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COUPLED IMMOBILIZED ENZYME - IMMOBILIZED CELL SYSTEM FOR CONTINUOUS PRODUCTION OF ETHANOL

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Abstract

For the continuous production of ethanol from thinned starch, a column reactor filled with covalently immobilized glucoamylase was coupled with a vertical reactor segmented with perforated plates supporting Saccharomyces cerevisiae cells entrapped in calcium alginate. The operation of the system was characterized by a fermentation efficiency of 84.5 ± 4.1%, and an ethanol concentration of 38.8 ± 0.3 g/l,

Key words: glucoamylase immobilized, yeast immobilized, ethanol production, bioreactor

Introduction

Starch is one of the most important raw materials for industrial ethanol production.

Two enzymes are generally used for the production of glucose from starch. α - Amylase is employed in soluble form since the molecular weights of its substrates, amylose and amylopectin, are too high for satisfactory hydrolysis with immobilized enzymes (HARTMEIER, 1986). In contrast, glucoamylase can be applied in immobilized form for the continuous saccharification of starch previously thinned by α-amylase. The continuous production of ethanol is performed by immobilized microbial cells. Different vertical packed-bed and fluidized-bed reactors are preferentially used (GODIA et al., 1987). Successful pilot plant and industrial operations are known (NAGASHIMA et al., 1983; 1987; NAJIMA et al ., 1987).

The present paper reports a coupled immobilized enzyme - immobilized cell system for the continuous production of ethanol from thinned starch as substrate.

Materials and methods

Chemicals. Glucoamylase was isolated from Aspergillus niger with a specific activity of 900-1500 units g^{-1} protein. Akrilex C-100, a polyacrylamide bead polymer containing carboxylic functional groups (6.4 meq g^{-1} dry wt) was a commercial product of Reanal. Its molecular exclusion limit was 100,000 daltons. 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-4-toluene sulfonate was purchased from Serva Feinbiochemica GmbH (Heidelberg, FRG). Soluble starch was a preparation of E. Merck AG (Darmstadt, FRG).

Corn starch was a gift from the Research Institute of the Alcohol Industry. All other chemicals were reagent grade commercial preparations (Reanal).

Microorganism and culture medium. Commercial baker's yeast was used. The cells were grown in a water bath shaker at 30 °C in a culture medium containing 100 g l^{-1} sucrose and diffrent nutrients, as described by WADA et al. (1979). The pH was adjusted to 4.0. Cells were harvested by centrifugation at 2500 x g for 10 min.

Immobilizations. Glucoamylase was covelently immobilized on a polyacrylamide support (Akrilex C-100) containing carboxylic groups activated by 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-4-toluene sulfonate as described earlier (SZAJÁNI et al., 1985). The activity was 10.2 units g^{-1}. One unit is defined as the amount of enzyme required for the liberation of 1 g of D-glucose from soluble starch per hour at pH 3.8 and 60 °C. For immobilization, baker's yeast cells were suspended in a sterile sodium alginate solution (Protanal SF 120, protan and Fragertun A.S., Drammen, Norway). The final cell density was 1x 106 cells ml^{-1}. Beads (∅ 4 mm) were formed by dripping the suspension through a syringe into sterile 1% calcium chloride solution. The cells were grown in a water bath shaker at 30 °C for 24 h.

Thinning of starch. Technical grade corn starch (700 g) was suspended in 2000 ml water, and 4 ml Optitherm LT α-amylase (Miles Laboratories Ltd.) was added. The suspension was incubated at 60 °C for 10 min. The temperature was then raised to 80-90 °C, a further 4 ml α-amylase was added and the incubation was continued for 20 min. This treatment was repeated twice. The suspension was next boiled to stop the action of α-amylase and was filtered. The pH of the filtrate was adjusted to 4.0 - 4.2 with 1 M hydrochloric acid.

Analytical methods. D-Glucose was measured iodometrically (ERDEY, 1956) or with glucose oxidase. Ethanol was determined by gas chromatography, with a Chrom 4 gas chromatograph (Laboratomi Pristroje, Prague, Czech Republic) equipped with a flame ionization detector and a Porapak Q (80-100 mesh) column (250 cm long and 3 mm i.d.). Nitrogen was used as carrier gas and methanol as internal standard.

Results and discussion

For the continuous production of ethanol from thinned starch as substrate, two bioreactors were coupled together. The first was a column reactor (4 x 1.5 cm) filled with glucoamylase (125 mg dry) covalently immobilized on a polyacrylamide support activated by water-soluble carbodiimide (SZAJÁNI et al., 1985). The second reactor was a vertical one segmented with perforated plates supporting Saccharomyces cerevisiae cells entrapped in calcium alginate (BUZÁS et al., 1990). The reactor volume and the length/diameter ratio were 143 ml and 4.2, respectively. The total gel volume of 73 ml with a cell density of 1 x 108 cells ml^{-1} gel, was equally divided onto 3 perforated trays.

Thinned corn starch (glucose content 102 g l^{-1}, pH 4.0) was passed through the first, immobilized enzyme reactor at a flow rate of 2.8 ml h^{-1}. The column was maintained at 60 °C. In a reservoir, the effluent was cooled and diluted to about 9% glucose content, and calcium chloride solution was added to it to give a final concentration of 1%. The immobilized cell reactor was fed with this medium at a flow

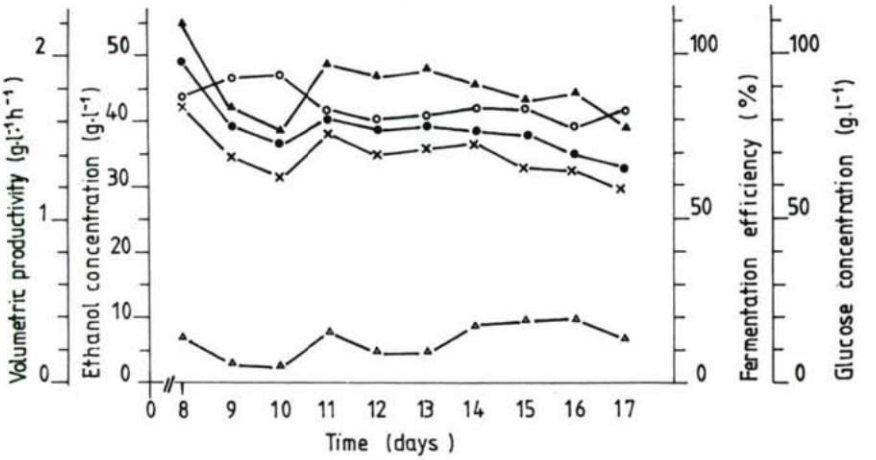

Fig. 1. Progress curves of the fermentation of thinned corn starch. Fermentation efficiency, O ; ethanol concentration, • ; volumetric productivity, x ; glucose concentration in the fermentation medium, Δ; and in the effluent, ▲.

rate of 5 ml h^{-1} at 30 °C. The two reactors were operated separately for 7 days to reach a steady state, after which they were coupled together and operated continuously. The progress curves of the fermentation are presented in Fig. 1.

The average values characterizing the process were found to be: fermentation efficiency, 84.5 ± 4.1%, ethanol concentration in the mash, 38.8 ± 0.3 g l^{-1} and volumetric productivity, 1.40 ± 0.12 g l^{-1} h^{-1}.

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