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A Perspective
Bioproduction of xylitol by enzyme technology and future prospects

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Abstract: Xylitol is a high value sugar alcohol with anticariogenic properties that is used as an ideal sweetener for diabetic patients. Industrially, xylitol is manufactured by catalytic reduction of pure xylose, which has some disadvantages. The fermentation process has been studied as an alternative, but its viability is dependent on the optimization of several variables. This fermentation process on an industrial-scale is not feasible due to decreased productivity. Compared to the fermentation process, enzymatic method is expected to make a substantial increase in productivity. Enzymatic xylitol production from xylose exist in lignocellulosics is an attractive and promising alternative method to the chemical process. The enzymatic method might be able to overcome the disadvantages of the chemical process. This article reviews the literature on the processes for xylitol production and identifies further ways for improved xylitol production to compete with the current chemical process.

Keywords: Xylitol, xylose, yeast, fermentation, xylose reductase

Introduction

Xylitol is a five-carbon sugar alcohol that is naturally found in some fruits and vegetables. The most significant application of xylitol is its use as an ideal sweetener for diabetic patients (Ylikahri, 1979). Other potential uses of xylitol are: as an anticariogenic agent in toothpaste formulations, as thin coatings on vitamin tablets, in chewing gum, soft drinks, mouthwashes, beverages, and in bakery products (Hyvönen and Koivistoinen, 1982; Mäkinen, 1992). Xylitol has received global demand mainly due to its insulin-independent metabolism, anticariogenecity, high sweetening power, and pharmacological properties. Xylitol is currently approved for usage in foods, pharmaceuticals, and oral health products in more than 50 countries (Povelainen, 2008). Xylitol can be produced either by chemical hydrogenation of pure xylose or by biotechnological processes. Currently, xylitol is industrially produced by chemical reduction of pure xylose in the presence of a nickel catalyst at high temperature and pressure (Melaja and Hämäläinen, 1977; Granström *et al.*, 2007). The yield of xylitol is about 50–60% of the xylan fraction and the resultant product is very expensive due to extensive purification procedures (Nigam and Singh, 1995; Parajó *et al.*, 1998). The chemical process is laborious, and cost- and energy-intensive. In view of alternatives to the conventional process, two biotechnological approaches seem promising: the fermentation process and the enzymatic approach. These biotechnological processes are highly attractive alternatives that are able to produce a high-quality and cost-effective product. The fermentation process uses bacteria, fungi, and yeast for xylitol production from xylose or hemicellulosic hydrolysate. Yeasts are considered as the best xylitol producers among the microorganisms investigated. In the fermentation process, the yield of xylitol obtainable from xylose is in a range of 65–85% of the theoretical value (Nigam and Singh, 1995). The application of the fermentation process on an industrial-scale is time-consuming because of some preparatory activities such as sterilization and regular inoculum development. An advantage of the fermentation process over chemical procedures is its lower cost due to the non-necessity of xylose purification (Parajó *et al.*, 1998). However, the fermentation method has not yet been able to accumulate the advantages of the chemical process.

Xylitol production from xylose using enzyme technology can be an attractive alternative to both fermentation and chemical processes. Compared to the fermentation process, the enzymatic approach employing xylose reductase (XR) for xylitol synthesis is expected to obtain a substantial increase in productivity. There are scarce reports on the enzymatic conversion of synthetic xylose to xylitol using XR (Kitpreechavanich *et al.*, 1984; Nidetzky *et al.*, 1996; Neuhauser *et al.*, 1998). The conversion of D-xylose to xylitol is more than 95% by the NADH-dependent XR from yeast (Nidetzky *et al*, 1996). Despite a wide range of applications, the use

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of xylitol as sweetener is limited due to its high price. This has inspired researchers to work toward the development of improved technologies to lower the production costs. In this field, the enzymatic approach to xylitol production from xylose present in the lignocellulosic biomass may provide an alternative for the chemical process. This review attempts to describe the current literature on the processes involving xylitol production, taking into account the chemical and biotechnological processes, microorganisms involved and tries to identify ways to improve enzymatic xylitol production so that it can compete with the chemical process.

Processes for producing xylitol

Chemical process

Xylitol is manufactured industrially by reducing pure xylose, obtained from hardwood or hemicellulosic hydrolysate in the presence of a Raney nickel catalyst. The chemical synthesis of xylitol starts with the extraction of xylose from hemicellulose by acid-catalyzed hydrolysis. After color removal and purification, xylose-rich hemicellulosic hydrolysate can be employed for xylitol production through hydrogenation of xylose at 80–140°C and hydrogen pressures up to 50 atmospheres in the presence of metal catalysts (Raney nickel). The xylitol solution formed requires further purification by chromatography, and then concentration and crystallization of the product to obtain pure xylitol (Melaja and Hämäläinen, 1977). The xylitol yield is only about 50–60% of the xylan fraction and thus the xylitol production process is expensive due to the extensive separation and purification stages (Parajó *et al.*, 1998; Nigam and Singh, 1995).

Biotechnological processes

Fermentation process

The fermentation process uses bacteria, fungi, and yeast for xylitol production from commercial pure xylose or hemicellulosic hydrolysate. The production of xylitol using bacteria and fungi has been studied to a lesser extent compared to that using yeast strains. A few bacteria, such as *Enterobacter liquefaciens* (Yoshitake *et al.*, 1973), *Corynebacteriurn* sp. (Rangaswamy and Agblevor, 2002), and *Gluconobacter oxydans* (Suzuki *et al.*, 2002), have been reported to produce xylitol. There are very few studies regarding xylitol production from D-xylose using filamentous fungi (Ueng and Gong, 1982; Dahiya, 1991). Yeasts are considered as the best xylitol producers among the microorganisms. As a result, yeasts have been studied extensively in the last few decades by several researchers (Gong *et al.*, 1981; Horitsu *et al.*, 1992; Nigam and Singh, 1995; Sampaio *et al.*, 2008). Forty-four yeast strains from the five genera were screened by Barbosa *et al.* (1988) for their ability to convert D-xylose to xylitol. *Candida guilliermondii* and *C. tropicalis* were found to be the best xylitol producers and these yeasts produced 77.2 g l^{-1} xylitol from 104 g l^{-1} xylose using high cell densities and a defined medium under aerobic conditions. The fermentation conditions were optimized by da Silva and Afschar (1994) during continuous cultivation of *Candida tropicalis* for xylitol production. *C. tropicalis* produced xylitol at a yield of 77–80% of theoretical value (0.91 g g^{-1}) in a medium containing 100 g l^{-1} D-xylose.

The screening of different xylose-assimilating yeast has confirmed that the best xylitol producers belong to the genus *Candida*. In the fermentation process using yeast, the yield of xylitol obtainable from D-xylose is in a range of 65–85% of the theoretical value (Nigam and Singh, 1995). The production of xylitol through the fermentation process is limited by certain factors, such as precise control of culture conditions, expensive nutrients, huge water consumption, and the type of process (Sampaio *et al.*, 2008). Thus, the application of the fermentation process on an industrial level is time-consuming, being associated with some preparatory activities such as sterilization and regular inoculum development involving input of energy, labor, and time, leading to decreased productivity. The advantage of the fermentation process over chemical procedures is its lower cost due to the non-necessity of extensive xylose purification (Parajó *et al.*, 1998). The fermentative xylitol production has been studied as an alternative, but its viability is dependent on the optimization of the various fermentation variables such as nutritional composition (substrate, nitrogen source, and micronutrients), the culture and process conditions, as well as the genetic nature of the microorganisms (Prakasham *et al.*, 2009; Branco *et al.*, 2009).

Enzymatic process

The production of xylitol from xylose by using enzyme technology is an alternative and promising approach. The enzymatic conversion of D-xylose into xylitol using xylose reductase (XR) of *Candida pelliculosa* coupled with the oxidoreductase system of *Methanobacterium* sp. has been reported by Kitpreechavanich *et al.* (1984). The authors observed that the xylose was stoichiometrically converted to xylitol with an equivalent consumption of NADPH

and that an almost quantitative conversion of xylose to xylitol was achieved using a $NADP^+$-to-xylose ratio of over 1:30, whereas the coenzyme was successfully regenerated and retained using a membrane reactor. About 90% conversion of xylose to xylitol could be achieved at 35°C and pH 7.5 after a 24 h reaction period. Nidetzky *et al.* (1996) optimized the production of xylitol from xylose by XR from *Candida tenuis* coupled with glucose dehydrogenase from *Bacillus cereus* for regenerating the NADH in an enzyme reactor. In this system, the substrate was converted at concentrations of 300 g l^{-1} xylose, with a 96% yield and xylitol productivity of 3.33 g l^{-1} h^{-1}. Neuhauser *et al.* (1998) reported on the *C. tenuis* XR-mediated NADH-dependent xylose reduction coupled with formate dehydrogenase (FDH) from *C. boidinii* for the byproduct-free recycling of NADH used in a pH-controlled enzyme reactor. In this process, a fed-batch conversion of 0.5 M xylose to xylitol using yeast XR yielded productivities of 2.8 g l^{-1} h^{-1}. To optimize the performance of the XR-catalyzed reactions for xylitol synthesis, the effect of several process variables on productivity needs to be studied: pH, temperature, initial substrate, and coenzyme concentration.

Conclusion and future prospect

Despite the yield of microbiological conversion of xylose to xylitol could be increased by 65–85% using different production methods, the chemical process would still be very competitive in terms of industrial-scale manufacture. The synthesis of xylitol from xylose using XR from yeast is an attractive alternative to chemical and microbial processes. The application of XR constitutes an alternative of economic interest regarding both the chemical reduction of pure xylose and the fermentation of xylose present in the hemicellulosic hydrolysate. During enzymatic production, all experiments were performed using commercial xylose-containing medium. It is certainly still necessary to study the enzymatic conversion of xylose in the lignocellulosic biomass to xylitol and to optimize the reaction conditions. The production of xylitol through the chemical process is expensive due to difficult separation and purification steps. On the other hand, the fermentation process on an industrial-scale is not feasible due to reduced productivity. Hence, it is important to explore alternative methods for the effective production of xylitol using XR enzyme. The enzymatic approach might be able to overcome the disadvantages of the chemical process that is largely being used at present and also the fermentation process that is under investigation.

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