Bio-production of a Polyalcohol (Xylitol) from Lignocellulosic Resources: A Review

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Written for presentation at the
CSBE/SCGAB 2006 Annual Conference
Edmonton Alberta
July 16 - 19, 2006

ABSTRACT

Lignocellulosic materials being supplied from a variety of resources at low price can be used as feedstock for chemicals and bio-products. Xylitol is a high value polyalcohol produced by the reduction of D-xylose (from hemicellulose fraction of lignocellulose) and is employed in food and pharmaceutical industries. The large number of advantageous properties, such as its low-calorie sweetening power and anticariogenicity justifies the high industrial interest for xylitol. Biotechnological production of this substance is lately becoming more attractive than the chemical method of catalytic hydrogenation due to the higher yield and because downstream processing is expected to be less costly. Studies about the bio-production of xylitol, in which microorganisms or enzymes are involved as catalysts to convert xylose into xylitol under mild conditions of pressure and temperature, have been mostly focused on establishing the operational parameters and the process options that maximize its yield and productivity in free cell system. However, some gaps in knowledge exist regarding this bioconversion process in immobilized cell system and selection or making an appropriate carrier (support) for biocatalysts in fermentation medium.

Keywords: Xylitol, xylose, hemicellulose, lignocellulosic hydrolyzates, fermentation, hemicellulose
1. Introduction

Recent studies indicate that xylitol can reduce the occurrence of dental caries in young children, schoolchildren, and mothers, and in children via their mothers. *Streptococcus mutans* levels in saliva and plaque are decreased by short-term consumption of xylitol. Xylitol may also decrease the transmission of *Streptococcus mutans* from mothers to children (Lynch 2003). Xylitol is compatible and complementary with all current oral hygiene recommendations. Its appealing organoleptic and functional properties enhance a wide array of applications that promote oral health (Peldyak 2002). It also promotes remineralization and thickening of mineral crystals at deep layers of the tooth enamel (Miate 2003).

Xylitol can also be used in the diet of diabetics, because it is slowly absorbed through the intestinal system, its initial metabolic steps are independent of insulin, and it does not cause rapid changes in blood glucose concentration (Förster 1974). It can be metabolized in the absence of insulin and can replace sugar (sucrose) on a weight for weight basis making it a suitable sweetener for diabetic patients (Cao et al. 1994). Obesity can also be restricted by regular consumption of xylitol (Parajo et al. 1998a).

According to some recent studies, xylitol also appears to have potential as treatment for osteoporosis (Mattilla et al. 2001) because the chelators, which form complexes with cations with intermediate stabilities, seem to be the most suitable promoters of the absorption. This is also the case concerning complexes with calcium and xylitol (Hämäläinen & Mäkinen 1989). Xylitol has many advantages as a food ingredient or additive. It does not undergo a detrimental Maillard reaction which is responsible for both darkening and reduction of nutritive value of proteins due to the destruction of amino acids. It limits the tendency to obesity when continuously supplied in the diet and the sensory properties of products are enhanced if it is involved in formulations without causing undesirable changes in physical and chemical properties during storage (Parajo 1998a).

Many extensive researches on the dietary and technological properties of xylitol have been fostered because of its growing market and high added-value (Parajo 1998a). Agricultural and forestry products consist mainly of lignocellulosics as organic materials. Availability and abundance of forestry products and renewable agricultural residues offer advantage to use D-xylose as a major carbon source. D-xylose and L-arabinose constitute 95% of arabino-xylan hemicelluloses in plant tissues on dry basis and pentosans constitute around 19-33%, 10-12% and about 40% (dry weight basis) in hardwoods, softwoods and agricultural residues, respectively (Winkelhausen and Kuzmanova 1998). Some sources of xylose from agricultural residues include rice straw (Roberto et al. 1996), barley straw (du Preez 1994), and seed coats of wheat, corn, rice, soybeans, and oats (Whistler, 1993).

Recently, some biotechnological activities have been focused on the production of xylitol from D-xylose. It has been closely related to bio-ethanol and for years it was considered only as a by-product in ethanol fermentation process from D-xylose. It prepared the background for production of ethanol and polyols using microbial method from aldopentoses. Screening microorganisms based on their potential for xylitol production was the first activities carried out by researchers, but due to its unique properties, increasing demand and growing awareness of environment protection, the real scientific interest began in the last few years (Winkelhausen 1998).
In this paper, the metabolism of xylose by microorganisms, variables and process parameters affecting bioconversion of xylose to xylitol in defined media and complex media of lignocellulosic hydrolysates using free and immobilized cell systems are reviewed.

2. Natural Occurrence, Chemical Structure, and Physical Properties of Xylitol

Xylitol is a naturally occurring five-carbon sugar alcohol (Figure 1) that has the same sweetness and one-third the caloric content of conventional sugar (Heikkila et al. 1992). Xylitol can be found in small quantities in various plants, fruits, and vegetables. Xylitol can be produced by chemical or microbial reduction of D-xylose or xylan-rich hemicellulose hydrolysates (Figure 2). Sugarcane bagasse, corn fiber, corn cobs, birch wood, oats, cotton-seed hulls, rice straw, and nut shells, are xylan-rich substrates, which can be used as feedstock for commercial xylitol production (Counsell 1978).

![Chemical structure of xylitol.](image)

Figure 1. Chemical structure of xylitol.

3. Methods of Production

Theoretically, xylitol can be produced by extraction, chemical and biotechnological methods which are explained in the following sections and are shown in Figure 2.

3.1 Solid-liquid extraction

Xylitol is found naturally in fruits and vegetables, as well as in yeast, lichens, seaweed, and mushrooms. It can be recovered from these sources by solid-liquid extraction, but its small quantity in the raw materials (less than 900 mg/100 g) is a major economic drawback for this method (Pepper and Olinger 1988).

3.2 Chemical production of xylitol

This process begins with the acid-catalyzed hydrolysis of xylan (a polysaccharide belonging to hemicellulosic fraction of plant biomass) to produce xylose (Counsell 1977). However, existence of other polymers different from xylan in the hemicellulosic fraction of the biomass produces hydrolysates which contain various impurities including glucose, arabinose, galactose and mannose because of hydrolysis of related polymers. When pure xylose is necessary, costly purification steps are required (Hyvonen et al. 1982). A considerable portion of impurities consists of monosaccharides that are not removable by ion exchange chromatography and activated carbon (Winkelhausen and Kuzmanova 1998). After purification and removal of proteins, color, metal ions, and other
impurities, the xylose-containing hydrolysate is subjected to hydrogenation at 80-140°C and hydrogen pressures of around 5000 kPa in the presence of metal catalysts. The xylitol solution produced by reduction process requires further purification by chromatographic fractionation and then concentration and crystallization of the product to obtain pure xylitol (Hyvonen et al. 1982). Among all the steps, purification and separation are the most expensive. Xylitol yield is around 50-60% of the initial xylose (Nigam and Singh, 1995). The chemical method of xylitol production based on the catalytic hydrogenation of xylose-rich hemicellulose hydrolysate is briefly shown in Figure 2. The major drawbacks of conventional production method, especially high pollution levels and waste-treatment concerns motivated researchers to find alternatives for xylitol production and one of the most interesting procedures is microbial production (Winkelhausen and Kuzmanova 1998).

![Chemical Method Diagram](image)

Figure 2. Xylitol production methods (Parajo et al. 1998a).
3.3 Microbial production of xylitol

Xylitol is produced from D-xylose by NADPH-dependent xylose reductase as a metabolic intermediate in microorganisms that are able to utilize xylose (Saha 1997). A number of yeasts and molds can produce xylitol because they possess the enzyme xylose reductase. *Candida guilliermondii*, *Candida tropicalis*, *Candida pelliculosa*, *Candida boidinii*, and genera of *Saccharomyces*, *Debaryomyces*, *Pichia*, *Hansenula*, *Torulopsis*, *Kloeckera*, *Trichosporon*, *Cryptococcus*, *Rhodotorula*, *Monilia*, *Kluveyromyces*, *Pachysolen*, *Ambrosiozyma*, and *Torula* are some of the yeasts with xylitol production capability (Saha 1997).

Some bacteria species such as *Enterobacter liquefaciens*, *Corynebacterium* sp., and *Mycobacterium smegmatis* can also produce xylitol (Horitsu et al. 1992). The conversion of D-xylose to xylitol by microorganisms is important for industrial production and it has been extensively studied in yeasts compared to the other microorganisms. In the biochemical pathway, D-xylose is converted into xylitol as an intermediate material (by xylose reductase activity) to be transformed to xylulose or directly is converted to xylulose. Then, xylulose-5-phosphate is generated by the activity of xylulose kinase and goes through pentose phosphate pathway.

3.3.1 Production of xylitol by bacteria

Most bacteria possess the enzyme xylose isomerase, which is specifically able to transform xylose into xylulose. In the second stage, it is phosphorylated into D-xylulose-5-phosphate (a common intermediate in the prokaryotes and eukaryotes' metabolism) by xylulokinase. D-xylulose-5-phosphate can be incorporated into the pentose-phosphate pathway or converted into glyceraldehyde-3-phosphate and acetyl-phosphate by xylulose-5-phosphate phosphoketolase. Similar to the metabolism of glucose by yeasts, this step produces an intermediate product of glucolysis without production of nicotinamide adenine dinucleotide phosphate (NADPH) (Evans and Ratledge 1984). In addition to xylose-isomerase, bacteria have oxido-reductive enzyme system that reduces xylose into xylitol, with further oxidation to xylulose. Using an *Enterobacter* strain in experiments leading to xylitol, Yoshitake et al. (1973) obtained a yield of 33.3 g xylitol L⁻¹ with volumetric productivity of 0.35 g L⁻¹ h⁻¹ in a medium containing 100 g initial xylose L⁻¹. In contrast, while performing fermentation with *Corynebacterium* sp., the addition of gluconate hindered xylitol production.

3.3.2 Production of xylitol by molds

Bio-production of xylitol by molds has been studied to a less extent compared to production by bacteria and yeasts. *Petromyces albertensis* was used as the biocatalyst for xylitol reduction by Dahiya (1991) and a yield of 0.4 g g⁻¹ xylose resulted after 10 days of fermentation. Ueng and Gong (1982) observed low yield of xylitol in fermentation with *Mucor* sp. on a hydrolysate from sugarcane bagasse hemicellulose. Chiang and Knight (1961) reported that *Penicillium*, *Aspergillus*, *Rhizopus*, *Byssochlamys*, *Glicoladium*, *Myrothecium*, and *Neurospora* sp. have the capability of producing low amount of xylitol from xylose.

3.3.3 Production of xylitol by yeasts

Some strains of yeasts are able to convert xylose into D-xylulose through an oxido-reductive transformation consisting of two consecutive reactions. In the first stage, D-xylose is transformed into the intermediate xylitol with xylose-reductase (XR), in the presence of nicotinamide adenine dinucleotide (NADH) or NADPH (Taylor et al. 1990).
In the second stage, xylitol is transformed (oxidized) into D-xylulose by either NAD⁺-linked or NADP⁺-linked xylitol dehydrogenase (XDH) (Girio et al. 1990; Prior et al. 1989).

![Diagram of D-xylose metabolism in yeasts](attachment:image)

Different yeast species show different abilities for fermenting xylulose to ethanol or xylitol (Jeffries 1981). When oxygen levels are too low, a redox imbalance occurs as the NADH produced in the xylitol dehydrogenase step cannot be re-oxidized back to NAD. This low-oxygen induced imbalance is due to a decreased respiration rate, which limits production of NAD⁺. Therefore, the alternate ethanol route is selected at the end of the pentose phosphate pathway. However, as the pentose phosphate pathway is still active, NADP⁺ conversion to NADPH continues (Hahn-Hagerdal et al. 1994). The resulting redox imbalance improves the accumulation of xylitol in the XR/XDH system, due to the limited supply of NAD⁺ for utilization by XDH. For higher xylitol accumulations in *D. hansenii*, semi-anaerobic conditions were essential to lower the NAD/NADH ratio in the system.
oxidation step of xylitol to xylulose. So non-regeneration of NAD in oxygen-limited conditions results in accumulation of xylitol and its subsequent excretion into the medium (Saha and Bothast 1997). Yeasts with XR activity linked to both NADH and NADPH, like *Pichia stipitis*, under anaerobic and oxygen-limited conditions can regenerate the NAD\(^+\) consumed in the second step of the xylene metabolism. Therefore, no xylitol accumulation occurs between the cofactors of XR and XDH and the major product in this situation is ethanol. In contrast, yeasts, like *Debaryomyces hansenii*, that consume xylene by XR activity, which is only dependent on NADPH (with complete absence of NADH-linked XR) in the first step of the xylene conversion, can produce xylitol as the major product (Girio et al. 1990). In the second step, xylitol usually in the presence of NAD\(^+\) is oxidized to xylulose (Vandeska et al. 1995b). The presence of either high XR or low XDH activities is considered as a criterion for selecting xylitol-producing microorganisms (Parajo 1998a).

Barbosa et al. (1988) calculated xylitol yield when NADH\(^2+\) is used as cofactor for XR under aerobic conditions in nutritionally balanced media as follows:

\[
126 \text{ xylose} + 3 \text{ O}_2 + 6 \text{ ADP} + 6 \text{ Pi} + 48 \text{ H}_2\text{O} \rightarrow 114 \text{ xylitol} + 6 \text{ ATP} + 60 \text{ CO}_2
\]

with a theoretical product yield of 0.905 mol xylitol/mol xylene. Under similar hypotheses, the same researchers reported the following mechanism for anaerobic conditions:

\[
48 \text{ xylose} + 15 \text{ H}_2\text{O} \rightarrow 42 \text{ xylitol} + 3 \text{ ethanol} + 24 \text{ CO}_2
\]

with a theoretical product yield of 0.875 mol xylitol/mol xylose. The dissolved oxygen concentration is a controlling parameter that affects the operational aspects of xylitol production by yeasts (du Preez 1994).

4. **Parameters of Fermentation**

Fermentation process as the main stage in xylitol production is controlled by several factors including substrate concentration, carbon source, salts and nitrogenous compounds, inoculum, aeration rate, temperature and pH that are explained in the following sections:

4.1 **Xylose concentration**

It has been proven that substrate concentration (D-xylose) is a critical parameter for yeast growth and fermentation. Xylitol cannot be produced in the absence of D-xylose and combination of aeration with xylitol concentration is an important and determinative factor for xylene reductase and xylitol dehydrogenase activities as well as xylitol formation in yeasts. The initial xylose concentration can influence its production by microorganisms. Increased xylose concentrations in the cultures of microorganisms which are able to tolerate high sugar and higher osmotic pressure lead to increased xylitol yields and production rates. Increase in initial xylose concentration usually led to decrease in growth rate, unless the aeration rate was increased (Nolleau et al., 1993). Horitsu et al. (1992) investigated the effect of D-xylose concentration on the production of xylitol for *C. tropicalis* by varying the concentration from 100 g l\(^{-1}\) to 300 g l\(^{-1}\). The maximum yield of xylitol was obtained at D-xylose concentration of 172 g l\(^{-1}\). Walther and co-workers (2001) reported that at high initial xylose concentrations and high aeration rate, *Canadida tropicalis* ATCC 96745 cells grew vigorously at the beginning of
fermentation and production rate was improved. However, xylitol yield was reduced at lower initial xylose concentration and high levels of dissolved oxygen. They also found that yield decreases at extremely high initial xylose concentrations which is attributed to the osmotic stress that could be induced in the microorganism at high concentration of sugar in the medium. It was also concluded that an appropriate manipulation of initial substrate concentration and aeration could probably result in desirable xylitol yields.

4.2 Carbon source

According to studies, carbon sources rather than xylose are effective in xylitol production. Yahashi et al. (1996) supplemented D-xylose with D-glucose for C. tropicalis cultivation. At the initial stage of fermentation, D-glucose was utilized for cell growth and then D-xylose was consumed. Xylitol yield and productivities increased 1.2 to 1.3 times by the addition of glucose to the fermentation media. This condition resulted in 104.5 g l\(^{-1}\) xylitol in 32 h and a yield of 0.82 g g\(^{-1}\) xylose. However, in an investigation by Silva et al. (1996) for Candida guilliermondii FTI 20037, addition of glucose to the fermentation medium decreased the yield of the product from 0.66 g g\(^{-1}\) to 0.45 g g\(^{-1}\).

A similar result was reported by Ooi et al. (2002) for the effect of glucose on xylitol yield and production rate of C. guilliermondii during fermentation. Furthermore, it was observed that using glucose as co-substrate prolonged the process. In a research by Lee et al. (2000) using a recombinant S. cerevisiae, fed-batch fermentation was proposed to overcome this problem. Glucose concentrations in the medium should be very low for Candida tropicalis to achieve effective xylitol production (Walther et al. 2001). In fermentation media containing substantial amount of glucose, aerobic conditions should be adopted to reach higher yields and productivities, while in the absence of glucose, microaerobic conditions improves yield of xylitol. In contrast to glucose as the co-substrate, arabinose appears to be an inducer of xylose reductase enzyme in that high arabinose concentrations enhanced both yield and productivity of xylitol in the experiments.

4.3 Nitrogen source

Yeast extract, an organic source of nitrogen, is an important nutrient for some xylitol-producing yeasts, while for some other yeasts has no significant effect on xylitol formation. These yeasts prefer urea or urea and Casamino acids for better yield (Winkelhausen and Kuzmanova, 1998).

Table 1. Effect of nitrogen source on xylitol yield and dry cell mass formed by Candida boidinii.*(Vandeska et al. 1995a).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nitrogen source</th>
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<tbody>
<tr>
<td></td>
<td>(NH(_4))(_2)SO(_4)</td>
</tr>
<tr>
<td>C(_{cm}) (g l(^{-1}))</td>
<td>5.88</td>
</tr>
<tr>
<td>Yx/s (g g(^{-1}))</td>
<td>0.06</td>
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C\(_{cm}\): Dry cell mass    Yx/s: Xylitol yield coefficient (g xylitol per g xylose used)
*Fermentation time was 4 days and each source yielded 1.06 g nitrogen/l. The medium contained (g l\(^{-1}\)): (NH\(_4\))2SO\(_4\), 5.00: KNO\(_3\), 7.65; NH\(_4\)NO\(_3\), 3.03; NH\(_4\)Cl, 4.05; and urea, 2.27.
Xylitol production and xylose utilization by the microorganism is influenced by the nature and concentration of the nitrogen source, and the effective factor on the two parameters mentioned previously is the yeast strain (Parajo 1998b). Vandeska et al. (1995a) observed increased xylitol yields with C. boidinii when the fermentation medium was supplemented with urea compared to those supplemented with ammonium sulfate (Table1).

4.4 Inoculum age and concentration

The rate and yield of fermentation is influenced by the age of inoculum which affects the metabolic activity and viability of the cells (Parajo et al. 1998b). Improvement the yield of xylitol using C. guilliermondii FTI 20037 by inoculum age, inoculum level (concentration) and hydrolysate composition was studied by Felipe et al. (1997). In this study, xylose concentration in the hydrolysate, inoculum level and inoculum age varied from 37.6 g l⁻¹ to 74.2 g l⁻¹, 0.1 to 6.0 g l⁻¹, and 16 to 48 hours, respectively. Maximum xylitol yield of 0.74 g g⁻¹ and productivity of 0.75 g l⁻¹ h⁻¹ was reached for 3.0 g l⁻¹ of 24-h old inoculum at an initial xylose content of 54.5 g l⁻¹. Pfeifer et al. (1996) obtained undesirable results for xylitol productivity and cell growth from C. guilliermondii inocula younger than 15 h or older than 24 h and the best conditions were achieved in the aforementioned range. Cao et al. (1994) examined the influence of initial cell concentration of Candida sp. B-22 on xylitol production from D-xylose and found that the rate of xylitol formation was linearly increased and the fermentation time was dramatically reduced with initial concentrations in the range of 3.8 to 26 g l⁻¹ of inoculum.

4.5 Aeration rate

Due to the diversity of effective parameters and the wide range of aeration levels considered in the literature, a detailed study of the influence of aeration on xylitol production is difficult to perform. Usually, adoption of intermediate values of the aeration rate provides the optimum condition for xylitol yield and productivity. For example, D. hansenii shifts its metabolism towards xylitol production under low aeration conditions (4-22 mmol l⁻¹ min⁻¹), leading to the highest productivity at oxygen transfer rate (OTR), higher than 2 mmol l⁻¹ h⁻¹. This study confirmed that oxygen is an essential component for xylose uptake, since the shift to anaerobic conditions stopped both xylose consumption and metabolic activity. In a related study, the maximum xylitol yield for Candida tropicalis under semi-aerobic conditions, was 0.62 g g⁻¹ xyllose, while under microaerobic conditions, the maximum yield was 0.36 g g⁻¹ substrate. In a medium containing glucose, higher yields and productivities were obtainable under aerobic conditions, while microaerobic conditions improved yields in the absence of glucose. These results can be attributed to increased oxygen demand by the high cell densities achieved in the presence of glucose (Walther et al. 2001).

4.6 Temperature and pH

The most suitable temperature has been reported to be around 30°C for xylitol production by yeasts. With C. tropicalis DSM 7524 small temperature variations above this level, do not significantly affect xylitol yield. When the cells were cultured in a temperature range between 30°C and 37°C the xylitol yield was not dependent on temperature, but at temperatures higher than 37°C the xylitol yield decreased drastically (De Silva and Afschar 1994). Similarly, xylitol formation in C. guilliermondii FTI 20037 was the same at 30 and 35°C, but it decreased with increase of temperature up to 40°C (Barbosa et al. 1988). The optimal initial pH for fermentation depends on the yeast
employed. The optimum pH for *Debaryomyces hansenii* and *Candida* sp. are 5.5 and 4-6, respectively (Cao et al. 1994). The best pH for *C. parapsilosis*, *C. guilliermondii* and *C. boidinii* are 4.5-5, 6.0 and 7.0, respectively. If pH is not controlled, it drops during the fermentation process, therefore, in such conditions, the initial pH values have to be higher than under controlled conditions. For example, the optimal initial pH value for *C. boidinii* under controlled condition is 5.5; while with no control, initial pH should be 7.0 (Winkelhausen and Kuzmanova 1998).

5. Production of Xylitol from Hemicellulose Hydrolysate

Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin and the composition vary according to plant species. The complex structure of lignocellulose in plants forms a protective barrier to cell destruction by bacteria and fungi. To make this structure suitable for conversion in fermentative processes, cellulose, and hemicellulose must be hydrolyzed into their corresponding monomers (sugars) for utilization by microorganisms (Iranmahboob et al. 2002). The main component of the hemicellulosic fraction of hardwoods and agricultural residues is xylan, a polymer made from xylose units that can be hydrolyzed to this sugar by mineral acids or xylanase. Under selected conditions, the solid residue from acid hydrolysis contains both the cellulosic and lignin fractions that can be separated in a further reaction step and utilized for different product applications. Most fermentation studies have focused on hydrolysates derived from acid hydrolysis although the hydrolysis of biopolymers can be performed enzymatically. Hemicellulose is much easier to hydrolyze compared to crystalline cellulose components of biomass because of its relatively low degree of polymerization and heterogeneous structure (Magee and Kosaric 1985). On the other hand, lignin (phenolic fraction), that remains as a solid residue in acid medium both of cellulose and hemicelluloses, can be hydrolysed by acids. Hemicelluloses are more susceptible than cellulose to the hydrolytic action of catalysts due to their open and branched structure. The polysaccharides of the raw material (lignocellulosic substances) have to be hydrolyzed to the corresponding sugars to obtain carbon source for microorganisms in fermentation processes. The most commonly employed catalysts for this purpose are H$_2$SO$_4$ and HCl.

The liquid phase (containing xylose, byproducts, and compounds derived from other fractions of the raw materials such as extractives or lignin) prepared by hydrolysis can be utilized for making fermentation media suitable for xylitol production. Like defined media formulated by chemicals according to the biocatalyst requirement, the concentration of xylose as substrate is an important factor that influences production of xylitol, such that increased concentration of xylose results in improved yield and productivity. However, when lignocellulosic hydrolysates are involved in making culture media, some additional effects related to the concentration of substrate must be considered. By increasing concentration of xylose using evaporation, an inhibition of microbial metabolism and reduction of cell growth occur which is dependent on a simultaneous increase in the concentration of other non-volatile compounds (Olsson and Hahn-Hagerdal 1996). Inhibitors in the hydrolysates resulting from acid catalysis can be classified into different groups including: minerals or metals resulting from the corrosion of the equipment or ions contained in the lignocellulosics, compounds like furfural and hydroxyl methyl furfural derived from degradation of sugars at high temperatures or acetic acid liberated from the acetyl groups in biopolymers, chemicals like aldehydes, phenolic compounds and aromatics which are derived from lignin degradation and compounds derived from extractives. Some other important and effective inhibitory compounds found in hydrolysates are organic acid like syringic, caproic, caprylic,
vanillic, pelargonic and palmitic acids. Some factors such as the microorganism utilized, adaptation potential of the microorganism, mechanism of fermentation process employed, and the simultaneous presence of several other inhibitors are the main parameters that determine the maximum allowable concentration for each process. (Parajo 1998c; Converti 2000; Mussato and Roberto 2001; Mussatto and Roberto 2004). Therefore, in order to make hydrolysates appropriate as fermentation media, acid hydrolysis should be carried out in a way that allows: high concentration of xylose as much as possible, concentrations of inhibitory byproducts in the ranges should be tolerable by the microorganism, and high selectivity towards cellulose degradation (Parajo 1998c).

5.1 Detoxification of hydrolysate

A number of detoxification procedures including biological, physical, and chemical ones have been proposed to convert inhibitors into inactive materials or to reduce their concentration in the culture. The type of the hemicellulose hydrolysate and the species of microorganism employed determine the effectiveness of a detoxification method. Composition of the hydrolysate, varying according to the raw material and the hydrolysis conditions employed, should be considered before choosing a detoxification method (Larsson et al. 1999).

5.1.1 Biological detoxification

Adaptation of a microorganism to the hydrolysate is an interesting biological method for improving the fermentation yield of hemicellulosic hydrolysate media (Felipe et al. 1996; Olsson and Hahn-Hagerdal 1996; Parajo et al. 1998c). In this method, which is based on consecutive fermentations, the microorganism of each experiment is involved as the inoculum for the next one. Adaptation of *Candida guilliermondii* to hemicellulose hydrolysate from rice straw for xylitol production is an effective and also an inexpensive method to alleviate the inhibitory effect of toxic compounds on the xylose-to-xylitol bioconversion (Silva and Roberto 2001). In some other biological methods of detoxification, specific enzymes (laccase and peroxidase enzymes of the white-rot fungus *Trametes versicolor*) or microorganisms are involved acting on the toxic compounds present in the hydrolysates and change their composition and properties (Jonsson et al. 1998).

5.1.2 Physical detoxification

Reducing or removing volatile compounds such as furfural, acetic acid, and vanillin, present in the hydrolysate by concentration using vacuum evaporation is a physical detoxification method. However, the concentration of some non-volatile toxic compounds like lignin derivatives and extractives increases and consequently, the degree of fermentation inhibition could be increased (Mussatto and Roberto 2004). Improving the fermentation process for xylitol production by removing acetic acid, furfural, and other volatile compounds from hemicellulose hydrolysates by evaporation is a suitable method of detoxification (Converti et al. 2000). Larsson et al. (1999) achieved a total removal of furfural from wood hemicellulosic hydrolysate reducing its volume by 90% through vacuum evaporation.

5.1.3 Chemical detoxification

Chemical methods include using ionization inhibitors at certain pH, changing the degree of toxicity of the compounds, and precipitation of toxic chemicals. In other chemical
methods, adsorbents like activated charcoal, diatomaceous earth, and ion-exchange resins are used to adsorb toxic compounds and filter out of the medium (Mussatto and Roberto 2004). Over-liming (increasing the pH to 9±10 with Ca(OH)₂), and readjustment to 5.5 with H₂SO₄, has been described as a chemical pretreatment method for detoxification of lignocellulosic hydrolysates known as alkali treatment. Dilute-acid treatment of hydrolysates from spruce with sodium sulfite, or using a large cell density inoculum have been proven to decrease the concentrations of some toxic compounds, especially furfural and HMF (Palmqvist and Hahn-Hagerdal 2000).

For detoxifying willow hemicellulose hydrolysate before fermentation by recombinant E. coli, a combination of sulfite and over-liming methods was shown to be the most effective (Olsson et al. 1995). Activated charcoal is widely used to remove compounds from the liquid phase by adsorption, and also to purify or recover chemicals. The effectiveness of activated charcoal used for adsorption treatment depends on some factors including: contact time, pH, temperature, and concentration of activated charcoal (Mussatto and Roberto 2004). However, Canilha et al. (2004) demonstrated that xylitol production by C. guilliermondii FTI 20037 using eucalyptus (acid catalyzed) hemicellulosic hydrolysate treated with ion-exchange resins was more successful than the same hydrolysate treated with activated charcoal with pH adjustment. Also, xylitol production from brewer’s spent grain hydrolysate by C. guilliermondii was severely influenced by the concentration of toxic compounds present in the medium in that the untreated concentrated hydrolysate medium, which contained the highest concentration of inhibitors (3.83 g l⁻¹ acetic acid and 10.38 g l⁻¹ total phenolics), the lowest fermentative parameters values were attained (Mussatto et al. 2005). Altogether, since each method of detoxification could be specific to certain types of impurities or toxic materials, better results are achievable by combining two or more different methods (Mussatto and Roberto 2004).

6. Immobilized-cell Fermentation

The choice of a suitable technology for microbiological production of xylitol is fundamental to the optimization and viability of this bioprocess. Among the existing technologies, immobilized cell systems stand out, because they result in high cell concentrations in the reactor and consequently high productivity and yield. Moreover, immobilized cell systems make it possible for cells to be easily recovered for later use (Corcoran 1985). In such systems, biocatalysts are cross-linked together or adsorbed on the surface of a carrier or maybe entrapped in a hydrogel system. The use of a repeated-batch fermentation system for producing xylitol from hemicellulosic hydrolysates, due to promotion a good adaptation of the cells with the medium, is an efficient way to cope with the toxic effects of these inhibitors (Sene et al. 1998). To investigate the efficiency of immobilized cells and the effect of stirred tank reactor (STR) parameters on fermentation parameters, Ca-alginate entrapped Candida guilliermondii FTI 20037 cells were used for xylitol production from sugarcane bagasse hemicellulosic hydrolysate. Optimization of the process was performed by screening design and response surface methodologies. Using a five-fold concentrated hydrolysate, values of 47.5 g l⁻¹, 0.81 g g⁻¹ and 0.40 g l⁻¹ h⁻¹ were obtained for xylitol production, xylitol yield, and volumetric productivity, respectively, in 120 h of fermentation at air flow rate of 1.30 l min⁻¹, agitation speed of 300 rpm, initial cell concentration of 1.4 g l⁻¹, and initial pH of 6.0 (Carvalho et al. 2002). The metabolic behavior of Candida guilliermondii cells entrapped in Ca-alginate beads for batch xylitol production in stirred tank reactor from sugarcane bagasse hydrolysate was investigated by Carvalho et al. (2005). Conditions including agitation speed (300 rpm), air flow rate (1.3 l min⁻¹), initial cell concentration, and starting
pH (6.0) resulted in xylitol production of 47.5 g l\(^{-1}\), yield of 0.81 g g\(^{-1}\) and a productivity of 0.40 g l\(^{-1}\) h\(^{-1}\) from a five-fold concentrated hydrolysate during 120 h of fermentation. Therefore, the lower xylitol productivity in this system compared to that reported for free \textit{C. guilliermondii} using different modes of fermentation, maybe attributed to intra-particle mass transfer limitations.

Throughout the fermentation process, energy requirement increased from 2.1 to 6.6 mol.ATP Cmol\(^{-1}\)DM, demonstrating appearance difficulty by the time for the microbial system. For good performance of the systems using immobilized cells, the right choice of bioreactor is very important. This is possible only after extensive experimental work due to the large number of influencing factors. Some advantages of fluidized bed reactor (FBR) over fixed bed reactors are better mass transfer rates, easier control of operating parameters and lower shear stress compared to stirred tank reactor and lower damage of immobilized cell system (Béjar et al. 1992).

Santos et al. (2005a) studied the influence of aeration rate and carrier concentration on bioproduction of xylitol using \textit{Candida guilliermondii} cells immobilized on porous glass in a fluidized bed reactor (FBR). The results indicated that aeration rate had a positive influence on volumetric productivity (Q\(_p\)) and a negative effect on product yield (Y\(_{p/x}\)), while carrier concentration had a negative influence on both of the fermentative parameters. The highest xylitol concentration (13.1 g l\(^{-1}\)) and the highest product yield (0.38 g g\(^{-1}\)) was attained at an aeration rate of 0.031 min\(^{-1}\) and a carrier concentration of 62.5 g l\(^{-1}\), but the xylitol productivity exhibited the lowest value (0.18 g l\(^{-1}\) h\(^{-1}\)). By increase of only aeration rate to 0.093 min\(^{-1}\) yield value decreased to (0.25 g g\(^{-1}\)), but the volumetric productivity was highest (0.44 g/l h), maybe because of cell metabolism increase.

Xylitol yield and concentration were at the minimum level and the cell concentration was maximum when the highest levels of the aeration rate and carrier concentration were applied, because in this condition cell metabolism was towards cell regeneration. On the other hand, increase in carrier concentration caused the bubbles in the reactor to burst, reducing xylitol accumulation due to enhancing cell growth (Santos et al. 2005a). As is well known, good performance of systems using immobilized cells mainly depends on the right selection of the immobilization carrier. Santos et al. (2003), investigating xylitol production from hemicellulose hydrolysate in a fluidized bed reactor using \textit{Candida guilliermondii} cells immobilized in porous glass, have recently proposed that inhibiting effects and/or mass transfer limitations could affect the performance of such a biosystem, thus experimental optimization is required.

Santos et al. (2005b) studied different adsorbing supports for \textit{Candida guilliermondii} cells to be used for xylitol production from sugarcane bagasse hemicellulose hydrolysate. The carriers employed were: Syrane porous glass beads with 2.53mm diameter in average and pore diameter in the range 60–300 µm, and NaX zeolite. A xylitol concentration of 35.5 g l\(^{-1}\), yield of 0.72 g g\(^{-1}\) and volumetric productivity of 0.49 g l\(^{-1}\) h\(^{-1}\) was achieved in free cell fermentation system, while in immobilized cell systems using porous glass and zeolite lower xylitol concentration (28.8–29.5 g l\(^{-1}\)), yield (0.52–0.53 g g\(^{-1}\)) and volumetric productivity (0.32–0.33 g l\(^{-1}\) h\(^{-1}\)) were observed. The lower fermentative parameters for cells immobilized on porous glass probably was due to the limitation of substrate transfer toward cells compared with the free-cell system (Table 2). Results also indicated that 50% of total cells were entrapped in porous glass, while in zeolite beads (with smaller size pores on the surface) less than 30% adsorbed at the end of batch immobilization tests. Porous glass was saturated by a microbial population around three times higher than in zeolite, leading to detachment of the cells.
Table 2. Results of batch fermentations with cells of *C. guilliermondii* either suspended or immobilized in porous glass and zeolite (Santos et al. 2005b)

<table>
<thead>
<tr>
<th>Method</th>
<th>(P_f) (gl⁻¹)</th>
<th>EtOH (gl⁻¹)</th>
<th>(X_f) (gl⁻¹)</th>
<th>Immob. (%)</th>
<th>(Y_{P/S}) (gg⁻¹)</th>
<th>(Q_P) (gl⁻¹h⁻¹)</th>
<th>(q_P) (gg⁻¹h⁻¹)</th>
<th>(Q_x) (gl⁻¹h⁻¹)</th>
<th>(Q_s) (gl⁻¹h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free cells</td>
<td>35.5</td>
<td>3.97</td>
<td>5.32</td>
<td>–</td>
<td>0.72</td>
<td>0.49</td>
<td>0.092</td>
<td>0.48</td>
<td>0.69</td>
</tr>
<tr>
<td>Porous glass immobilized cells</td>
<td>29.5</td>
<td>4.92</td>
<td>10.5</td>
<td>49.6</td>
<td>0.53</td>
<td>0.33</td>
<td>0.031</td>
<td>0.10</td>
<td>0.62</td>
</tr>
<tr>
<td>Zeolite immobilized cells</td>
<td>28.8</td>
<td>5.18</td>
<td>6.65</td>
<td>28.9</td>
<td>0.52</td>
<td>0.32</td>
<td>0.048</td>
<td>0.063</td>
<td>0.61</td>
</tr>
</tbody>
</table>

\(P_f\) = final xylitol concentration; EtOH = final ethanol concentration; \(X_f\) = final concentration of total cells; Immob = immobilized cells; \(Y_{P/S}\) = xylitol yield factor; \(Q_P\) = xylitol volumetric productivity; \(q_P\) = xylitol specific productivity; \(Q_x\) = cell volumetric productivity; \(Q_s\) = volumetric xylose consumption rate.

7. Xylitol Recovery from Fermented Hydrolysate

One of the most complicated steps in industrial fermentative process is product recovery and purification (Gurgel et al. 1995). The impurities found in the xylitol fermentation broth have a range of molecular sizes. Most of these impurities are residual nutrients from the fermentation and include yeast extract, polypeptides, sugars, sugar alcohols, and inorganic salts. Knowing the characteristics of the xylitol molecule is critical in understanding the methods of recovery. The size of the xylitol molecule has been reported to be about 0.96-0.99 nm in length and 0.3-0.33 nm maximum radius (Kiyosawa 1991). Yeast extract, an impurity in the broth is composed of amino acids, peptides, oligo-peptides, and proteins. The major methods for xylitol recovery include ion-exchange resins, activated carbon, and chromatography. Gurgel et al. (1995) used activated carbon, anion and cation exchange resins to purify xylitol from sugar cane fermented medium. Xylitol had affinity for strong cation-exchange resin (Amberlite 200C) and weak anion-exchange resin (Amberlite 94S), which resulted in 40-55% loss of product because xylitol adhered to the surface of the resin. The best substance for clarifying medium was activated carbon (25 g in 100 ml fermented broth) at 80°C and pH 6.0 for 60 min. Also, the affinity of xylitol to activated carbon (20%) was less than that of resins. It was also demonstrated that the most important factor in color removal is concentration of the activated carbon. The influence of activated carbon concentration is lower at higher temperatures, and this effect is higher at pH 6.0 compared to pH 9.0. In another study, Silva and co-workers (2000) evaluated activated charcoal and aluminium polychloride for xylitol purification from fermented medium. The results showed that using 5.2 g l⁻¹ of aluminium polychloride associated with activated charcoal at pH 9, temperature of 50°C for 50 min promoted a 93.5% reduction in phenolic compounds and a 9.7% loss of xylitol from the fermented medium.

Liquid–liquid extraction is a simple, clean and fast method of purification used in numerous industrial processes to remove undesirable impurities or to recover dissolved substances. Solvents recovery is easy due to their low boiling points. Precipitation is another method that can be used to recover a substance from a fermentation broth, because when a solute is precipitated, it can be removed by simple solid/liquid separation (Glatz 1990). Both methods of liquid-liquid extraction and precipitation were used together by Mussatto et al.(2005) for xylitol recovery from a fermented medium by...
C. guilliermondii. Undesirable impurities were extracted from the broth using either ethyl acetate, chloroform or dichloromethane and the best results for clarification were obtained with ethyl acetate. In the second stage, the most effective chemical for precipitation of impurities was tetrahydrofuran (compared to ethanol and acetone), but xylitol loss was too high (≈30%).

8. Summary and Conclusion

Xylitol is being used as a highly valued ingredient with some interesting and useful properties in food and pharmaceutical products. It can be produced from xylose-rich (hemicellulose fraction of lignocellulose) resources by chemical or biotechnological method. Commercial production of xylitol is by chemical reduction of D-xylose in the presence of nickel as catalyst at high temperature and pressure. However, the high temperature and pressure requirements, low product yield, expensive separation and purification, in addition to usage of catalyst, make the chemical route expensive. Biotransformation of xylose to xylitol could be an alternative method in which microorganisms or enzymes are involved to convert xylose into xylitol under mild conditions of pressure and temperature. It is a reduction process that is mainly controlled by the activities of key enzymes NADPH or NADH-dependent xylose reductase reducing D-xylose to xylitol, and NAD-linked xylitol dehydrogenase oxidizing xylitol to D-xylulose. Xylitol production in yeasts utilizing D-xylose depends on various parameters including microorganism strain, inoculum age, culture conditions, fermentation system, composition of the medium, inhibiting compounds, temperature, and pH. Meanwhile, in complex media like lignocellulosic hydrolysates, the presence of toxic compounds due to their direct inhibitory effect and sometimes synergistic effects on other parameters, could be detrimental for cell metabolism. Therefore, to make hydrolysates appropriate as fermentation media, hydrolysis process should be performed in a way that allows inhibitory compounds be at a tolerable level for the microorganism as well as high concentration of substrate and then an appropriate detoxification method could be adopted to minimize concentration of inhibitory materials, thus maximizing bioconversion efficiency.

Purification and recovery of the product are the main challenges related to this process and a successful fermentation using immobilized cell system could be effective in cost reduction. Studies on the bio-production of xylitol have been mostly focused on establishing the operational parameters and the process options that maximize its yield and productivity in free cell system. However, some gaps in knowledge exist with bioconversion in immobilized cell system and selection or making an appropriate carrier (support) for biocatalysts in fermentation medium.

9. References


