BIOPROCESS ENGINEERING STUDIES ON THE BIOCONVERSION OF SORBITOL TO SORBOSE

By Acetobacter suboxydans

by

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Bioconversion
Bio Process Engineering.
Enzymes.

To

my wife Letha

and

sons Aravind and Rohit

CERTIFICATE

This is to certify that the thesis entitled, "**BIOPROCESS ENGINEERING STUDIES ON THE BIOCONVERSION OF SORBITOL TO SORBOSE** *By Acetobacter suboxydans*", being submitted by **Mr. R. GIRIDHAR** to the Indian Institute of Technology, Delhi for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him under my supervision. The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

Batch kinetic analysis of sorbitol to sorbose bioconversion by *Acetobacter suboxydans* was investigated at a constant temperature of 30°C and pH 6.0. Using initial sorbitol concentrations (S_0) of 100, 200, 300 and 400 g l^{-1} in batch fermentations, a sorbose productivity of 10.11, 14.28, 12.41 and 3.99 g l^{-1} respectively was obtained. Substrate inhibition studies revealed that the specific growth rate of the culture decreased with increase in initial sorbitol concentration. The culture exhibited a Luong type (Luong, 1985) inhibition by sorbitol with a concave relationship (inhibition exponent 'a' = 0.81) between μ and S. The growth was completely inhibited at $S_0 = 510$ g l^{-1}.

The effect of increasing initial sorbose concentration (P) on specific growth was also investigated. The specific growth rate (μ) decreased with increasing initial sorbose concentrations. Extrapolation of the above correlation indicated that no growth would be observed at P = 700 g l^{-1}.

Unstructured batch mathematical models for sorbose fermentation was developed. Their parameters were identified for S_0 = 100 g l^{-1} and 200 g l^{-1} . For optimal estimation of model parameters, a non-linear regression technique assisted by a computer program was used to minimize the deviations between the model predictions and the experimental observations. The model simulations and experimental results were found to be in good agreement, when optimized parameters

were used. The statistical validity of the identified models were demonstrated with an accuracy of 99% using 'F' tests.

The batch kinetic models were then extrapolated to develop nutrient feeding strategies for fed-batch fermentations for productivity improvement. The adequacy of the fed-batch models were further demonstrated by conducting fed-batch fermentations at constant feed rate (S_0 = 500 g l^{-1}, 0.2 l h^{-1}) and variable feed rate (pseudo-steadystate w.r.t S) to maintain a constant sorbitol concentration (S = 36.2 g l^{-1}) in the reactor.

Several nutrient feeding strategies for fed-batch fermentations were designed and tested to improve the sorbose concentration and/or productivity in the fermenter. Higher sorbose concentration and productivity were obtained by feeding sorbitol at constant feed rates (500 g l^{-1} @ 0.2 l h^{-1}, 600 g l^{1} @ 0.36 l h^{-1}), multiple feeding and feeding at a linearly decreasing rate. Fed-batch fermentations initiated with S_0 = 100 g l^{-1} were found to be more productive than the fermentations initiated with S_0 = 200 g l^{-1}.

Continuous fermentations were done at different dilution rates (0.05, 0.10, 0.15 and 0.3 h^{-1}) and the effect of dilution rate on culture growth and sorbose formation was elucidated. A dilution rate of 0.10 h^{-1}, for continuous fermentation without cell recycle, demonstrated a maximum sorbose concentration of 176.90 g l^{-1} with the highest conversion efficiency of 88.5%. A sorbitol productivity of 17.69 g l^{-1} h^{-1} was also obtained. However, when the dilution rate was increased to 0.3 h^{-1} the sorbose concentration decreased to 73.20 g l^{-1} mainly due to culture washout.

Eventhough the productivity increased to 21.96 g l^{-1} h^{-1} the outflow of unconverted sorbitol was also found to be higher (130 g l^{-1}) at D = 0.3 h^{-1}.

With total cell recycle, high sorbose concentration (181.38 g l^{-1}) alongwith a sorbose productivity of 18.14 g l^{-1} h^{-1} and 90.6% conversion was obtained at D = 0.10 h^{-1}. Lower specific rates of sorbose formation in continuous fermentation with cell recycle as compared to without cell recycle, indicated severe oxygen limitation since the sorbose concentration did not increase in proportion to the increase in biomass concentration achieved by cell recycle.

The possibility of enhancing sorbose concentration by using oxygen vector (n-hexadecane) in shake flask fermentations was investigated. Addition of n-hexadecane improved the sorbose accumulation in shake flask fermentations as compared to fermentation without n-hexadecane. Addition of 4% n-hexadecane resulted in a maximum sorbose accumulation of 82.12 g l^{-1} in 24 hours as against 64.83 g l^{-1} without n-hexadecane. Batch sorbose fermentation with 4% n-hexadecane demonstrated an increase in productivity from 14.3 g l^{-1} h^{-1} (without n-hexadecane) to 16.7 g l^{-1} h^{-1}. Fed batch fermentation using 4% n-hexadecane reduced the processing time from 26 hours (without n-hexadecane) to 20 hours increasing the sorbose productivity from 12.6 g l^{-1} h^{-1} (without n-hexadecane) to 15.9 g l^{-1} h^{-1}.

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