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Ammonium lactate from deproteinized alfalfa juice by *Streptococcus faecium*

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SUMMARY

Deproteinized alfalfa juice is a by-product of the mechanical fractionation of alfalfa to obtain protein. In this work the juice was used as the substrate for the production of ammonium lactate (L-lactic acid) by a strain of *Streptococcus faecium*. Batch fermentation with a constant pH of 5.8 gave 27.2 g/l of lactic acid (90% conversion and 1.1 g/l/h productivity) and 6×10^{12} cells/l after 24 h. Semicontinuous fermentation allowed the conversion of 3-times the volume of deproteinized juice after 44 h, finally giving 29.7 g/l of ammonium lactate (99% conversion and 2.5 g/l/h productivity) and $4–6 \times 10^{12}$ cells/l.

INTRODUCTION

Different technologies have been used to fractionate alfalfa [2,5], to obtain a pressed fiber cake suitable for feeding ruminants and a juice from which to extract protein suitable for human alimentation [14,15]. The by-product of the process is a deproteinized juice with a high BOD/COD level requiring intensive treatment before discharge as waste. The economic advantage of fractionating alfalfa depends, in part, on finding a commercial use for the resultant deproteinized juice [6,9,17]. Previous studies indicated that the deproteinized juice, supplemented with phosphate or sulphate salts, could be used for the production of *Candida* sp. biomass [11,12].

In this work, use of the deproteinized juice as culture medium for fermentative production of ammonium lactate by a bacterium is examined. Ammoniated salts of short-chain organic acids have been shown to be superior to urea and comparable to soybean meal as a nitrogenous feed supplement for ruminants [4]. Lactic acid bacterial biomass is useful in animal feedstuff as a growth promoter [1,13].

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MATERIALS AND METHODS

Microorganism

A strain of *Streptococcus faecium* isolated from fermented vegetables was used in this study. This microorganism was routinely maintained in MRS broth [8], incubated at 28°C for 24 h, and stored at 10°C.

Deproteinized alfalfa juice

The deproteinized alfalfa juice (DAJ) was obtained by the mechanical extraction process shown in Fig. 1.

The juice, after pH correction to 6.2 with ammonium hydroxide, was employed directly as the culture medium.

The total sugar content of the juice, expressed as glucose, was determined by the phenol-sulphate method [3]. Reducing sugars were determined by the Somogyi procedure [16] and sugar composition by gas-liquid chromatography according to Oades [10]. Protein was evaluated according to Lowry et al. [7].

Fermentation conditions

Fermentation was carried out in a 14-liter fermenter (Chemap, Volketswil, Switzerland) with a working volume of 10 litres equipped with a pH control unit. A 2% (v/v) inoculum from a 36-h culture of *S. faecium* grown on DAJ was used; the culture was incubated at 28°C with 20 rpm impeller speed. Two different conditions of batch fermentation were tested: (a) uncontrolled pH; (b) pH controlled at 5.8 by automatic addition of ammonium hydroxide solution (10%).

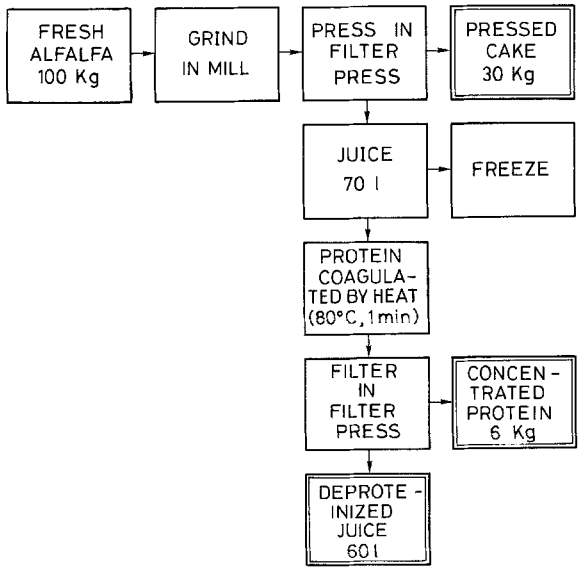

Fig. 1. Unit operation for the fractionation of alfalfa.

A three-stage semicontinuous fermentation with controlled pH was carried out with culture times of: 24 h, first stage; 10 h, second stage; 10 h, third stage. At 24 h (end of first stage) the impeller was stopped and cells were allowed to settle; 8 litres of supernatant were then decanted from the fermenter. The 2-liter residual culture, with 1.3×10^{13} cells/l, was used as the inoculum of the second stage of fermentation, 8 litres of fresh deproteinized juice being added. The procedure for the second stage was also employed for the third stage.

Analytical procedures

Samples of culture broth were taken at regular intervals and centrifuged at $2000 \times g$ for 20 min at 4°C. The supernatants were analyzed for the presence of L-lactic acid and residual sugars were determined as reducing sugars expressed as glucose. The L-lactic acid concentration was determined enzymatically according to the L-lactic kit (Boehringer, Mannheim, F.R.G.).

RESULTS

Deproteinized juice composition

A typical analysis of the unit operation products by which DAJ is produced is shown in Table 1.

The sugar composition of DAJ (% of total sugars) is as follows: rhamnose 4.6; fucose 1.5; ribose 0.6; arabinose 10.0; xylose 3.0; mannose 3.0; galactose 19.0; glucose 53.0; inositol 5.3.

From these results it appears that the concentration of the principal elements is enough to make DAJ an attractive nutrient source for use as a substrate in the production of L-lactic acid by *S. faecium*.

Table 1

Chemical composition of the alfalfa fractionation products (% dry weight)

Fractions	Dry wt. (g)	Protein	Total sugars
Fresh alfalfa	22.0	4.5	9.0
Pressed cake	45.0	8.5	n.d.[a]
Juice	10.5	3.0	28.3
DAJ	7.5	2.0	36.0
Concentrated protein	45.0	20.0	n.d.[a]

[a] n.d. = not determined.

Fermentation

The pH of the medium at the start of batch fermentation was 6.2. Five hours after inoculation the pH began to fall; no correction was made, and after 15 h the pH dropped to 5 and lactic acid production stopped. The yield of lactic acid was 8.3 g/l (27% conversion of sugars to lactic acid), and cell yield was 1×10^{12} cells/l.

A much higher yield of L-lactic acid, preferably in its ammonium form, was desirable, therefore in a second batch fermentation (Fig. 2) the pH was adjusted to 5.8 at 17 h by the addition of ammonium hydroxide. In this fermentation, the lactic acid concentration at 24 h was 17.7 g/l (59% conversion of sugars to lactic acid). The production rate at 24 h was 1.8 g/l/h. The cell yield was 3.3×10^{12} cells/l. At 40 h lactic acid concentration reached 23 g/l (76.6% conversion) but the production rate fell to 0.3 g/l/h; cell content was 4.3×10^{12}/l. In a third batch fermentation (Fig. 2) the pH was maintained at 5.8 from the beginning. In this fermentation the lactic acid concentration at 24 h was 27.2 g/l (90% conversion and productivity 1.1 g/l/h); cell yield was 6×10^{12} cells/l. At 24 h the production rate was 2.9 g lactic acid/l/h.

A semicontinuous three-stage fermentation with pH controlled at 5.8 from the start produced the best results (Fig. 3). At the end of the first stage (24 h) lactic acid (as ammonium lactate) concentration was 27.2 g/l (productivity 1.1 g/l/h) and cell concentration was 4×10^{12} cells/l. At 24 h the production rate was 2.9 g/l/h. Percent conversion of sugars to lactic acid was 90%. At the end of the second stage (34 h, total) the lactic acid (expressed as ammonium lactate) was 35.5 g/l (productivity 3.6 g/l/h); cell concentration was 6.6×10^{12} cells/l. The calculated percentage conversion of sugars was greater than the theoretical value (118%). At the end of the 10-h

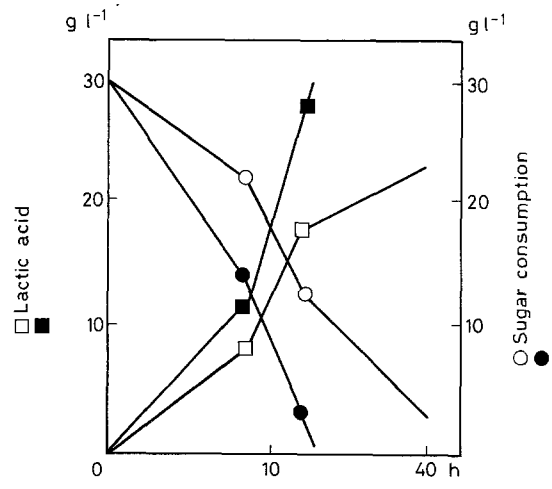

Fig. 2. Lactic acid production and sugar consumption by *S. faecium* in a fermentation with pH controlled at 5.8. pH controlled after 17 h: lactic acid produced (□), sugar consumed (○); pH controlled throughout fermentation: lactic acid produced (■), sugar consumed (●).

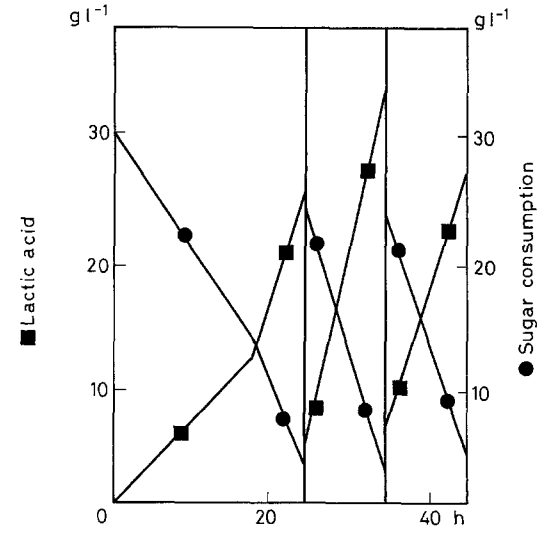

Fig. 3. Lactic acid production and sugar consumption by *S. faecium* in a three-stage semicontinuous fermentation.

second stage, the production rate was 2.9 g/l/h. At the end of the third stage (44 h, total) the lactic acid (as ammonium lactate) concentration was 28 g/l (productivity 2.8 g/l/h) and cell concentration was 6 x 10^{12} cells/l. Percent conversion was 93.3%. At the end of the 10-h third stage, the production rate had fallen to 2.1 g/l/h.

The semicontinuous three-stage fermentation, with a total volume of 26 litres and a fermentation time of 44 h, yielded – on average – 29.7 g/l of ammonium lactate (productivity 2.5 g/l/h). Conversion of sugars to lactic acid was 99% and cell concentration 4 × 10^{12} cells/l (40 g dry weight).

DISCUSSION

This study demonstrates that lactic fermentation of DAJ is comparable with whey fermentation, both in sugar concentration of the substrate and in biomass yield and conversion [4].

The product derived from the lactic fermentation of DAJ as the substrate is a syrup containing ammonium lactate and has a high content of lactic acid bacteria which are of particular interest for the nutrition of ruminants; such a syrup has no toxicity. The optimized fermentation process described in this paper for the production of ammonium lactate is an attractive alternative to the disposal of agroindustrial waste. This anaerobic process does not call for high-cost aeration equipment as does the production of single cell protein from the same substrate. The final whole broth, used directly or after dry-down, may find application as an animal feed. Preliminary acceptability studies have shown excellent palatability for ruminants.

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