Repeated Fed-batch Sorbose Fermentation by *Gluconobacter oxydans*

R. Giridhar and A. K. Srivastava*
Department of Biochemical Engineering & Biotechnology,
Indian Institute of Technology, Delhi, Hauz Khas,
New Delhi – 110 016, INDIA, e-mail: ashokks@dbeb.iitd.ernet.in

Original scientific paper
Received: 1. 2. 2001.
Accepted: 1. 4. 2001.

Gluconobacter oxydans was cultivated in repeated fed-batch culture for the bioconversion of D-sorbitol to L-sorbose. Batch culture with an initial concentration of 200 kg m^{-3} sorbitol was converted to repeated fed-batch by harvesting one-third of the reactor volume and recharging with fresh nutrient medium having the same sorbitol concentration. Four cycles of harvesting and recharging were done. At the end of fourth cycle (26 h) 2.26 kg of sorbose was produced with an increased average productivity of 19.31 kg m^{-3} h^{-1}.

Keywords:
Gluconobacter oxydans, fed-batch, sorbitol, sorbose

Introduction

Sorbose fermentation, involving the microbial oxidation of D-sorbitol to L-sorbose by *Gluconobacter oxydans*, is an intermediate step in the industrial production of Vitamin-C. The batch fermentation process is severely inhibited by sorbitol.[1–3] Therefore, it is not possible to ferment high concentrations of sorbitol in batch process, leading to lower productivity. Fed-batch mode of operation provides an excellent means of regulating the nutrients feed rate to optimize the productivity while at the same time preventing the over and underfeeding of nutrients. Improvement in sorbose productivity has been attempted by using fed-batch fermentation strategies like intermittent feeding of sorbitol[4], gradient fed-batch[1], exponential fed-batch[3] and pulse/multiple feeding.[5]

Another processing technique to enhance the sorbose productivity is to extend the life of the batch fermentation by converting it into a repeated fed-batch fermentation. In repeated fed-batch fermentation, a portion of the reactor content is periodically withdrawn and the residual growing culture in the reactor is used as the starting point for further fed-batch process. This ensures the benefit of high inoculum ratio at the time of fresh feed. Typically, repeated fed-batch cultivation consists of three stages: filling, batch processing and harvesting. The cycles described above are repeated. Ideally, after a steady cyclic operation has been achieved, the cell and substrate concentrations at the beginning of a cycle will be equal to those at the end of the previous cycle.[6] The theory & applications of repeated fed-batch culture has been well discussed in the literature.[7–10]

*Corresponding author

The present study focuses on the utility of repeated fed-batch strategy of bioprocessing to reduce the unproductive down time, process high amount of sorbitol in relatively shorter time, and thereby improve the productivity of sorbose fermentation.

Materials and Methods

Chemicals

w = 70 % D-sorbitol solution was supplied by M/s Anil starch products, Ahmedabad, India. Yeast extract powder, ammonium dihydrogen phosphate and magnesium sulfate were obtained from M/s Qualigens fine chemicals, Mumbai, India. All chemicals were of Analytical Reagent (AR) grade.

Microorganism and maintenance

Gluconobacter oxydans NRRL B-72 strain was used in this work. The culture was maintained on agar slants having the composition (kg m^{-3}): D-sorbitol, 5.0; yeast extract powder, 5.0; ammonium dihydrogen phosphate, 3.0; magnesium sulfate, 1.0; agar 20. The pH of the medium was adjusted by the automatic addition of 3 mol dm^{-3} NaOH/HCl. A 48 hour growth at 30 °C was preserved at 4 °C.

Inoculum development

A loop of microorganism from the slant was transferred into 0.01 dm^3 liquid medium in test tubes having the same composition as above (as mentioned in the Microorganism and maintenance section, less the agar). The test-tubes were incubated at 30 °C for 72 hours. The growth was characterised by the appearance of a thick pellicle on the surface and uniform turbidity. It was then trans-

ferred into 1.0 dm^3 capacity flasks containing 0.1 dm^3 of liquid medium (as mentioned in the Microorganism and maintenance section, less agar). The flasks were incubated in a rotary shaker (Adolf Kuhner, Germany) at 30 °C and 250 rpm. Subsequent transfer into the bioreactor was done when the biomass concentration in the flasks was about 2.5 to 3.0 kg m^{-3}.

Fermentation

Repeated fed-batch fermentation

Repeated fed-batch fermentation was initiated as a batch with an initial sorbitol concentration of 200 kg m^{-3} sorbitol and a working volume of 4.5 dm^3 in a 7.0 dm^3 of total volume (Bioengineering AG, Switzerland) fermenter. The concentrations (kg m^{-3}) of other components used in the fermentation medium were as follows: yeast extract, 5.0; ammonium dihydrogen phosphate, 3.0 and magnesium sulphate, 1.0. The pH was maintained at 6.0 by the automatic addition of 6 mol dm^{-3} NaOH/HCl. An aeration flow rate of 2.2 dm^3 dm^{-3} min^{-1} and an agitation speed of 700 rpm were maintained throughout the fermentation.

The batch fermentation was converted into repeated fed-batch mode by rapidly withdrawing one-third (= 1.5 dm^3) of the volume of the culture from the reactor when the dissolved oxygen concentration, as indicated by an Ingold dissolved oxygen probe, showed an abrupt increase. The same volume of nutrient medium containing 200 kg m^{-3} of sorbitol alongwith other nutrients (whose concentrations are given in the Microorganism and maintenance section, less agar) was fed to the residual growing culture in the reactor. Four cycles of harvesting and nutrient feeding were done.

The sorbose productivity (time-average value) in the repeated fed-batch culture was calculated as described by Mori et al.[10] as follows:

$$\text{Sorbose productivity} = \frac{\text{Total sorbose drawn out and produced at the end of cultivation}}{(\text{final culture volume in the reactor})(\text{total time of culture operation})}$$

Analytical Techniques

Biomass concentration

Optical density (OD) of suitably diluted samples was measured at 600 nm in a UVIKON 930 spectrophotometer (Kontron Instruments, USA). Biomass was estimated from a OD vs concentration (kg m^{-3}) correlation which was determined *a priori* as follows:

$$\text{Biomass (kg m}^{-3}) = 0.73 \times \text{OD}_{600}$$

Sorbitol and sorbose concentrations

Sorbitol and sorbose concentrations were estimated by HPLC (Waters Associates, USA) using a Supelcosil LC-NH_2 (Supelco, USA) column (25 cm x 4.6 mm ID) equipped with RI detector and using acetonitrile/water (ψ = 75 : 25) as eluent with a flow rate of 0.06 dm^3h^{-1} at ambient temperature.

Results and Discussions

The variation of biomass, sorbose and sorbitol concentrations with time course of repeated fed-batch fermentation is shown in Fig. 1. Four cycles of culture withdrawal and fresh nutrient medium addition were done when the dissolved oxygen concentration in the bioreactor indicated an abrupt increase at hours 13, 16, 19 and 23. The biomass concentration obtained at the end of each cycle were 6.0, 6.2, 6.5 and 6.6 kg m^{-3} respectively. Whereas, sorbose concentrations at the end of each cycle were respectively, 206.50, 208.50, 205.82 and 207.00 kg $m^{-3}h^{-1}$. A sorbose productivity of 19.31 kg m^{-3} h^{-1} was obtained.

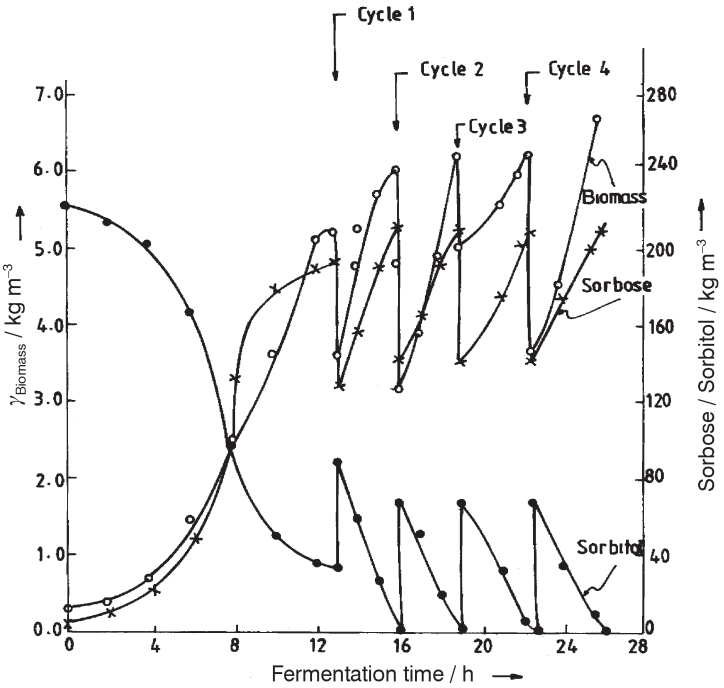

Fig. 1 – *Repeated fed-batch sorbose fermentation by Gluconobacter oxydans: 0 – 13 h batch (Initial substrate concentration, γ_{S0} = 200 kg m^{-3} sorbitol), 13 – 26 h fed-batch (4 cycles of 1.5 dm^3, 200 kg m^{-3} sorbitol) fermentation*

No literature report exists for the use of repeated fed-batch culture for productivity improvement in sorbose fermentation. However, some feeding strategies for fed-batch sorbose fermentation had been reported for fed-batch sorbose fermenta-

tion. Mori et al.[4] used intermittent addition of sorbitol powder for fed-batch sorbose fermentation and obtained a productivity of 44.85 kg m^{-3} h^{-1}. Pure oxygen was supplied to the fermenter by these investigators to maintain 2–3 ppm level of oxygen during cultivation. *Bull* et. al.[11] used partial recycling of microbial cells in chemostat and could enhance the product formation rates to a level of 91 kg $m^{-3}h^{-1}$. However, unusual design of cell retention device[11] pure oxygen[4] sorbitol powder usage[4] and high rpm, utilized[11] in their investigations, restricted the industrial applicability of their production strategies. *Bošnjak* et al.[1] using gradient fed-batch cultivation, obtained a sorbose productivity of 11.62 kg m^{-3} h^{-1}, while Srivastava and Lasrado[3] obtained a productivity of 12.6 kg m^{-3} h^{-1} using exponential fed-batch culture. *Giridhar* et. al.[5] initiated a fed-batch fermentation with initial sorbitol concentration of 100 kg $m^{-3}$and added 4 pulse feeds of 0.5 m^3 containing 600 kg m^{-3} sorbitol. This led to an overall sorbose productivity of 13.4 kg $m^{-3}h^{-1}$ and a final sorbose concentration of 320.48 kg m^{-3}. However fed-batch fermentation (with initial sorbitol concentration of 100 kg m^{-3}) with multiple feeding (two pulse feed of 0.5 m^3 nutrient medium containing 600 kg m^{-3} sorbitol followed by constant feed flow rate of 0.36 m^3h^{-1} till full working capacity of the reactor) led to an overall sorbose productivity of 15.09 kg $m^{-3}h^{-1}$ and sorbose concentration of 332.60 kg m^{-3}. The productivity of 19.31 kg m^{-3} h^{-1} obtained in this investigation is the highest compared to the above literature reports except for *Mori* et al.[4] and *Bull* et. al.[11], who used unusual oxidation conditions. Moreover, by converting batch cultivation into repeated fed-batch mode, the non-productive downtime for cleaning, filling and sterilization can be eliminated thereby increasing the overall reactor productivity and reducing the cost of production. In addition, this technique of semicontinuous cultivation can be easily adopted to the existing industrial condition without any additional expenditure on the equipment. Furthermore, in conventional fed-batch cultivation techniques, the volume of sorbitol that can be processed is limited by the maximum working capacity of the bioreactor. While, in repeated fed-batch fermentation an infinite number of cycles of culture withdrawal and nutrient medium addition can be done provided there is no contamination.

Conclusions

Batch culture, using *Gluconobacter oxydans,* with an initial concentration of 200 kg m^{-3} was converted to repeated fed-batch by harvesting one-third of the reactor volume and recharging with fresh nutrient medium having the same sorbitol concentration. Four cycles of harvesting and recharging were implemented. At the end of the fourth cycle (26 hours) 2.26 kg of sorbose was produced with an increased average productivity of 19.31 kg $m^{-3}h^{-1}$.

Notation

γ – mass concentration, kg m^{-3}
ψ – volume ratio, m^3 m^{-3}

References

1. *Bošnjak, M., Pintar, P., Gomerčić, K.*, Med. Fac. Landbouww. Rijksuniv. Gent. **56** (1991) 1723
2. *Bonomi, A., Augusto, E. F. P., Barbose, N. S., Mattos, M. N., Magossi, L. R., Santos, A. L.*, J. Biotechnol. **31** (1993) 39
3. *Srivastava, A. K. and Lasrado, P. R.*, Bioproc. Eng. **18** (1998) 457
4. *Mori, H., Kobayashi, T., Shimizu, S.*, J. Chem. Eng. Jpn. **14** (1981) 65
5. *Giridhar, R., Srivastava, A. K.*, Biotechnol. Bioprocess Eng. **5** (2000) 340
6. *Weighand, W. A.*, Biotechnol. Bioeng. **23** (1981) 249
7. *Pirt, S. J.*, Principles of microbe and cell cultivation, Blackwell Scientific, Oxford, 1975
8. *Bošnjak, M., Topolovec, V. Johanides, V.*, Biotechnol. Bioeng. Symp. **9** (1979) 155
9. *Peringer, P. Blachere, H. T.,* Biotechnol. Bioeng. Symp. **9** (1979) 205
10. *Mori, H., Yamane, T., Kobayashi, T. Shimizu, S.*, J. Ferment. Technol. **61** (1983) 391
11. *Bull, D. N., Young, M. D.*, Biotechnol. Bioeng. **23** (1981) 373