Characterization of two substrates fermentation processes for xylitol production using recombinant *Saccharomyces cerevisiae* containing xylose reductase gene

이우종, 유연우[1], 서진호[2]
삼양제넥스 생명공학연구소, [1] 아주대학교 생물공학과, [2] 서울대학교 식품공학과
전화 (0331) 290-2583, FAX (0331) 293-4789 (서진호)

Abstract

Fermentation characteristics of recombinant *Saccharomyces cerevisiae* containing the xylose reductase gene from *Pichia stipitis* were analyzed in an attempt to convert xylose to xylitol, a natural five-carbon sugar alcohol used as a sweetener. Xylitol was produced with a maximum yield of 0.95 (g xylitol/g xylose consumed) in the presence of glucose that is used as a cosubstrate for cofactor regeneration. However addition of glucose caused inhibition of xylose transport and accumulation of ethanol. Such problems were solved by adopting glucose-limited fed-batch fermentation. This process done with *S. cerevisiae* EH13.15:pY2XR at 30 ℃ resulted in 105.2 g/L xylitol concentration with maximum productivity of 1.69 g L^{-1} hr^{-1}.

Introduction

Xylitol, a five-carbon sugar alcohol, is a reduction product of xylose and naturally found in plants, fungi, microorganisms and the human body. It has sweetness equivalent to sucrose with an extreme cooling effect and has been used as a sweetening agent in human foods. It is gaining interest as an alternative sweetener due to its functional properties such as reducing the development of dental caries and no need for insulin to regulate its metabolism [1].

On an industrial scale, xylitol is currently produced from xylose derived from hemicellulose hydrolysates by a chemical hydrogenation with metal catalysts under extreme conditions. This process requires an intensive separation process to remove by-products and the yield of xylitol from this process is as low as 50-60% [2].

Research efforts have been focused on biological production of xylitol using yeasts, especially species of genus *Candida* [3]. These xylose-fermenting yeasts use a fraction of xylose for cell growth and endogenous metabolism, resulting in decreased xylitol yield. Xylose can be converted to xylitol by a single-step enzymatic reaction catalyzed by xylose reductase. Since this enzyme requires NAD(P)H as cofactor, an enzymatic conversion of xylose to xylitol is not feasible. However a recombinant *S. cerevisiae* strain transformed with the xylose reductase gene (*XYL1*) could allow the efficient conversion of xylose to xylitol with high yields over 95% because xylitol is not further metabolized to xylulose [4].

In this study the fermentation characteristics of a genetically engineered *S. cerevisiae* strain containing the xylose reducing gene was analyzed and two substrates fermentation process was developed : glucose is used as energy source for cell growth and cofactor regeneration and

xylose used as substrate for conversion to xylitol.

Materials and methods

1) Microorganism

A recombinant yeast strain able to express the xylose reductase gene from *Pichia stipitis* was constructed by using *S. cerevisiae* EH13.15 [*Mat* α□ *trp1*] as host and by introducing plasmid pY2XR [Figure 1]. The xylose reductase gene is expressed by the constitutive glyceraldehyde dehydrogenase promoter.

2) Media

YNB medium [0.67% (w/v) yeast nitrogen base w/o amino acid, 2% (w/v) glucose] was used for batch cultivation of the recombinant *S. cerevisiae* strain and defined medium [5] was used for fed-batch cultivation.

3) Fermentations

Flask cultures were carried out in 500 mL baffled flasks containing 100 mL culture media at 30 ℃ in a shaking incubator at 200 rpm. Fermenter cultures were carried out in a 2.5 L fermenter (Korea Fermenter Co., Korea) with an 1 L working volume. Fermentation was started with 750 mL of the defined medium. An agitation speed was set at 400 rpm and aeration rate at 1 vvm. pH was controlled automatically at 5.0 by addition of 2 N NaOH and 2 N HCl. Growth temperature was maintained at 30 ℃. Foam was removed by the addition of Antifoam 289 (Sigma, U.S.A.). After glucose in the medium was depleted, 250 mL of mixture solution of glucose and xylose was added and feeding of glucose and xylose solution was started.

Results and discussion

Batch fermentation with *S. cerevisiae* EH13.15:pY2XR resulted in 4.34 g/L cell mass, 8.03 g/L xylitol and 0.71 g L^{-1} hr^{-1} xylitol productivity. A xylitol yield was as high as 0.90 g xylitol/g xylose but xylitol production was ceased upon depletion of glucose that is an essential co-substrate for supplying energy sources necessary for endogenous metabolism and cofactor regeneration. So two substrates fed-batch fermentation process feeding glucose was adopted to avoid co-substrate depletion during the bioconversion period. This process increased maximum dry cell mass to 8.02 g/L and xylitol was produced up to 20.8 g/L with increased productivity of 1.57 g L^{-1} hr^{-1}. Product yield was also as high as 0.95 g xylitol/g xylose. However large amount of ethanol, which is a toxic by-product converted from glucose was accumulated to 38.2 g/L.

Ethanol inhibited cell growth and thereby xylitol production, prevention of ethanol formation is important for bioconversion process. As ethanol formation might be due to the limited oxidation capacity of sugar catabolism it can be prevented by reducing glucose uptake [6]. On the other hand, xylose transport should be increased to enhance xylitol productivity. Glucose and xylose are known to be transported by the membrane transporter through facilitated diffusion and it seemed that they share the same membrane transporter and inhibit the transport of each other

competitively [7]. However the membrane transporter has much higher affinity for glucose than xylose and such a difference might result in ethanol accumulation and low xylitol productivity in the two substrates fed-batch fermentation. So xylose to glucose ratio in the fermentation medium should be raised to facilitate xylose transport rate and decrease that of glucose.

Fed-batch fermentation maintaining xylose concentration 10 times higher than glucose was perfomed. Ethanol productivity decreased to 1.27 g L^{-1} hr^{-1} and 57.7 g/L xylitol was accumulated with improved productivity of 1.85 g L^{-1} hr^{-1}. However high concentration of ethanol (48.2 g/L) was accumulated. Fed-batch fermentation maintaining even higher molar ratio than previous fed-batch culture was carried out [Figure 6]. Maximum cell mass increased to 11.7 g/L and xylitol was accumulated up to 88.9 g/L with a productivity of 1.66 g L^{-1} hr^{-1}. But still high amount of ethanol (27.7 g/L) was accumulated. This result suggests the presence of another transport system which has very high affinity for glucose or transport glucose only and glucose could be efficiently transported by the high affinity glucose transporter without inhibition of xylose even under such a severe glucose-limited condition.

Glucose feed rate was controlled to reduce ethanol formation and glucose was maintained around 0.35 g/L throughout the fermentation. Ethanol productivity decreased to 0.36 g L^{-1} hr^{-1}. but cell mass increased to 12.7 g/L and 105. g/L xylitol was produced with a productivity of 1.69 g L^{-1} hr^{-1} [Figure 2]. It seemed that a sufficient amount of glucose required for cell growth and cofactor regeneration was transported even under very high xylose to glucose ratio condition.

Acknowledgement

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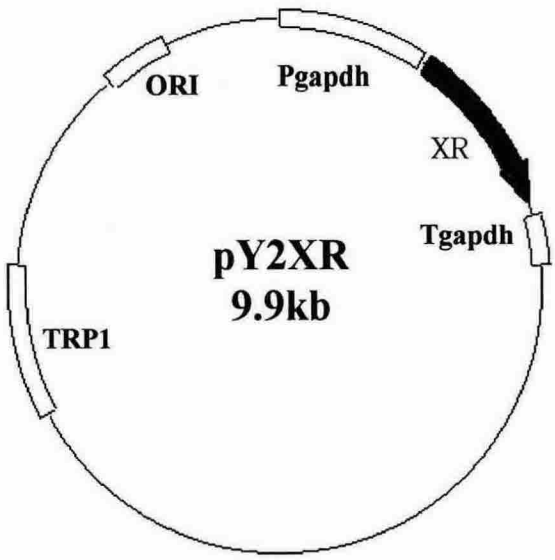

Figure 1. Genetic map of plasmid pY2XR

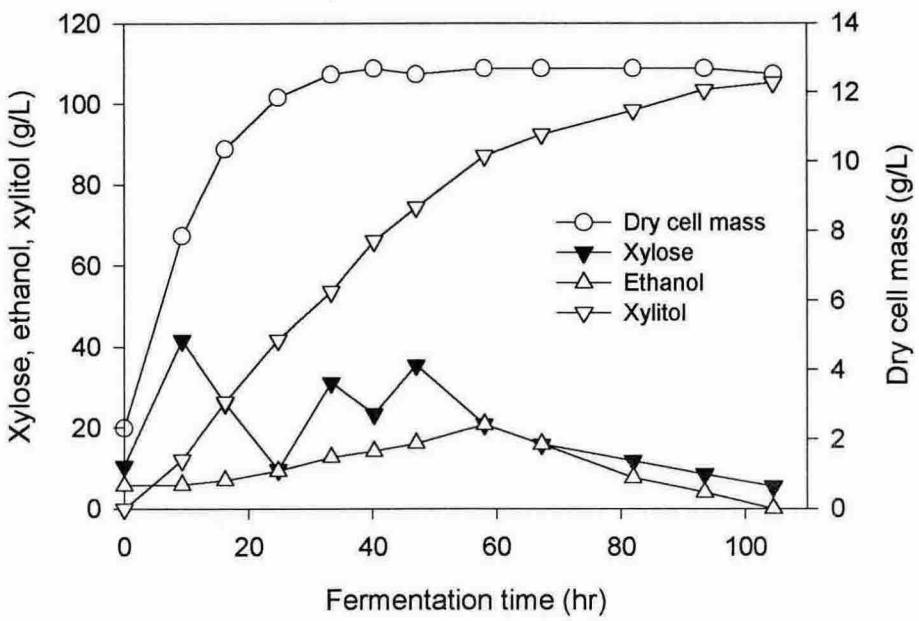

Figure 2. Fed-batch fermentation profiles of *S. cerevisiae* EH13.15:pY2XR in O'Connor's medium at 30 ℃