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The effect of whey protein hydrolyzates on the lactic acid fermentation

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SUMMARY

The batch fermentation of whey permeate to lactic acid was improved markedly by the addition of enzyme-hydrolyzed whey protein. Acid concentrations greater than 90 g/l were achieved at a productivity of 4.3 g/l per h and a 98% substrate use. Cell mass concentration reached 6 g/l. The acid productivity achieved is somewhat higher than that typical for fermentation of whole whey. The process economics, based on in-house hydrolyzate preparation, look promising. Presented in this paper are the experimental results showing the effects of hydrolyzate concentration on acid and cell mass production.

INTRODUCTION

The permeate stream resulting from the whey protein recovery process is high in lactose and presents a significant waste treatment problem. One means of permeate disposal is via the fermentation to produce lactic acid. However the acid, a product of cell growth and maintenance metabolism, inhibits the fermentative microorganism, *Lactobacillus* sp., resulting in slow permeate fermentation rates and incomplete substrate utilization.

Additives such as yeast extract and corn steep liquor (CSL) have been used to enhance cell growth and accelerate fermentation rates [8,12,18], but the concentrations required are high, and the results are variable. In addition, the inconsistent quality of CSL and the high cost of yeast extract make these supplements undesirable.

Cell growth also is stimulated by various peptides which supply growth-limiting amino acids [2–4,13] that may not be transported readily into the cell as free residues [1,5,9,14,15]. Because these peptides must be transported actively [3], their bioactivity may be a function of peptide molecular weight – not just sequence and/or composition [19].

The purpose of this work was to determine the feasibility of improving the lactic acid fermentation

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of whey permeate by using whey protein hydrolyzate to supply such peptides. To make the study as realistic as possible, we used an industrial-grade enzyme as described previously [6] and components and operating conditions that would be found in a 'typical' commercial whey fermentation plant (see Fig. 1). We address here primarily our results for a hydrolyzate having an average molecular weight (AMW) of 700. Preliminary screening [6] had shown this molecular weight to be optimal for acid productivity.

MATERIALS AND METHODS

Hydrolyzate preparation

Reverse-osmosis (RO)-concentrated whey and RO-ultrafiltered whey permeate were obtained from Roy's Dairy (Monroe, WI). All whey products were pH-adjusted to 7.2 with concentrated KOH then pasteurized at 78°C for 30 min. Pasteurized products were stored frozen for subsequent use.

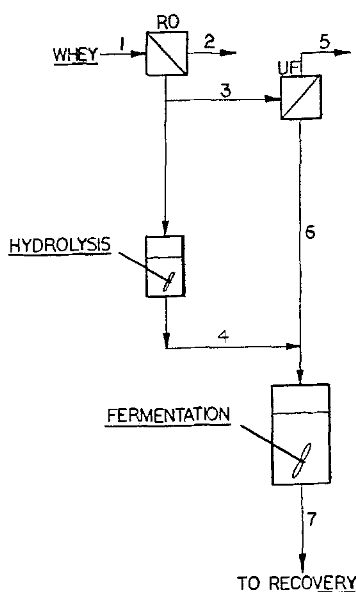

Fig. 1. Hydrolysis fermentation process. Stream composition: (1) whey: 50 g/l lactose, 8.3 g/l protein; (2) water: to waste; (3) 90 g/l lactose, 15 g/l protein; (4) as (3), hydrolyzed; (5) 500 g/l protein to market; (6) 90 g/l lactose, 0.5 g/l protein; (7) cell mass, protein, lactic acid. UF, ultrafiltration.

A protein hydrolyzate of AMW 700 was prepared by hydrolyzing pasteurized whey protein with the bacterial endoprotease HT Proteolytic (Miles Labs, Elkhart, IN). The protein was treated with 1000 units/l of protease, and was incubated at 50°C for 420 min. Concentrated KOH was used to control the pH at 7.2.

The protease was inactivated by adjusting the hydrolyzate pH below 4.0 with concentrated HCl. Insoluble matter was removed by membrane filtration (0.2 μ Millipore).

Hydrolyzate AMW was estimated according to the method of Myers et al. [11]:

$$\mathrm{AMW} = \frac{\text{total } \alpha\text{-amino nitrogen} \cdot 120}{\alpha\text{-amino nitrogen generated during hydrolysis}} \quad (1)$$

where total α-amino nitrogen [16] is given by

$$\alpha\text{-amino nitrogen} = \frac{\text{total nitrogen}}{1.31 \text{ nitrogen/amino acid}} \quad (2)$$

and 120 = AMW of an amino acid residue. Total nitrogen was determined by the Kjeldahl test (by ASW Consultants, Allentown, PA) and hydrolyzate α-amino nitrogen by the ninhydrin assay [17].

Seed development

One milliliter of *Lactobacillus bulgaricus* culture (Chris Hansen's Lab, Milwaukee, WI) was inoculated into 9 ml of sterile, 10% skim milk broth and incubated at 45°C until the milk clotted ($\approx$6 h). Fifty milliliters of the clotted skim milk culture was inoculated into 450 ml of pasteurized whey permeate. The seed culture was then pH-adjusted to 5.5 with KOH, N_2-blanketed and incubated at 45°C for 2 h. Agitation was provided by magnetic stirring.

Fermentation

A 10% seed inoculum was used in all fermentations. One liter fermentations were conducted at 45°C; the pH was controlled at 5.5 by automatic addition of 18% NH_3. The fermentors were N_2-blanketed to insure anaerobiosis, and agitation was provided by magnetic stirring. The compositions of the hydrolyzate/permeate broths tested are presented in Table 1.

Table I

Media compositions

Fermentation designation (%)	Permeate (ml/l)	Hydrolyzate (ml/l)
0	1000	0
25	750	250
50	500	500
75	250	750
100	0	1000

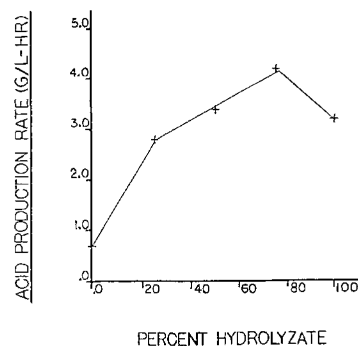

Fig. 2. Overall acid productivity as a function of hydrolyzate concentration.

Analytical

Cell mass was determined by filtering the broth through predried, tared 0.2 μ filters, washing with 0.05 M phosphate buffer (pH 6.0) and water, then drying the filters at 100°C for 24 h. Lactic acid and protein assays were conducted on the filtered broth.

Lactic acid analyses were performed on a Beckman HPLC (model 331) equipped with a refractive index detector and peak integrator. The assays were conducted according to the procedure of Ohleyer et al. [12]. Lactose was determined by the dinitrosalicylic acid reducing sugar assay [10], and protein was determined by the Lowry assay [7].

RESULTS AND DISCUSSION

Fermentation studies

By supplementing permeate with whey protein hydrolyzate, overall acid productivity was increased nearly 7-fold over that obtained with pure whey permeate (see Fig. 2). In addition, acid production rates similar to that of whole whey (4 g/l per h) were achieved by supplementing the permeate to 75% with the hydrolyzate. Clearly, as a fermentation of pure hydrolyzate is comparable to a whole whey fermentation on a gram protein/gram sugar basis, approximately 30% of the whole whey protein may be marketed without sacrificing acid production rates.

The improved fermentation performance can be correlated directly with the effect of hydrolyzate concentration on final cell mass obtained. Fermentation results indicate that the whey protein hydrolyzate supplies the complex nitrogen source required for cell growth: Fig. 3a illustrates that cell mass increases linearly with increasing initial hydrolyzate concentration. Also, Fig. 3b shows specifically that in all fermentations (except 100%) the total cell mass produced is a function of total protein consumed; the yield coefficient ($Y_{x/pr}$) is 1.0 g cell/g protein, and the correlation coefficient is 0.997. This indicates that the fermentation is nitrogen-limited, and that the nitrogen in readily assimilable form is supplied by the protein hydrolyzate. In addition, the lactose concentration measured at the time cell growth ceased was more than 100 times the reported Monod constant for lactose [18]. This shows that, for these fermentations, the limiting substrate for cell growth is protein alone. The decrease at 100% hydrolyzate concentration is believed to be due to a metabolic shift caused by very high nitrogen concentration or by some inhibitor which begins to cause significant effects only at very high concentration. The effect must be investigated further to determine the exact cause.

The economics of the proposed process indicate that using the hydrolyzate as a supplement is cost-effective if 40% of the whey protein side stream can be marketed [6] (based on the average composition of dairy whey as seen in industry). The economics might be improved further if enzyme costs are reduced. For example, use of an immobilized enzyme may be advantageous. This and other process alternatives remain to be evaluated.

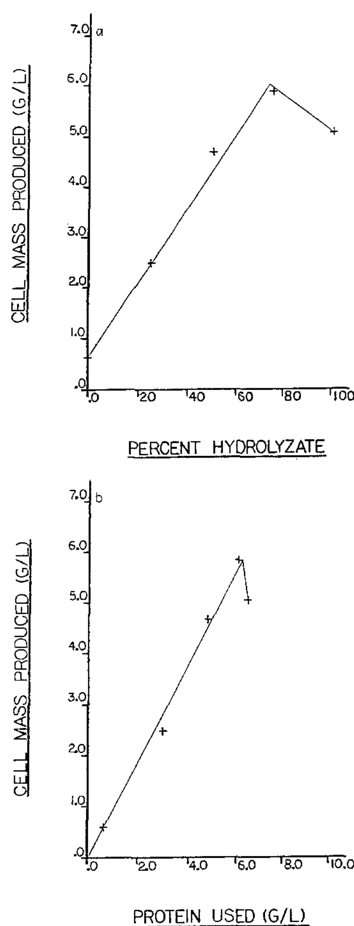

Fig. 3. (a) Total cell mass produced as a function of initial hydrolyzate concentration. (b) Total cell mass produced as a function of total protein used.

CONCLUSIONS

(1) Whey protein hydrolyzate can increase markedly the productivity of the batch lactic acid fermentation of whey permeate. It appears to have the potential of being a convenient, economic additive. (2) The hydrolyzate supplies growth-limiting peptides. (3) The kinetics of the fermentation are dependent on the concentration of the hydrolyzate. (4) There have been several reports [8,12,18] of continuous fermentation giving much higher lactic acid productivities than possible by batch fermentation, but lower concentrations and significant operating problems tend to outweigh the productivity advantage. Whether use of the hydrolyzate can help to overcome these problems remains to be seen.

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