 16 h were comparable in all fermentations, irrespective of the level of CSL added.

In other experiments a combination of 0.2% yeast extract and 0.1% CSL with or without mineral solution (composition and percentage as in reference 8) added to the whey medium did not increase the rate of fermentation any higher than that obtained with either yeast extract or CSL added individually.

Effect of initial lactose concentration. Although whey normally contains about 5% lactose, higher lactose whey can be obtained by concentrating the raw whey as it comes from the cheese vat. If such concentrated whey could be fermented efficiently, transportation and handling costs, the size of the fermentors, evaporation costs, and the cost per ton of FACW could be significantly reduced than when whey containing 5% lactose was used. Therefore, the effect of initial lactose concentration on fermentation was investigated. Figure 6 shows that at initial lactose concentrations up to 7%, the fermentation proceeded well, as determined by the rate of ammonia input with time. When 11.5% lactose whey was fermented, ammonia consumption during the fermentation was no greater than that observed with 7% lactose whey. An analysis of the lactose levels at the end of fermentation showed that only trace amounts of residual lactose (0.05 to 0.10%) were present when 3, 5.4, or 7% lactose whey was fermented, whereas the residual lactose level was close to 4% when whey with an initial
lactose concentration of 11.5% was fermented. These results suggested that lactose levels up to 7% in whey could be fermented efficiently and that lactose levels of 11.5% were fermented incompletely.

The incomplete fermentation observed with 11.5% lactose whey may be due, either wholly or in part, to the buildup of undissociated lactic acid and/or inhibition by the ammonium ion used for neutralizing the lactic acid. To find out more about the nature of this inhibition, we did an experiment in which ammonium lactate, sodium lactate, or calcium lactate was added to normal whey (containing 5% lactose) to give a final concentration of 5% (wt/vol) added lactate ion. The medium was inoculated as before, and the rate of utilization of lactose during the fermentation was measured. There was complete inhibition of fermentation as evidenced by the lack of utilization of lactose. In the control fermentor, without any added lactate, the fermentation proceeded normally. These results suggested that, irrespective of the cation involved, exogenous lactate, added at a 5% level at the beginning of the fermentation, completely inhibited whey fermentation. However, there was a distinct possibility that different cations (Ca$^{2+}$, Na$^+$, or NH$_4^+$) added in the above experiment may also have contributed to the inhibitions. Therefore, another experiment was conducted in which the amount of cation required to neutralize the 5% level of lactate ion was calculated and was added to the fermentor in the form of ammonium sulfate, sodium sulfate, or calcium sulfate. We found no significant inhibition of fermentation with any of the three cations. This again suggested that it was lactic acid and not the concentration of cation that played a primary role in inhibiting the fermentation when whey with a high lactose content was fermented. However, we do realize that these results are not conclusive, and it is possible that other interpretations may be equally valid.

Large-scale fermentations. The basic information obtained from the above series of experiments was utilized in scaling up fermentations to a pilot plant level. FACW, described above, was a thick, tan-colored, syrupy liquid and had a mild dairy flavor and salty taste. The compositions of unfermented whey, FAW, and FACW are given in Table 1. A comparison of normal whey and FACW shows that there is a 50- to 60-fold increase in crude protein and about a 100-fold increase in lactic acid content in FACW as compared with unfermented whey. Approximately 75 to 77% of the crude protein in FACW was in the form of ammonium lactate, 17% was in the form of whey proteins (lactalbumin and lactoglobulins), and the remaining 6 to 8% was contributed by the bacterial cells. Lactic acid was generally the only detectable acid produced during the fermentation. However, traces of acetic acid were occasionally observed. About 54% of the lactic acid in FACW is D(-)-lactic acid and 46% is L(+)lactic acid. FACW is stable and resistant to microbial spoilage at temperatures of 4, 25, 37, and 60°C for more than 12 months.

**DISCUSSION**

The results show that the batch fermentation of lactose in whey could be carried to near completion in 24 h or less by using *L. bulgaricus*. This is a considerable improvement over previous whey fermentation methodology, which in most cases required 42 to 72 h for the complete fermentation of lactose to lactic acid (4, 36; Czarnetzky, U.S. Patent 2,094,437, 1959). Also, the fermentation process described here is rather unique in that it is a means to an end rather than an end in itself; that is, lactose is converted to lactic acid primarily as a means to trap ammonium ion and thereby enrich the crude protein level in the product.

**Table 1. Composition of whey, FAW, and FACW**

<table>
<thead>
<tr>
<th>Component</th>
<th>Whey (g/100 g)</th>
<th>FAW (g/100 g)</th>
<th>FACW (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>5.0</td>
<td>0.10</td>
<td>1.0</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.5</td>
<td>5.10</td>
<td>49.0</td>
</tr>
<tr>
<td>Ammonia nitrogen</td>
<td>0.05</td>
<td>0.69</td>
<td>6.6</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>0.17</td>
<td>0.90</td>
<td>8.8</td>
</tr>
<tr>
<td>Crude protein</td>
<td>1.1</td>
<td>5.63</td>
<td>55.0</td>
</tr>
<tr>
<td>equivalent*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Crude protein equivalent is defined as total N × 6.25.*
It was previously reported that by using restrictive fermentation temperatures of 43 to 44°C, pH 5.5, and acid-tolerant organisms such as *L. bulgaricus*, unsterilized and unpasteurized whey could be fermented to produce lactic acid without any putrefactive spoilage (36, 44; Weisberg et al., U.S. Patent 2071368, 1937). This was the case in this study where several hundred batches of whey were fermented both in laboratory and pilot scale fermentors without encountering putrefactive spoilage. The lack of the requirement for sterilization or pasteurization of whey would reduce the operational costs involved in any industrial scale fermentation.

Our results indicate that the fermentation of whey proceeds at a faster rate at pH 5.5 but less efficiently at pH 5.7 to 6.1 or at 5.0. Our results are, thus, practically identical to those obtained by Finn et al. (18) for the fermentation of glucose by lactobacilli. These workers observed maximum bacterial densities at a pH of 5.2 to 5.6 and reduced bacterial densities at a higher or lower pH level. Luedeking and Piret (30) also obtained essentially similar results with batch fermentations of glucose conducted between pH 5.2 to 6.0. He reported a significant inhibition of fermentation at pH 4.8. In contrast with the above results, Kempe et al. (29) observed a pH optimum of 4.5 for the production of lactic acid from wheat grit mash supplemented with nutrient salts and yeast extract. They found that the conversion of sugar to lactic acid varied inversely with the pH of the fermentation. More recently, Keller and Gerhardt (28) observed lower residual lactose concentrations when the continuous fermentation of whey was conducted at pH 6.0 instead of pH 5.5. It is somewhat difficult to reconcile the differences in results obtained by different investigators, and we have no clear explanation for these discrepancies at this time.

To have successful fermentation with unsterilized carbohydrate-rich substrates, the inoculum size should be high enough to produce rapid fermentation and thus reduce the chances of contamination by undesirable bacteria. At the same time, from an economic standpoint, the inoculum should be small enough so as not to significantly increase the operational costs. In this study we found that a 2.5% inoculum produced just as rapid and contamination-free fermentation as did that obtained with a 10% inoculum. This is a considerably smaller-sized inoculum than that used by previous investigators (2, 4, 6, 36, 43; Czarnecky, U. S. Patent 2,094,437, 1969; Jansen, Dutch Patent 57848, 1945) and is one of the strengths of this process.

The results indicate that high-lactose whey is fermented incompletely under the particular set of experimental conditions used in this study. Our results suggest that the high concentration of lactic acid produced during the fermentation may be a primary factor responsible for the incomplete fermentation observed with 11% lactose whey. Whittier and Rogers (44) and others (19, 28, 37) have previously indicated that in a high-lactose whey medium, the concentration of undissociated lactic acid is the primary factor limiting the fermentation. In fact, it has been shown recently (Gerhardt, personal communication) that whey containing lactose concentrations up to 15 to 20% could be fermented efficiently if the lactate produced during the fermentation could be removed by dialysis. It must be emphasized, however, that further work needs to be done to understand more clearly the factor(s) limiting the fermentation of high-lactose whey.

To assess the value of FACW as a protein feed supplement to ruminant animals, extensive toxicity and feeding trials have been conducted with feedlot steers (13, 22-24, 37). Results of feeding trials showed that there are no palatability problems with FACW and there was no decrease in daily dry matter consumption, even when FACW was fed to the animals at two times the daily requirement for supplemental protein. Growth response and feed efficiency were superior to urea and comparable to soybean meal. Feedlot steers can consume at least twice as much FACW nitrogen as urea nitrogen before reaching toxic levels. More recently FACW has been shown to be equivalent to soybean meal as a supplemental nitrogen source for dairy cows (J. T. Huber, unpublished data). A preliminary cost analysis indicated that FACW could favorably compete with soybean meal as a cattle feed supplement, and the process is being commercially adopted now.

The process described has several obvious advantages. It is a simple and economical process for the fermentative recycling of waste whey into a nitrogenous feed supplement. There are no wastes generated in this process. The product is stable and nontoxic, thus eliminating the need for complicated storage equipment and prefeeding conditioning periods that are required for other nonprotein nitrogen products such as urea. The substrate used does not compete with man's foodchain. The product contains bacterial protein and milk proteins that, when separated from ammonium lactate, have a potential as a protein source for humans.

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