Utilization of Collagenous By-Products from the Meat Packing Industry: Production of Single-Cell Protein by the Continuous Cultivation of *Bacillus megaterium*

WAYNE A. BOUGH, W. L. BROWN, JULES D. PORSCHE, AND D. M. DOTY

*American Bacteriological and Chemical Research Corporation, Gainesville, Florida 32601, and Jules D. Porsche and Associates, Chicago, Illinois 60514*

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The conditions for continuous cultivation of *Bacillus megaterium* on a collagen-derived substrate (SP-100) were determined. The optimum conditions of temperature, pH, and dilution rate were 34 °C, pH 7.0, and 0.25/hr, respectively. Increasing the substrate concentration in plain tap water resulted in proportional increases in the productivity of cell mass from 0.6 g per liter per hr at 1% substrate to 1.8 g per liter per hr at 10% substrate; however, the protein content of the biomass decreased from 60 to 36%, and the protein yield decreased from 91 to 50% at substrate concentrations of 1 and 10%, respectively. These effects (decreases) were reversed up to 7.5% substrate by mineral supplementation of the medium. The productivity of biomass increased from 0.6 to 1.9 per liter per hr; the protein content of the biomass, from 43 to 54%; and the protein yield, from 60 to 93%, respectively, as the substrate concentration (with mineral supplementation of the medium) was increased from 1 to 7.5%. Spent medium could be refortified and recycled as often as five times. The amino acids in the substrate protein appeared to be utilized for growth and metabolism more or less uniformly. Analysis of the *B. megaterium* biomass indicated considerable enrichment of the essential amino acids and reduction of proline, glycine, and hydroxyproline as compared to the collagen-derived substrate. The Protein Efficiency Ratios obtained on the collagen-derived substrate (SP-100) and on the *B. megaterium* biomass, expressed as percentages of the casein reference protein, were 14 and 74%, respectively. Thus, considerable improvement in nutritional value was effected by bacterial conversion of the collagen-derived substrate into single-cell protein.

Collagen, the insoluble protein of connective tissue and of the hides and skins of most mammals, is the most abundant protein in animal tissue. It comprises approximately 30% of the total protein and accounts for approximately 6% of the body weight. Thus, a large amount of collagen is produced from animal slaughter operations in the United States where the total liveweight of animals slaughtered was 27.8 × 10⁶ tons in 1966 (14). The primary source of collagen is cattle hide, which is 7 to 8% of the body weight, and approximately 10⁶ tons of cattle hides are produced annually (16). Most are made into leather with minor quantities going into the production of gelatin and glue. Collagen is also an ingredient of meat meal and tankage, the production of which was approximately 1.4 × 10⁶ tons in 1957 (17).

The value of collagen as a human food or as a feed for nonruminant animals is limited because of its poor amino acid balance; collagen is low in methionine and contains no tryptophan (16). Accordingly, the protein efficiency ratio (PER) of gelatin derived from collagen is reported to be negative (15).

One approach to improving the nutritional value of collagen and utilizing collagenous by-products is by converting these materials into microbial biomass or single-cell protein. The
objective of this research was to produce microbial protein of high nutritional quality by using collagen-derived material for the primary substrate.

Continuous microbial fermentation techniques already have been investigated for the utilization of other agricultural and forestry by-products such as sugar cane bagasse (2), potato waste (9), soy whey (3), and newspaper (13). Processes have also been studied for the microbial utilization of gas oil (7), and acid-hydrolyzed municipal waste by yeasts (6). Table 1 presents a summary of the information on these various processes for production of single-cell protein and gives the reported productivity of biomass for each process. Clearly, the yeast fermentations have achieved the highest productivity which is 1.7 g per liter per hr. The higher productivity figure of 3.66 g per liter per hr for yeast as suggested by Meller (6) seems optimistically high and is discounted since it was not verified by experimental studies.

The present study presents the results of experiments on the utilization of collagen-derived substrate in the continuous culture of Bacillus megaterium for the production of single-cell protein. The productivity of this system exceeds the results reported for other continuous bacterial fermentations and equals those reported for the continuous cultivation of yeasts.

MATERIALS AND METHODS

Culture. A pure culture of B. megaterium obtained from Carolina Biological Supply Company, Burlington, N.C., item number 15-4900, was used in all the continuous culture experiments. Inocula were prepared by shake flask culture at 35 C in medium containing 5% (w/v) of the collagen-derived substrate. A 10-ml inoculum of a 5-hr log-phase culture was injected into the continuous fermentation vessel containing 2.3 liters of fresh medium.

Media. The collagen-derived substrate chosen for this study was SP-100, a product of Oscar Mayer and Co., Madison, Wis. This product, essentially the soluble-protein fraction remaining from the wet rendering process of pork operation, typically contained 4 to 6% moisture, 15-16% nitrogen, and 5-6% ash. SP-100 is obtained by partial hydrolysis and solubilization of collagen by steam and acid during the rendering process. An amino acid analysis of SP-100 conducted by WARF Institute, Inc., Madison, Wis., is shown in Table 2. The pH of a 5% solution of SP-100 was 6.1 to 6.2.

Except where mineral supplementation is indicated, the fermentation medium was composed of SP-100 in tap water.

The mineral supplement, where indicated, was adapted from the mineral components of the medium of Tannenbaum et al. (12) for the growth of B. megaterium (in milligrams per liter): KH$_2$PO$_4$, 1,000; MgSO$_4$·7H$_2$O, 200; CaCl$_2$, 15; FeCl$_3$·6H$_2$O, 3; ZnSO$_4$·7H$_2$O, 2; CuSO$_4$·5H$_2$O, 2; and MnSO$_4$·H$_2$O, 2. The mineral supplement was added to the solution of substrate in tap water prior to sterilization. The initial pH of the medium was adjusted to pH 6.9 upon entering the fermentation vessel.

Automatic pH control. During fermentation, the pH of the medium was controlled in the range of pH 6.9 to 7.1 by the automatic addition of 1 N NaOH to raise the pH and by either 1 N phosphoric acid when specified or sterile 50% glucose to effect a decrease in the pH of the culture. The automatic dual limit pH controller was set for a minimum time delay of 1.0 to 1.5 min between additions of sodium hydroxide or glucose.

Equipment. A magnetic drive fermentor (Virtis model 40-100) was used in all continuous culture experiments. The volume of the culture was maintained at 2.3 liters and agitation was 400 rev/min. The temperature of the fermentation was 34 C.

The control systems for automatic control of pH and foam production (Alton Electronics Co., Gainesville, Fla.) were adapted to the fermentor. Contact of foam with a probe of the antifoam controller resulted in the immediate operation of the antifoam stroke pump (series SV-500, Valcor Engineering Corp.). Silicone antifoam (10%, FD-62, Hodag Chemical Corp., Skokie, Ill.) was used.

Table 1. A summary of information on the productivity of continuous microbial fermentations

| Investigator       | Microorganism                          | Substrate                              | Productivity (g/liter/hr) |
|--------------------|----------------------------------------|----------------------------------------|---------------------------|
| Church and Nash    | Gliocladium deliquescentes             | Soy whey (protein and carbohydrate)   | 0.06                      |
| Updegraff (13)     | Myrothecium verrucaria (mold)          | Newspaper (carbohydrate)              | 0.11                      |
| Reiser (9)         | Candida utilis (yeast)                 | Potato waste (protein and carbohydrate) | 1.7                      |
| Munk et al. (7)    | C. lipolytica (yeast)                  | Gas oil (hydrocarbon)                 | 1.7                      |
| Meller (6)         | C. utilis (yeast)                     | Acid-hydrolyzed municipal waste       | 3.66 (Theoretical)        |
| Bewersdorf and Donálek (1) | Mixed bacterial culture (a)              | Methane (hydrocarbon)                 | 0.15                      |
| Callihan and Dunlap (2) | (b) Cellulomonas and Alcaligenes faecalis (bacteria) | Alkaline-treated (a) 0.03-0.096 |
|                    |                                        | Bagasse (carbohydrate)                | (b) 0.512                |

Calculated from batch data
The pumps for glucose and antifoam were connected to the fermentor with Teflon 909 hose covered with bronze-wing braid (Parker Hannifin, Wickliffe, Ohio), permitting steam sterilization of pumps and tubing.

The fermentor components, including the fermentation vessel, pH probe, pumps, and feed lines, were steam-sterilized by a procedure suggested by the Biolafitte Company of France (personal communication, May 7, 1971): (i) Each unit was steamed for 30 min. (ii) The steam was shut off and the vessel was aerated for 2 hr. (iii) Each unit was again steamed for 30 min.

Sterile medium was fed continuously into the fermentation vessel through autoclavable silicone tubing by a Variable Speed Masterflex Tubing Pump (Cole-Parmer, Chicago, Ill.).

The culture was aerated with compressed air sterilized by filtration through a column of sterile packed cotton. The aeration rate was 3 volumes of air per volume of culture medium per min.

**Definitions.** The dilution rate, expressed per hour, is the fraction of the culture volume which was replaced by fresh medium per hour of continuous cultivation.

The productivity, expressed as grams per liter per hour, is the dry weight of biomass produced per liter of culture medium per hour of continuous cultivation.

The protein content, expressed as percent, represents the amount of protein per 100 g (dry weight) of biomass. Protein was determined by Kjeldahl or biuret analyses as indicated.

The biomass concentration, expressed as grams per liter, is the dry weight of cells per liter of culture medium.

The protein yield, expressed as percent, is the dry weight of protein in biomass that was produced from 100 g (dry weight) of the collagen-derived substrate (SP-100) depleted or utilized during fermentation.

The nitrogen utilization ratio is the ratio of the amount of Kjeldahl nitrogen found in biomass to the amount of ammonical nitrogen found in spent medium and is used as a measure of the efficiency of substrate nitrogen utilization.

**Analytical measurements and yields.** The amount of substrate protein depleted (utilized) was determined by the differences between Kjeldahl analyses (N x 6.25) of the whole-culture medium and the spent medium; the latter was the supernatant fluid, which resulted from sedimentation of the bacterial cells at 22,000 x g for 10 min. The biomass pellet was suspended in distilled water and sedimented as before in a tared centrifuge tube. The tube and biomass pellet were dried overnight at 100 C for determination of the concentration of biomass as grams (dry weight) of cells per liter.

The calculation of "crude protein yield" was based upon the grams of crude protein (N x 6.25) found in biomass per gram of substrate protein utilized. The calculation of "protein yield" was based upon the grams of protein found in biomass by the biuret method of Herbert et al. (5) per gram of substrate protein utilized. These analyses were performed in duplicate on biomass pellets, which had been washed with distilled water and resedimented at 22,000 x g for 10 min. The dry weight concentration of biomass was determined on duplicate samples of the same culture medium taken for determination of the protein content by the biuret method.

Protein yields were determined on steady-state continuous fermentations. The values reported are averages obtained during 2 to 3 days of steady-state operation. The protein yield is the percentage of the amount of substrate protein depleted or utilized that was converted to biomass protein.

The population of viable cells, expressed as log number of cells per milliliter, was determined by a spread-plate technique. Samples of culture medium were diluted in sterile tap water. A 0.1-ml sample of appropriate dilution was spread on plates of standard methods agar (Difco) and incubated for 12 to 16 hr at 35 C.

**Recycling of refortified spent medium.** The effluent from the fermentor was collected in 12- to 15-liter batches in an ice bath. The bacterial cells were sedimented by centrifugation either with a Sorvall SS-3 continuous-flow centrifuge operated at 27,000 x g or with a Sharples continuous-flow electric centrifuge. The spent medium thus collected was refortified by adding 10% (0.5 g/liter) of the original

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### Table 2. Amino acid analyses of substrate, recycled spent medium, and biomass from the continuous cultivation of Bacillus megaterium

| Amino Acid | Grams per 16 g of nitrogen |
|------------|---------------------------|
|            | Substrate, SP-100<sup>a</sup> | Recycled<sup>c</sup> spent medium | Biomass<sup>b</sup> B. megaterium |
| Lysine     | 4.1 | 4.9 | 6.3 |
| Histidine  | 1.5 | 1.3 | 1.8 |
| Arginine   | 6.7 | 5.9 | 3.9 |
| Aspartic   | 6.5 | 7.7 | 7.4 |
| Threonine  | 2.3 | 1.9 | 3.2 |
| Serine     | 3.3 | 3.1 | 2.4 |
| Glutamic   | 14.3 | 9.6 | 16.9 |
| Proline    | 8.7 | 7.0 | 3.8 |
| Glycine    | 18.7 | 18.0 | 5.8 |
| Alanine    | 8.7 | 8.3 | 8.4 |
| Valine     | 2.8 | 2.3 | 4.2 |
| Methionine | 1.2 | Neg. | 3.2 |
| Isoleucine | 1.8 | 1.4 | 3.4 |
| Leucine    | 4.1 | 3.5 | 5.4 |
| Tyrosine   | 1.2 | 1.5 | 2.5 |
| Phenylalanine | 2.3 | 2.4 | 3.0 |
| Tryptophan | 0.13 | 0.15 | 0.52 |
| Hydroxyproline | 9.0 | 9.0 | 1.8 |
| Cystine    | 0.24 | 0.25 | 0.35 |

<sup>a</sup>The continuous cultures were fed 5% SP-100 in tap water at a dilution rate of 0.25/hr.

<sup>b</sup>Contained 11.5% N, 5.3% ash.

<sup>c</sup>Spent medium collected after four stages of recycling (cf. Fig. 6).

<sup>d</sup>Contained 11.5% N, 7.3% ash.
concentration of SP-100 substrate. The refortified spent medium was sterilized by autoclaving at 15 psi for 3 hr, cooled overnight, and then recycled as substrate for the continuous fermentation. The amount of protein utilized (depleted from the medium) was determined daily by Kjeldahl analysis (N × 6.25) as the difference between the amount of substrate protein fed and the amount recovered in the spent medium. The total amount of glucose used to control the pH was also determined to calculate the total yield of the fermentation.

**Amino acid analyses.** Samples for amino acid analyses were sent to WARF Institute, Inc., Madison, Wis. These were run on an amino acid analyser by the method of Spackman et al. (10). Hydroxyproline was determined by the colorimetric method of Neuman and Logan (8). Tryptophan and cystine were determined by the microbiological method of Henderson and Snell (4).

**PER.** Samples of the collagen-derived protein substrate, SP-100 (90.4% protein, 5.3% ash), and *B. megaterium* biomass (44.3% protein by biuret, 7.3% ash) were sent to Kel Farm, Alachua, Fla., for determination of PER, the average ratio of total weight gain to total protein consumption. The biomass sample had been dried overnight in a forced-air oven at 100 C and ground in a Wiley mill. These samples were from the same lot as those sent for amino acid analysis.

Weanling (24-day-old) male Sprague-Dawley rats, obtained from Charles River Breeding Laboratories, Wilmington, Mass., were divided into groups of 10 animals each. Animals were housed in individual screen-bottom cages and fed water and test diet ad libitum.

Body weights and food consumption data were collected at weekly intervals throughout a 4-week test period. The PER was calculated for each group.

Test rations were formulated as follows: reference protein or samples, to contain 10% protein; cottonseed, 8%; salt mixture USP XIV, 4%; vitamin mixture, 1%; alphacel, 1 to 7%; sucrose, 35%; corn starch, 35%. The ANRC casein reference protein was obtained from Sheffield Chemical Co., Union, N.J. The vitamin mixture provided the following components per 100 g ration: vitamin A, 2,000 IU; vitamin D, 200 IU; vitamin E, 10 IU; menadione, 0.5 mg; choline chloride, 200 mg; p-aminobenzoic acid, 10 mg; inositol, 10 mg; niacin, 4 mg; calcium p-pantothenate, 4 mg; riboflavin, 0.8 mg; thiamine-hydrochloride, 0.5 mg; pyridoxine-hydrochloride, 0.5 mg; folic acid, 0.2 mg; biotin, 0.04 mg; vitamin B12, 0.003 mg.

**RESULTS AND DISCUSSION**

**Evaluation of organisms.** Growth of various microorganisms on slants of collagen agar and SP-100 agar at 37 C indicated that *Bacillus subtilis*, *B. megaterium*, *Torulopsis kefir*, and a *Rhodotorula* sp. (Presque Isle Microbiological Associates, Presque Isle, Pa.) were likely prospects for production of biomass on the collagen-derived medium SP-100. These organisms were further screened in shake flask experiments. Table 3 shows the concentration of biomass and the final pH of the medium resulting from growth of these organisms on increasing concentrations of the SP-100 substrate (1, 2, and 5%) in tap water. The highest concentration of biomass, 9.2 g/liter, was observed with the growth of *B. megaterium* on 5% SP-100 in tap water. These shake flask experiments did not employ pH control or glucose supplementation.

A *Pseudomonas* sp., isolated from soil at a meat by-product rendering plant, grew quite well on collagen and SP-100, and was nontoxic to rats when fed as the sole source of protein for 6 days. However, the cell size of this organism was small and the maximum stationary-phase biomass concentration was only 20 to 30% of the amount obtained with *B. megaterium*. Growth of mixed cultures of the *Pseudomonas* sp. and *B. megaterium* under batch conditions resulted in a 50% reduction in the stationary-phase biomass concentration as compared to growth of pure cultures of *B. megaterium* (unpublished data, American Bacteriological Chemical Research Corp.).

On the basis of these batch experiments and on considerations such as higher digestibility and biological value as compared to yeast (11), *B. megaterium* was chosen for further study under conditions of continuous cultivation.

**Growth conditions.** Batch experiments on pH control indicated that a higher concentration of biomass could be obtained if glucose rather than phosphoric acid was added to control the rise in pH. Table 4 shows similar results obtained under conditions of continuous culture. The productivity of the fermentation was increased from 0.2 to 0.3 g per liter per hr by adding glucose rather than phosphoric acid. Likewise the crude protein yield was increased from 37 to 65%. Obviously, in controlling the pH, glucose was being utilized as a supplemental carbon source by the *B. megaterium* culture, which accounts in part for the increased productivity and protein yield. Another effect of controlling the pH of the continuous fermentation was that the nitrogen utilization ratio was increased from 0.4 to 1.5 by changing from phosphoric acid to glucose, respectively, as a means of pH control. Less ammonia was excreted into the medium when glucose supplementation was employed.

Growth of *B. megaterium* in batch on 5% SP-100 under controlled conditions demonstrated that the optimum temperature was 34 C and the optimum pH was approximately 7.0 as shown in Fig. 1 and 2, respectively. The pH
TABLE 3. Production of biomass on a collagen-derived substrate (SP-100) by various organisms

| Organism            | 1% SP-100 | 2% SP-100 | 5% SP-100 |
|---------------------|-----------|-----------|-----------|
|                     | Final pH  | Biomass   | Final pH  | Biomass   | Final pH  | Biomass   |
|                     |           | dry wt    |           | dry wt    |           | dry wt    |
| Bacillus megaterium | 8.6       | 1.6       | 8.9       | 4.0       | 9.0       | 9.2       |
| B. subtilis         | 8.2       | 0.1       | 8.4       | 1.0       | 8.2       | 2.7       |
| Torulopsis kefyr    | 6.5       | 0.2       | 6.9       | 0.8       | 7.3       |           |
| Rhodotorula         | 6.6       | 0.3       | 6.1       | 0.4       | 6.9       | 2.2       |

*a Grown in shake flask culture, 100-ml culture volume, at 37°C for 24 hr. These results were derived from batch experiments separate from those described in Materials and Methods.

TABLE 4. Effects of controlling the pH of the continuous fermentation with glucose rather than phosphoric acid during growth of Bacillus megaterium on 1% SP-100

| Dilution rate/ hr | pH Control agent | Productivity (g/liter/hr) | Crude protein yield (%) | Nitrogen utilization ratio* |
|-------------------|------------------|---------------------------|-------------------------|----------------------------|
| 0.17              | Phosphoric acid, 1 N | 0.2                       | 37                      | 0.4                        |
| 0.17              | Glucose, 50%      | 0.3                       | 65                      | 1.5                        |

*a The nitrogen utilization ratio is defined as the ratio of the amount of Kjeldahl nitrogen found in biomass to the amount of ammonical nitrogen found in the spent medium.

![Fig. 1. Determination of optimum temperature. The relationship of temperature to biomass concentration at 1 and 2 days of batch growth of B. megaterium on 5% SP-100 at pH 7.0.](image)

![Fig. 2. Determination of pH optimum. Relationship of pH to biomass concentration at 1 and 2 days of batch growth of B. megaterium on 5% SP-100 at 34 C.](image)

of these fermentations was controlled to within 0.1 unit of the present pH value by the automatic addition of 50% glucose or 1 N sodium hydroxide. The dry weight concentration of biomass after 24 hr of fermentation at pH 7.0 and 34 C was 15.3 g/liter as compared to 9.2 g/liter for the growth of B. megaterium on 5% SP-100 without pH control and glucose supplementation.

Optimum dilution rate and nitrogen balance. The optimum dilution rate for the continuous culture of B. megaterium on SP-100 was estimated from the results of a series of experiments where the dilution rate of a 1%
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SP-100 medium was varied from 0.17 to 0.29/hr. The dilution rate is the fraction of the culture volume replaced by fresh medium per hour of continuous feeding. Figure 3 shows the effects of changing the dilution rate on the crude protein yield, productivity, and biomass concentration. The values plotted are averages of daily measurements made during 2 to 3 days of steady-state continuous fermentation. The values for crude protein yield are excessively high because protein in biomass was estimated by Kjeldahl analysis (N x 6.25) and thus includes nitrogen contained in nucleic acids, proteins, cell walls, and other organic nitrogen compounds.

The highest productivity shown in Figure 3 was observed at a dilution rate of 0.25/hr. The crude protein yield was also quite high; thus, a dilution rate of 0.25/hr appeared to be optimum for the production of single-cell protein (biomass) by this system.

The nitrogen utilization ratio, shown in Table 5, was also observed to be maximum at a dilution rate of 0.25/hr, which is consistent with the results discussed above on the maximum productivity and protein yield. It appears that the efficiency of substrate nitrogen utilization was greatest at a dilution rate of 0.25/hr. The amount of nitrogen excreted into the spent medium increased with dilution rates lower or higher than 0.25/hr as shown in Table 5. A mass balance of nitrogen during the continuous fermentation of 1% SP-100 fed at a dilution rate of 0.25/hr revealed that 19.8% of the total nitrogen was found in biomass, 7.1% in ammonia in spent medium, and 73.1% in organic compounds (unused substrate) in spent medium. All of the nitrogen was accounted for by the spent medium and biomass.

**Substrate concentration and mineral supplementation.** Using the previously determined optimum dilution rate of 0.25/hr, the effects of increasing the substrate concentration on the productivity and protein yield were studied in a series of experiments. The substrate was made up in tap water; no mineral

![Figure 3](image-url)

**Fig. 3.** Determination of the optimum dilution rate of the continuous cultivation of B. megaterium. Effects of changing the dilution rate of a 1% SP-100 medium on the crude protein yield, productivity of biomass, and biomass concentration.
supplement was added. Figure 4 shows the results obtained. The productivity of biomass increased from 0.6 g per liter per hr at 1% SP-100 to approximately 1.8 g per liter per hr at 10% SP-100. Foaming was critical at substrate concentrations greater than 5%. The protein content of the biomass decreased from 60 to 36% and the protein yield decreased from 90 to 50% at substrate concentrations of 1 and 10% SP-100, respectively. The highest substrate concentration that could be used without resulting in excessive foam production was 5% SP-100, which supported a biomass productivity of approximately 1.6 g per liter per hr.

The effects of mineral supplementation had been investigated in shake-flask experiments and no effect on the biomass concentration at a substrate concentration of 5% SP-100 was noted. However, Fig. 5 shows the positive effects of mineral supplementation on the fermentation at increasing substrate concentrations. The productivity was improved approximately 5 to 10% relative to the results shown in Fig. 4 for unsupplemented medium containing only substrate and tap water. The productivity of biomass obtained as a result of mineral supplementation of 5% SP-100 in tap water was approximately 1.7 g per liter per hr as compared to 1.6 g per liter per hr obtained without mineral supplementation. As before, foam production from cultures growing on substrate concentrations higher than 5% SP-100 was difficult to control in this system.

The most striking effects of mineral supplementation were that the protein content of the biomass and the protein yield increased with increasing substrate concentrations up to 7.5% SP-100 as shown in Fig. 5. However, protein content and protein yield decreased with increasing substrate concentrations when not supplemented with minerals as shown in Fig.

### Table 5. Effect of dilution rate on the nitrogen-utilization ratio during continuous fermentation of 1% SP-100—a measure of nitrogen excreted into spent medium during growth on a protein substrate

| Dilution rate/hr | Biomass concn. (g/liter) | Nitrogen utilization ratio |
|------------------|------------------------|----------------------------|
| 0.17             | 1.7                    | 1.5                        |
| 0.23             | 2.5                    | 2.0                        |
| 0.29             | 2.4                    | 2.9                        |

![Graph](image_url)

**Fig. 4.** Effects of increasing substrate concentrations (without mineral supplementation) on the protein yield, productivity of biomass, and protein content of biomass in the continuous cultivation of B. megaterium. The medium consisting only of the SP-100 substrate in tap water was fed at a dilution rate of 0.25/hr.
4. The maximum protein yield was 93% and the protein content of the biomass was 54% at a substrate concentration of 7.5% with mineral supplementation. The protein yield and protein content of the biomass at a substrate concentration of 10% were 86 and 43%, respectively, which represents a decrease from the maximum observed at 7.5% SP-100 with mineral supplementation.

The highest substrate concentration that could be used with mineral supplementation without resulting in excessive foam production was 5% SP-100, which supported a biomass productivity of approximately 1.7 g per liter per hr. The biomass produced had a protein content of approximately 53%. The protein yield for the conversion of substrate protein to biomass protein was approximately 87%.

**Recycling of refortified spent medium.** The effects of recycling refortified spent medium without mineral supplementation in the continuous cultivation of *B. megaterium* is shown in Fig. 6. The concentration of biomass resulting from growth on this spent medium decreased steadily as the number of stages of recycling increased. Accordingly, the productivity decreased from 1.7 to 1.0 g per liter per hr from the 1st to the 10th day, respectively.

The total amount of biomass collected was 504 g, and it contained 260 g of protein by the biuret method of Herbert et al. (5). The total amount of substrate protein used was 629 g; thus, an overall protein yield of 41% was achieved for the conversion of substrate protein into biomass protein. The total amount of glucose used for pH control during the fermentation was 450 g; thus, an overall cell yield of 47% was achieved for the conversion of substrate protein and glucose into bacterial cells or biomass.

In experiments such as those shown in Fig. 1 to 5, where fresh substrate was fed throughout and steady-state levels of biomass concentration and productivity were observed, the biomass concentration did not fluctuate more than 10%. Thus, the decrease in biomass concentration and productivity observed in Fig. 6 is attributed to the adverse effects of repeated
autoclaving and recycling of refortified spent medium as a growth substrate. Nevertheless, the results shown in Fig. 6 do illustrate that spent medium can be recycled and used more than once. Recycling of spent medium is important in minimizing the waste load from a continuous process for the production of single-cell protein.

**Amino acid analyses.** Table 2 shows amino acid analyses of the SP-100 substrate, recycled spent medium, and biomass. The biomass was collected from several continuous culture experiments where a substrate concentration of 5% SP-100 in tap water was fed at a dilution rate of 0.25/hr. The presence or absence of the mineral supplement was a variable in these experiments. Also, the biomass collected during recycling of refortified spent medium (see Fig. 6) was included in this collection of bacterial cells. This same lot of biomass was used in a rat feeding study to determine the PER.

The amino acid analysis of the spent medium obtained after four stages of recycling (cf. Fig. 6) is shown in Table 2 for comparison with the amino acid profile of the SP-100 substrate. The amino acids contained in the substrate protein appeared to be utilized for growth and metabolism more or less uniformly since no particular amino acids accumulated in the spent medium during recycling. All of the amino acid residues in the SP-100 protein appear to be of similar collective value as a substrate for the production of biomass.

The amino acid profile of the *B. megaterium* cells shown in Table 2 shows considerable evidence of enrichment over the substrate profile. The concentration of three amino acids typically high in collagen were significantly reduced when the SP-100 substrate is compared to the biomass: proline, from 8.7 to 3.8 g/16 g of N; glycine, from 18.7 to 5.8 g/16 g of N; and hydroxyproline, from 9.0 to 1.8 g/16 g of N, respectively. The concentrations of essential amino acids in the *B. megaterium* biomass were all increased in comparison to the substrate. Notably, the isoleucine concentration was increased from 1.8 to 3.4 g/16 g of N; va-
line, from 2.8 to 4.2 g/16 g of N; tryptophan, from 0.13 to 0.52 g/16 g of N; and lysine, from 4.1 to 6.3 g/16 g of N by the conversion of SP-100 substrate into biomass. Accordingly, the nutritional value of the *B. megaterium* biomass was, as expected, considerably enhanced over that of the collagen-derived substrate.

**PER.** The results of a 4-week rat feeding study to determine the PER of the collagen-derived substrate, SP-100, and *B. megaterium* biomass confirmed the superior nutritional value of the *B. megaterium* protein, as shown in Table 6. The PER values of the collagen-derived substrate (SP-100) and of the *B. megaterium* biomass, expressed as percentages of the casein reference protein, were 14 and 74%, respectively.

The low PER of the SP-100 substrate, as in the case of collagen discussed previously (15, 16), can be attributed to its poor amino acid profile. The amino acid analyses shown in Table 2 indicate that the collagen-derived substrate was particularly deficient in valine, methionine, isoleucine, tyrosine, and tryptophan. The PER obtained on the *B. megaterium* biomass reflects the increased content of essential amino acids, as discussed previously.

This study has shown that microbial protein of high nutritional quality can be produced from a collagen-derived material as the primary substrate by the continuous cultivation of *B. megaterium* for the production of single-cell protein.

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**LITERATURE CITED**

1. Bewerdorff, M., and M. Dostálek. 1971. The use of methane for production of bacterial protein. Biotechnol. Bioeng. 13:49-62.

2. Callihan, C. D., and C. E. Dunlap. 1971. Construction of a chemical-microbial pilot plant for production of single-cell protein from cellulotic wastes, p. 96 and 99. U.S. Environmental Protection Agency. Sup. of Documents, U.S. Government Printing Office, Washington, D.C.

3. Church, B. D., and H. A. Nash. 1970. Use of fungi imperfecti in waste control, p. 68. Water Pollution Control Research Series, 12060 EHT 07/70; Federal Water Quality Administration. Sup. of Documents, U.S. Government Printing Office, Washington, D.C.

4. Henderson, L. M., and E. E. Snell. 1948. A uniform medium for determination of amino acids with various microorganisms. J. Biol. Chem. 172:15-29.

5. Herbert, P., P. J. Phippe, and R. E. Strange. 1971. Chemical analysis of microbial cells, p. 244-249. In J. R. Norris and D. W. Ribbons (ed.). Methods in microbiology, vol. 5B. Academic Press Inc., New York.

6. Meller, F. H. 1969. Conversion of organic solid wastes into yeast: an economic evaluation, p. 15. Public Health Service Publication No. 1909. Sup. of Documents, U.S. Government Printing Office, Washington, D.C.

7. Munk, V., M. Dostálek, and O. Volfovká. 1968. Cultivation of yeast on gas oil. Biotechnol. Bioeng. 11:383-391.

8. Neuman, R. E., and M. A. Logan. 1950. The determination of hydroxyproline. J. Biol. Chem. 184:299-306.

9. Reiser, C. O. 1954. Torula yeast from potato starch wastes. Ag. Food Chem. 2:70-74.

10. Spackman, D. H., S. Moore, and W. H. Stein. 1958. Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. 30:1190-1206.

11. Tannenbaum, S. R. 1968. Factors in the processing of single-cell protein, p. 343-352. In R. I. Mateles and W. R. Tannenbaum (ed), Single-cell protein. The M.I.T. Press, Cambridge, Mass.

12. Tannenbaum, S. R., R. I. Mateles, and C. R. Capco. 1966. Processing of bacteria for production of protein concentrates, p. 254-290. In World protein resources, Advances in Chemistry Series 57. American Chemical Society, Washington, D.C.

13. Updegraff, D. M. 1971. Utilization of cellulose from waste paper by *Myrothecium verrucaria*. Biotechnol. Bioeng. 13:77-97.

14. U.S. Department of the Interior. 1967. The cost of clean water, vol. III. Industrial waste profiles No. 8: meat products, p. 3. Sup. of Documents, U.S. Government Printing Office, Washington, D.C.

15. Vincent, W. A. 1969. Algae for food and feed. Process Biochem. 4(6):45-47.

16. Whitmore, R. A., H. W. Jones, W. Windus, and J. Naghski. 1970. Preparation of hide collagen for food. J. Amer. Leather Chemists Ass. 65:382-388.

17. Wilder, O. H. M. 1960. By-product feeds, p. 403. In American meat institute foundation, the science of meat and meat products. W. H. Freeman and Company, San Francisco.

**Table 6. Protein efficiency ratios of Bacillus megaterium protein and the collagen-derived substrate (SP-100)**

| Protein source | Protein intake (g/day) | Body-weight gain (g/day) | PER, uncorrected | % of casein |
|----------------|------------------------|--------------------------|------------------|------------|
| Casein         | 19.02                  | 3.91                     | 2.06             | 100        |
| Substrate (SP-100) | 8.46                  | 0.24                     | 0.28             | 14         |
| *B. megaterium* | 10.95                  | 1.66                     | 1.52             | 74         |