High xylitol production rate of osmophilic yeast *Candida tropicalis* by long-term cell-recycle fermentation in a submerged membrane bioreactor

Seun-Gyu Kwon, Seung-Won Park[1], Deok-Kun Oh*

Department of Bioscience and Biotechnology, Sejong University, Seoul 143-747, Korea

[1]Sugar, Flour & Oil BU, CJ Cooperation, Seoul 152-050, Korea

Tel: (02) 3408 3767, Fax: (02) 3408 3899

Abstract

Candida tropicalis, an osmophilic strain isolated from honeycomb, produced xylitol at a maximal volumetric production rate of 3.5 g l^{-1} h^{-1} from an initial xylose concentration of 200 g l^{-1}. Even with a very high xylose concentration, *e.g.*, 350 g l^{-1}, this strain produced xylitol at a moderate rate of 2.07 g l^{-1} h^{-1}. In a fed-batch fermentation of xylose and glucose, 260 g l^{-1} of xylose was added, and xylitol production was 234 g l^{-1} for 48 h, corresponding to a rate of 4.88 g l^{-1} h^{-1}. To increase the xylitol production rate, cells were recycled in a submerged membrane bioreactor with suction pressure and air sparging. In cell-recycle fermentation, the average concentration of xylitol produced per recycle round, total fermentation time, volumetric production rate, and product yield for ten rounds were 180 g l^{-1}, 195 h, 8.5 g l^{-1} h^{-1}, and 85%, respectively. When cell-recycle fermentation was started with the cell mass contratrated two-fold after batch fermentation and was performed for ten recycle rounds, we achieved a very high production rate of 12 g l^{-1} h^{-1}. The production rate and total amount of xylitol produced in cell-recycle fermentation were 3.4 and 11 times higher than in batch fermentation, respectively.

Introduction

Xylitol, a natural functional sweetener, has sweetness equal to sucrose, and can replace sucrose on a weight-weight basis and it promotes oral health and caries prevention. Xylitol has found increasing use in the food industry due to these properties. Xylitol is also an important sugar substitute for diabetics because it does not need insulin to regulate its metabolism.

Xylitol is manufactured by the chemical reduction of the five-carbon sugar xylose, which is one of the major components of hemicellulose hydrolysates. For the industrial microbiological production of xylitol, it is very important to increase the volumetric production rate for a long period. The volumetric production rate can be improved both by the specific production rate and the cell concentration because the specific production rate multiplied by the cell concentration is the volumetric production rate. The increase of specific production rate is in the domain of microbial genetics such as screening and mutant selection of a high producing strain. The increase of cell concentration can be rapidly obtained by using cell recycle fermentation.

Candida tropicalis cells were recycled for 14 rounds by centrifugation. The long-term cell-recycle fermentation for the production of xylitol showed a 2.4 times higher volumetric production rate than did simple batch fermentation because shorter fermentation time and higher substrate consumption rates were obtained in cell recycle fermentation due to the higher cell concentration obtained by centrifugation. However, the centrifugation method is usually too complex to be economically on small scale and is difficult to maintain aseptic. The application of centrifugation method to commercial-scale production is also difficult due to the high cost of equipment.

Using isolated osmophilic *Candida tropicalis, we attempted to produce xylitol* at a high volumetric production rate by optimizing the initial xylose concentration and by fed-batch fermentation involving feeding xylose and glucose. Although many studies have examined the use of submerged membrane bioreactors with suction pressure and air sparging to treat wastewater, a submerged membrane bioreactor has not yet been applied to metabolite production. Therefore, the osmophilic yeast was recycled with non-concentrated and two-fold concentrated cells in a submerged membrane bioreactor using suction pressure and air sparging.

Results and Discussion

Cell-recycle fermentation in a submerged membrane bioreactor

To prevent membrane fouling and cell damage and to supply the cells with air, the cells in this study were separated using out-in vacuum suction and air sparging filtration (Fig. 1). In the submerged membrane bioreactor, xylitol was produced using cell-recycle fermentation. All the cells were re-used and the cell mass was either not concentrated or concentrated two-fold after batch fermentation. The xylitol production in both cell-recycle fermentation processes did not decrease until the cells had been recycled more than ten rounds. The protein concentration of the filtrate in the hollow fiber membrane system remained below 0.04 mg ml^{-1} after ten recycle rounds, and there was no fouling during cell concentration. When the number of cell-recycle rounds was increased, the fermentation time per round decreased due to the increased cell mass.

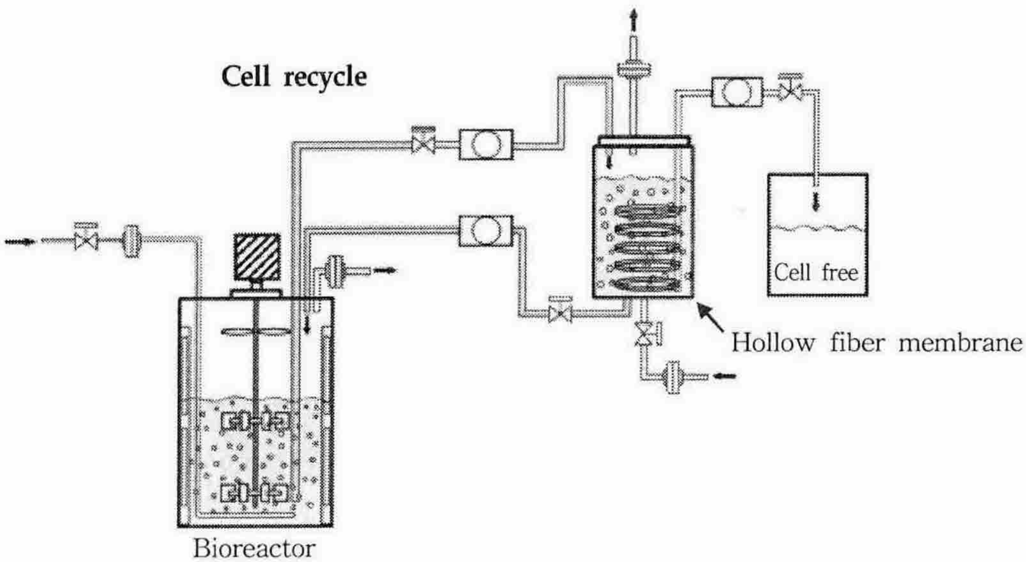

Fig. 1. Schematic diagram of a submerged membrane bioreactor with suction pressure and air sparging.

In cell-recycle fermentation, the average concentration of xylitol produced per recycle round, total fermentation time, and product yield for ten recycle rounds were 180 g l^{-1}, 195 h, and 84%, respectively (Fig. 2).

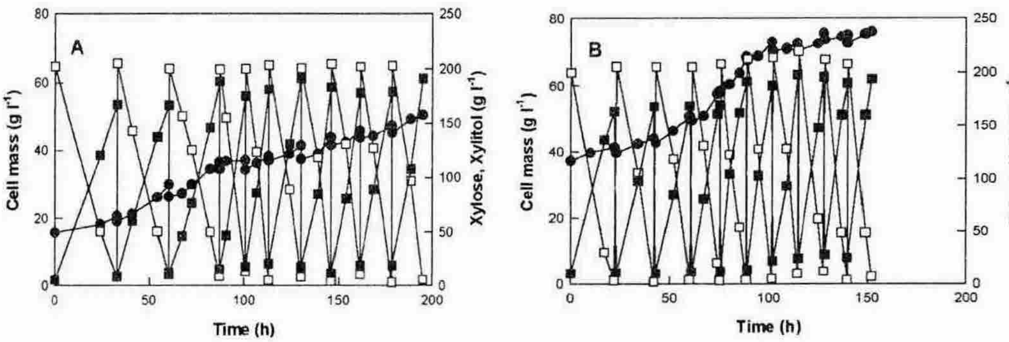

Fig. 2. Xylitol production by cell recycle fermentation in a submerged membrane bioreactor without the addition of glucose. (A) The re-use of all non-concentrated cells (B) and two-fold concentrated cells after batch fermentation. (cell mass, xylose, glucose, xylitol)

When the initial cell mass was concentrated two fold, the xylitol production rate increased from 9.26 to 12 g l^{-1} h^{-1}. The average concentration of xylitol produced per recycle round, fermentation time, and product yield for ten recycle rounds in cell-recycle fermentation with the cell mass concentrated two-fold were 182 g l^{-1}, 152 h, and 85%, respectively. The volumetric production rate and total amount of xylitol produced in the cell-recycle fermentation using a cell mass initially concentrated two-fold in a submerged membrane bioreactor increased 3.4 and 11 times, respectively, compared with batch fermentation. A 2.4-fold increase in the production rate was reported with cell-recycle fermentation using centrifugation as compared to batch fermentation. Since the cells were re-used to inoculate the next recycle round, a higher initial cell concentration was maintained in the submerged membrane bioreactor than with batch fermentation. Less time was required to bioconvert a given amount of xylose (initially, about 200 g l^{-1} per recycle round) into xylitol in the recycle-fermentation mode than in the batch-fermentation mode due to the increasingly greater initial biomass with recycle fermentation. The xylitol yield from xylose in cell-recycle fermentation (85%) was 2% greater than with batch fermentation (83%) because less xylose was needed to grow cells in the cell-recycle method. The volumetric production rate can thus be improved by increasing the cell concentration, as was rapidly obtained in this study by recycling all cells.

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