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Short Communication

High solids fermentation of hydrolysates of wheat starch B in a continuous dynamic immobilized biocatalyst bioreactor

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SUMMARY

A simple and efficient method of conversion of wheat starch B to ethanol was investigated. Employing a two-stage enzymatic saccharification process, 95% of the wheat starch was converted to fermentable sugars in 40 h. From 140 g/l total sugars in the feed solution, 63.6 g/l ethanol was produced continuously with a residence time of 3.3 h in a continuous dynamic immobilized biocatalyst bioreactor by immobilized cells of *Saccharomyces cerevisiae*. The advantages and the application of this bioreactor to continuous alcoholic fermentation of industrial substrates are presented.

INTRODUCTION

Recently, we have demonstrated the method of using a continuous dynamic immobilized biocatalyst bioreactor (CDIBB) for ethanol production [2,4]. This reactor was used for ethanol production from glucose, sucrose, cane molasses, corn wet milling waste [4] and wood hydrolysates [2]. The present study was undertaken to examine high solids fermentation of hydrolysate of wheat starch B to ethanol in the CDIBB.

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MATERIALS AND METHODS

Preparation of wheat starch B

Industrial wheat starch B slurry at 17.2% dry solids was obtained from Manildra Milling Corporation, Minneapolis; 98% of this feed stock was starch. A 17 litre batch of hydrolysed starch slurry was prepared in a 20 litre plastic tank. The pH of wheat starch was adjusted to 7.0 with $Ca(OH)_2$ powder. An enzyme dosage of 1 litre α-amylase

(Nova Termamyl 120 L) per ton of dry starch [7] was added and the temperature was raised to 85°C by direct steam injection. The liquefaction continued under constant stirring for 30 min.

The liquefied slurry was cooled to 55°C, the pH was lowered to 5.0 with 10 N HCl, and 1 litre amyloglucosidase per ton of dry starch [7] was added. Saccharification was continued at 55°C for 40 h. At the end of saccharification, the hydrolysate was heated to 85°C by direct steam and maintained at that temperature for 30 min to inactivate amyloglucosidase, which could otherwise catalyse the back-conversion of glucose to iso-maltose [7].

Fermentation

Dried bakers' yeast, *Saccharomyces cerevisiae* (Fleischman), was purchased from a local market, and immobilized in the CDIBB at a cell density of 40 g/l (based on total bioreactor volume of 3.4 litres), this being the total difference in the amount of cells added to the reactor for immobilization and the free cells in the effluent after immobilization. The experimental apparatus, cell immobilization procedure and the performance of the bioreactor have been described elsewhere [2,4]. The starch syrup, containing 140 g/l fermentable sugars, was used as the feed, enriched with 0.1% of yeast extract, 0.02% urea and 0.01% diammonium phosphate serving as nutrients. Continuous ethanolic fermentations were conducted under anaerobic conditions at 30°C, pH 5.0 and at different flow rates.

The performance of the reactor was assessed by determining ethanol produced, residual sugars, the volumetric ethanol productivity, fermentation residence time and yield.

Analysis

Ethanol was determined by gas chromatography (Hewlett Packard model 5700A) using *n*-propanol as the internal standard [5]. The glucose and oligosaccharides produced by the hydrolysis of starch were determined by HPLC using double-distilled water at 65°C as eluent in an Aminex HPX-87P column [3] and at 80°C for an HPX-42A column. Cell density was measured by a Spectronic 20 (Bausch and Lomb) at 660 nm [5,6]. Dry substance in the feed was determined by drying in a vacuum oven at 60°C for 48 h.

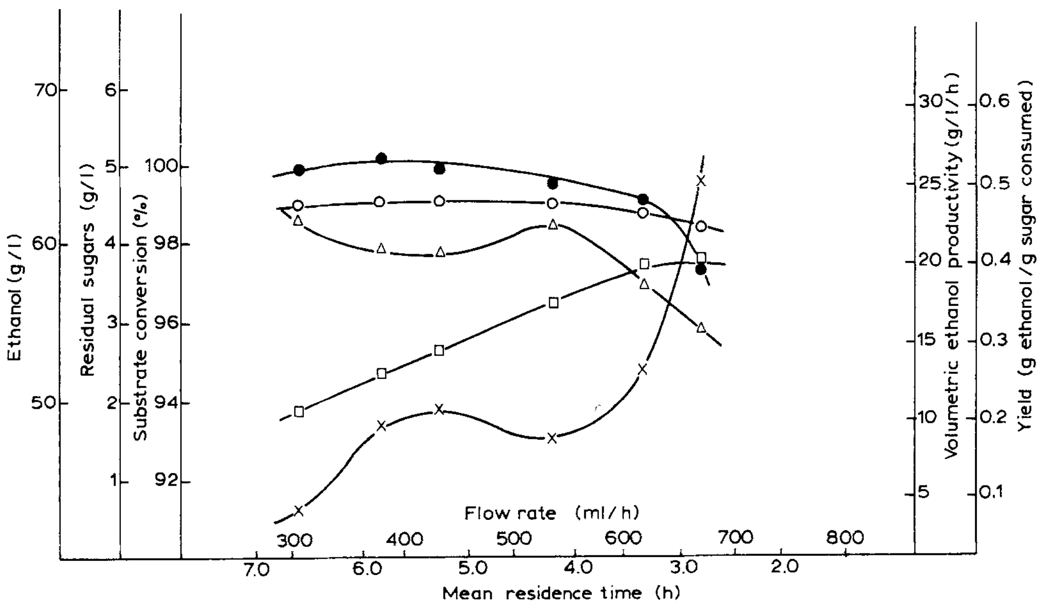

Fig. 1. Results of fermentation in CDIBB of starch hydrolysate at different feed flow rate (or residence time). ●, ethanol; ○, residual sugars; △, substrate conversion; □, volumetric ethanol productivity; ×, yield.

Table 1

Hydrolysis of wheat starch B

	Dry solids (%)	Glucose (g/l)	Fermentable sugars: glucose, maltose, and maltotriose (g/l)
Starch slurry	17.22	1.26	6.43
Liquefied starch		14.89	76.00
Saccharified starch syrup	17.57	172.11	175.02

RESULTS AND DISCUSSION

The results of the hydrolysis of starch and the fermentation of the hydrolysate at different flow rates of feed stock in the CDIBB are summarized in Table 1 and Fig. 1, respectively.

The present liquefaction step resulted in a rapid drop in the viscosity of the starch slurry and a quick fading of the typical starch iodine-blue color. 80% of starch was hydrolysed into oligosaccharides (from DP2 to DP9) during the liquefaction process. More than 95% conversion of the starch to fermentable sugars was achieved (Table 1).

From 140 g/l sugars in the feed solution, 63.6 g/l ethanol was produced continuously in 3.3 h residence time in the CDIBB reactor using immobilized cells of *S. cerevisiae* (Fig. 1). The maximum volumetric ethanol productivity achieved from this 3.4 litre reactor (based on liquid holdup) was 19 $g \cdot l^{-1} \cdot h^{-1}$ at the flow rate of 600 $ml \cdot h^{-1}$ and dilution rate 0.3 (throughput 14.4 l/day). Under these operating conditions 97% of substrate was fermented to ethanol giving a final yield of 0.47 g ethanol/g sugar, representing 93% fermentation efficiency. Since the continuous fermentations were performed under an anaerobic environment, yeast growth was limited. The effluent contained only 0.12 g/l dry cells.

To be cost-competitive and economically attractive, interest in the commercial fermentation of ethanol is presently geared towards the use of cheap waste streams from the food and starch processing industries. Wheat starch B is such a feed stock, being an industrial discard obtained during wet milling of wheat flour; it is abundantly available and represents a good source of glucose for ethanol production [1]. Conversion to ethanol represents an attractive source of income for a wheat refinery.

The results presented here show that wheat starch B was easily converted to high-yield fermentable sugars with a conventional dual enzyme process. Moreover, this substrate containing 14% dry solids was continuously fermented to ethanol in a CDIBB without any problems of clogging or fouling. This reactor is quite stable in continuous prolonged operations and has an estimated half-life of 100 days. Previously we have also demonstrated high-productivity continuous operations of CDIBB in fermenting industrial substrate rich in hexose and pentose and yielding over 10% (v/v) ethanol in the effluent [2,4]. It therefore has industrial potential, since it can handle substrates presently employed in the alcohol fermentation industry without the need for clarification or prefiltration and is economically attractive, offering short residence time coupled with high fermentation yield.

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