

CHAPTER 1

INTRODUCTION

1.1 Introduction

Xylitol is a five-carbon sugar alcohol, a natural carbohydrate which is widely distributed in land based plants, especially in certain fruits and vegetables. Xylitol has been used in a wide range of products. It can be used as an additive in food industry, in pharmaceutical industry, health food, beverages, cake, gums as sweetener and in light industry as raw material. Xylitol can also be used clinically as a sugar substitute for treatment of diabetes. However, the small amounts present in natural sources render its quantitative extraction difficult and uneconomical. The commercial process for xylitol production is now based on chemical reduction of xylose derived from hydrolysates of hemicellulose. The limiting step of this process, the xylitol purification and separation to remove other polyoles and sugars, again increases the production cost.

Xylitol can also be produced by fermentation in the D-xylose metabolism of microorganisms. A variety of microorganisms have been used for xylitol production including *Candida boiinii*, *C. guillermondii*, *C. parapsilosis*, *C. tropicalis*, *C. utilis*, *C. blankii*, *Debaryomyces hansenii*, *Kluyveromyces fragilis*, *K. marxianus*, *Saccharomyces* sp. and *Petromyces albertensis* (Skoog & Hahn-Hägerdal, 1988; Dahiya, 1991; Vandeska *et al.*, 1995a&b). Among them, the yeasts, especially those belonging to *Candida* species have been reported to produce xylitol with good yield either from synthetic sugar solutions or hydrolysates (Pfeifer *et al.*, 1996; Roberto *et al.*, 1996; Mayerhoff *et al.*, 1997; Kim *et al.*, 1999).

The key enzymes for xylitol production in yeasts are D-xylose reductase which uses either NADH or NADPH to reduce D-xylose to xylitol, and predominantly, NAD-linked xylitol dehydrogenase which re-oxidises xylitol to D-xylulose (Nishio *et al.*, 1989; Hahn-Hägerdal *et al.*, 1994; Vandeska *et al.*, 1995b). However, higher yields are difficult to achieve because part of the xylose consumed is utilised for the production of cell mass and by-products such as ethanol. Other problems with xylose-utilising yeasts are their poor resistance to inhibitors in non-detoxified hemicellulose

hydrolysates. Furthermore, xylitol accumulation in yeasts is sensitive to environmental conditions such as nutrition, temperature, pH, inoculum, substrate and aeration which is critical for yeast growth and fermentation (Nolleau *et al.*, 1993; Preez, 1994; Lu *et al.*, 1995; Nigam & Singh, 1995; Yahashi *et al.*, 1996; Kim *et al.*, 1997; Silva *et al.*, 1998; Saha & Bothast, 1999).

Lignocellulosic biomass, comprising cellulose, hemicellulose, and lignin, is one of the most abundant renewable resources and hemicellulose is a major fraction in such material which can be easily hydrolysed to a mixture of reducing sugars containing a high proportion of D-xylose. This pentose is a potential source for the production of useful xylitol by microbial fermentation (Roberto *et al.*, 1994; Domínguez *et al.*, 1996; Parajó *et al.*, 1996 & 1997). Also lignocellulosic sugarcane bagasse, the fibrous residue derived after the extraction of sugar from sugarcane, is a good source of D-xylose. It is an agricultural residue available in large quantities in Thailand (Agricultural Statistic Center of Thailand, 1999). This waste can be hydrolysed using acid to obtain a mixture of fermentable sugars with xylose as the major components. However, some by-products in the hydrolysate, such as acetic acid, furfural and phenolic compounds, can present and be potential inhibitors of microbial metabolism.

An attempt to overcome those above problems, a recombinant strain of *Saccharomyces cerevisiae* transformed with the xylose-reductase-encoding XYL1 gene from the yeast *Pichia stipitis* CBS 6054 was generated by Hallborn *et al.* (1991) and used as a xylitol producing yeast. This organism is able to grow anaerobically and show high tolerance to inhibitors presented in lignocellulosic hydrolysates (Hallborn *et al.*, 1991; Hallborn *et al.*, 1994; Meinander *et al.*, 1994; Roca *et al.*, 1996). In 1995, Billard *et al.* has isolated and characterised the gene encoding xylose reductase from *K. lactis*. Also, Lee *et al.* (2003) has studied and described the cloning of the *xyll* gene from *C. parapsilosis* mutant strain KFCC-10875 encoding an XR with unusual coenzyme specificity and its functional expression in *C. tropicalis*, an organism currently used for industrial xylitol production. Woodyer *et al.* (2005) has purified, and characterised of a highly active xylose reductase from *Neurospora crassa* which may be used for the production of xylitol. Furthermore, Fed-batch culture techniques also has been reported to provide better yields and productivity in the production of

xylose by yeast strains (Meinander *et al.*, 1994; Vandeska *et al.*, 1996; Bae *et al.*, 2004).

The aim of this work is to improve the productivity of xylitol from sugar cane bagasse with the biotechnological process. An appropriate physicochemical treatment of bagasse using acid hydrolysis was employed to provide a high content of xylose and to convert the hydrolysate as a suitable medium. The 3 strains of yeast; *Candida guilliermondii* 5068, *Kluyveromyces marxianus* 5057 and *Hansenula anomala* 5302 (purchased from MIRCEN, Thailand) were examined and selected for the maximal xylitol production based on the previously reported for efficiently xylitol production in sugarcane bagasse hydrolysate.

Experiments through genetic manipulation techniques using polymerase chain reaction (PCR) and oligonucleotide primers were carried out to increase xylitol productivity. The recombinant yeast was investigated for xylitol productivity compare with those wild types. Finally, fermentation conditions and various controlled process parameters, such as aeration and agitation rates, were also analysed and presented for further development to increase the productivity of xylitol from sugarcane bagasse hydrolysate using batch process.

1.2 Objectives of the research

1.2.1 To study the effect of acid hydrolysis on xylose concentration from sugarcane bagasse.

1.2.2 To select the efficiency pentose - fermenting yeast strains in Thailand for xylitol production.

1.2.3 To study the xylose reductase (XR) gene characteristics from the selected yeast and to increase the XR one using genetic manipulation technique.

1.2.4 To investigate the growth and xylitol production in sugarcane bagasse hydrolysate by the selected yeasts and the cloned yeasts.

1.2.5 To determine the productivity of xylitol in sugarcane bagasse hydrolysate by the cloned one under agitation and aeration in bioreactor.