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SIMULTANEOUS SACCHARIFICATION AND PROTEIN ENRICHMENT FERMENTATION OF SUGAR BEET PULP

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SUMMARY: A product with 40 % protein content was obtained from sugar beet pulp (1.25-2.0 mm) in 48 h one stage (simultaneous) saccharification/fermentation process under optimized conditions using a specific enzyme mixture and Candida tropicalis strain, also saving about 40 % enzymes in comparison to a 2-stage process.

INTRODUCTION

In addition to the production of liquid fuels from cellulosic biomass, bioconversion of cellulosic agricultural by-products to single cell protein (Cooney et al. 1980) has attracted world wide attention. Sugar beet pulp is one such raw material; it can be processed to obtain fermentable sugars using polysaccharide degrading enzymes (Voragen et al. 1980), and these may be utilized for SCP production or for other fermentations (Martinez et al. 1984).

In earlier unpublished studies on protein enrichment of sugar beet pulp, we found that a process with two separate phases of saccharification and then fermentation of saccharified filtrate is economically unattractive. The present work was undertaken to investigate and optimize the conditions for simultaneous saccharification and fermentation of sugar beet pulp for the protein enrichment.

Culture preparation: Candida tropicalis DSM 70151 was maintained on Sabouraud agar medium at 4°C. Active seed culture was started by inoculating a portion of the stock culture into Sabouraud liquid medium (3.0 % Oxoid) and incubating for 24 h at 30°C and 150 rpm. This whole culture broth was used as inoculum at 5 % v/v concentration.

Enzymes:
a) Enzyme source-1 (ES-1) - a Novo SP 249 product (NOVO Industry A/S, Denmark) from Aspergillus niger consisting mainly of pectinases and hemicellulases.
(b) Enzyme source-2 (ES-2) - an enzyme preparation from Trichoderma reesei Rut-C30 NRRL 11460 consisting mainly of cellulases.

Saccharification/fermentation: The whole process was carried out in 10 l fermentors (Biostat, B. Braun, FRG), minimum working volume 5 liter, equipped with a three blade stirrer, bottom air sparger and automatic control systems for pH, temperature and O_2 level. The medium comprised 50g/l (dry weight) beet pulp, 0.52 g/l $MgSO_4 \cdot 7H_2O$, 0.79 g/l KH_2PO_4 and 3.54 g/l $(NH_4)_2SO_4$, plus enzyme mixtures as specified. Sugar beet pulp (30% dry matter) was obtained from sugar factory after extraction of sugar. It was dried, milled and analysed; an average composition was found as: cellulose-20%, hemicellulose-30%, pectin-16%, lignin-4%, protein-9%, soluble carbohydrates-5% and ash-10%.

Assays: Sugars released in fermentation broth and in acid hydrolysed samples were measured by the method of Sinner & Puls (1978) using sugar analyzer (Biotronic LC-200, FRG). Fermented samples were centrifuged to recover the product containing yeast biomass and residual substrate. The products were washed, dried and weighed. These were analysed for protein content by Kjeldahl method (N x 6.25) and for cellulose, hemicellulose and pectin contents by estimating different sugars obtained by acid hydrolysis (Puls et al.

1985). Protein and other components are expressed as percent i. e. g per 100 g d. w. product and percent product recovery means g product recovered per 100 g substrate.

RESULTS AND DISCUSSION

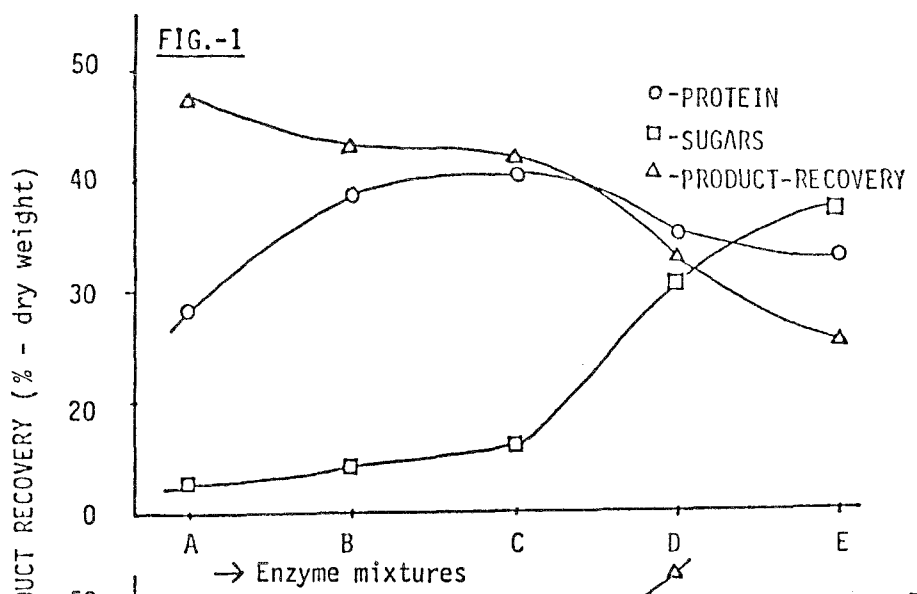
Conditions optimal for the separate cultivation of the yeast may not be similar to those required for simultaneous process. This process carried out at various temperatures and pH values showed 30°C and pH 5.2 to be optimal parameters (unpublished results) so all the fermentations were carried out at these conditions.

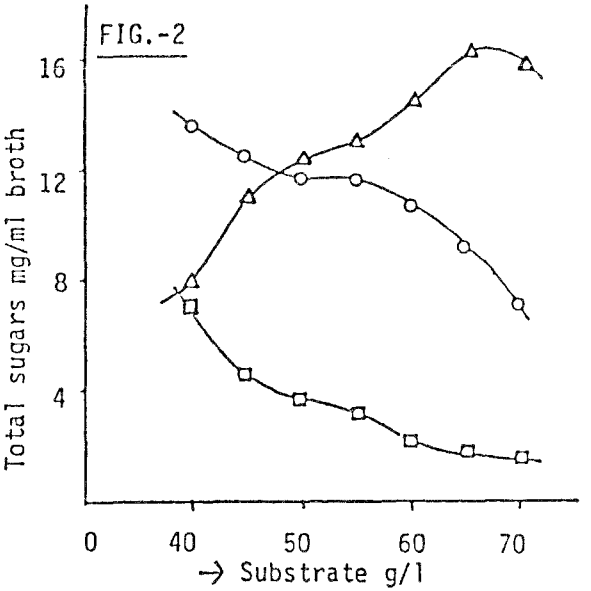
Enzymes - Enzyme mixtures (A-E) were prepared by taking both enzyme sources in different quantities, as
A - 0.05 % (v/v) ES-1 + 0.15 % (w/v) ES-2;
B - 0.075 % ES-1 + 0.225 % ES-2;
C - 0.1 % ES-1 + 0.3 % ES-2;
D - 0.125 % ES-1 + 0.375 % ES-2;
E - 0.15 % ES-1 + 0.45 % ES-2.
Fig.-1 shows that the enzyme quantities of mixtures B & C were more optimum for protein enrichment and also for the recovery of product. Higher enzyme concentrations (D & E) reduced product recovery without increasing its protein content, showing more saccharification of substrate than required for fermentation, so that after 48 h fermentation more unutilized sugars remained in the medium than when using lower concentrations of enzymes (fig.-1). Excess sugars in medium may cause interference with culture growth resulting in less protein formation. Here in simultaneous process optimum enzyme concentration (mixture B) is 60 % to the concentration, we required for saccharification step in a 2-stage process, giving same degree of solubilization, the reason may be that in the simultaneous process released sugars were being constantly utilized by yeast eliminating any inhibition for enzyme activity thus a lower amount of enzymes was as much as effective as a higher amount (1.7 times more) required for saccharification step.

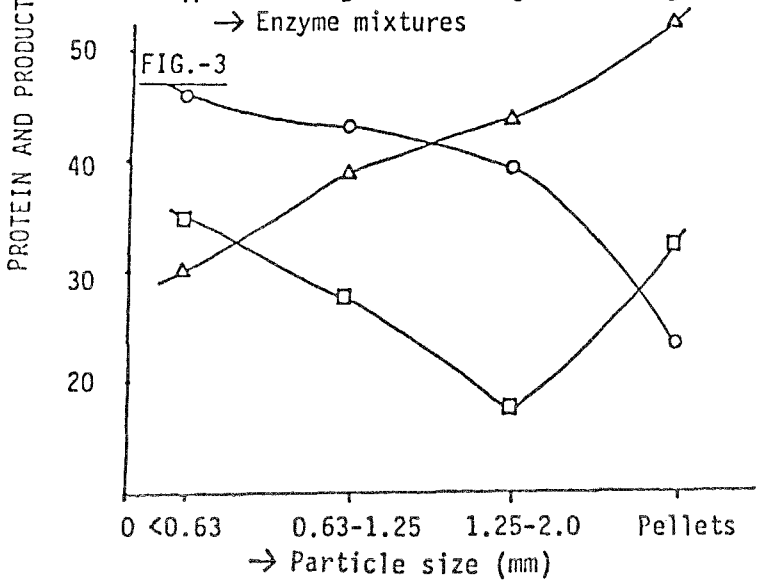
Substrate concentration - Fig. 2 shows the effect of substrate concentration in simultaneous process; 45-55 g/l substrate was found optimum for both the yield of product and for its protein content. Above 55 g/l concentration the protein level decreased due to the lower saccharification of the substrate. The reason may be that the enzyme quantity (mix. B) is not sufficient for higher substrate concentration, which was optimized for 50 g/l substrate. The rate of saccharification may be enhanced by increasing enzyme quantity. Another reason may be that the higher substrate concentration is not favourable for good mixing and aeration. At lower substrate concentrations, protein content was high but the product recovery decreased because of higher substrate solubilization.

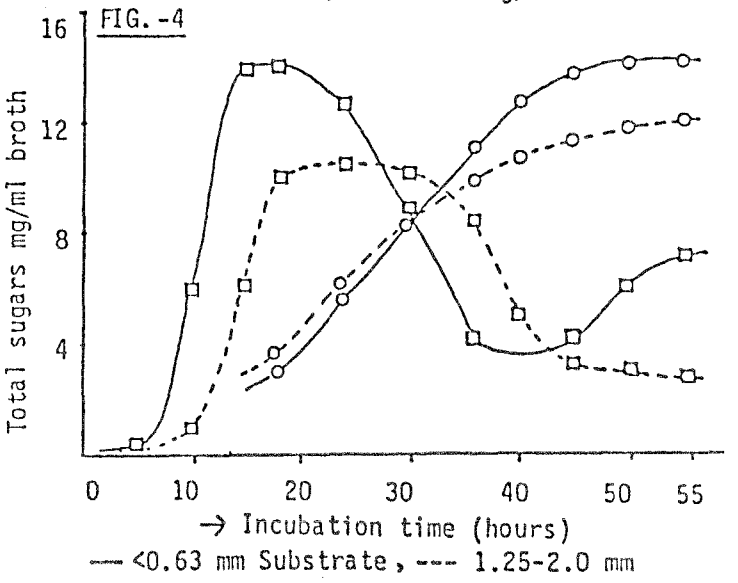
Particle size - A high degree of solubilization occured with particle size < 0.63 mm; although the protein content of the product was very good (47 %), a low level of product recovery (about 30 %) was obtained and a significant part of the released sugars also remained unutilised (fig. 3). Moloney et al. (1984) reported that extensive grinding of the pulp enhanced total polysaccharide hydrolysis by about 30 % and degradation of cellulose by 50 % by increase in surface area, but such extensive hydrolysis is not required for the single-stage process. The 1.25 - 2 mm particle size substrate showed solubilization optimum for utilization, and a low amount of sugars left unutilized after 48 h. The use of sugar beet pellets obtained directly after sugar extraction ($\sim$ 1.2 cm) led to considerable stirring problem, during the 48 h of the process, as initial 500 rpm and a final speed of 200 rpm being needed. Even under optimized and controlled conditions protein enrichment was not encouraging because the high speed of agitation caused damage of yeast cells.

Fig. 4 shows the saccharification and fermentation profile of < 0.63 mm and 1.25 - 2.00 mm particle size substrates. Sugars could be detected in the medium from the 10th hour of incubation. Maximum rate of saccharification was ob-









(Scales are same for protein, product recovery and sugar in fig. 1-4)

served in the period from 15 h to 24 h with < 0.63 mm substrate and with bigger particles upto 36 h. Protein content rapidly increased upto 40 h and then stabilized in 45 - 48 h incubation. Results in table-1 are given for the analysis of products obtained in 48 h with 50 g/l substrate concentration with enzyme mixture B.

Table-1 Characteristics of product I from particle size 1.25 mm - 2.00 mm and product II from particle size < 0.63 mm

Composition (%)	Product I	Product II
Protein	39.65	45.68
Cellulose	14.92	10.40
Hemicellulose	11.95	7.54
Pectins	1.38	0.57
Lipids	5.00	4.41
Ash	7.32	10.50
Productivity (%, based on total pulp utilized , exclusive of losses as dissolved carbohydrates in supernatants and washings)	43.33	30.01
Biomass yield (g cells dry wt./g pulp utilized)	0.35	0.27

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