Chapter 7
Fermentation Strategies Explored for Xylitol Production

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Abstract This chapter reviews different fermentation strategies for xylitol production using synthetic media or hemicellulosic hydrolyzates as carbon source. Most of the published works were carried out with free cells in batch operation because of its versatility and easy use in preliminary tests, where the age of inoculum, cell recycling, initial cell concentration, pH, temperature, type and concentration of nutrients in the culture medium, initial xylose concentration, presence of carbon sources other than xylose, and dissolved oxygen level were selected as the main variables. Conversely, continuous fermentation systems were shown to offer additional advantages such as high productivity for long periods of time, elimination of idle time for cleaning and sterilization and simplicity to perform an automated control. The most attractive equipment employed for this purpose included the continuous flow stirred tank, crossflow membrane and submerged membrane bioreactors. The use of cells immobilized by adsorption, entrapment, or covalent binding showed several advantages compared to the free ones, including higher cell density and possible biomass recycling for continuous operation. Repeated-batch fermentations were also investigated to evaluate the durability of immobilized cells with the aim of implementing the technology into a continuous process or scaling up the conversion of xylose to xylitol. Seeking long-term stability, the packed bed and fluidized bed bioreactors proved to be the most

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effective equipment; however, their hydrodynamic characteristics and the influence of aeration rate on fermentation performance still deserve further efforts. Finally, the fed-batch process, mainly with free cells, was also reported as an effective tool to keep the substrate at a suitable level throughout the whole fermentation process.

Keywords Batch systems · Continuous fermentation · Fed-batch systems · Immobilization · Packed bed reactors · Fluidized bed reactors

7.1 Introduction

In the last two decades, considerable efforts have been addressed to produce xylitol by biotechnological means, in spite of the fact that xylitol had been discovered and isolated in 1890 by the German chemist Fischer from beech wood, and produced for the first time in 1930 as an unstable form of xylitol by reduction of purified D-xylose (Mäkinen 2000). These discoveries set up the basis to produce primarily xylitol by microorganisms. Initially, the most outstanding papers were focused on screening of suitable microorganisms and optimum operating conditions (aeration, pH, temperature, age of inoculum, etc.) in batch fermentation. Advances in the applications of xylitol, increased demand of consumers for natural additives and awareness of environmental protection by reducing the release of residues have been stimulating xylitol production by using continuous or fed-batch processes either in traditional or novel systems. Therefore, the aim of this chapter is to review the most outstanding technologies available in literature on the microbial production of xylitol.

7.2 Production Technologies

Due to the shortage of sugar during the Second World War, Finland, Japan, Germany and the Soviet Union promoted research on the production of xylitol, which almost ceased after the war. In 1975 the Finnish company Oy began the xylitol production on a large scale, and in 2004, after several associations and incorporations, with the name of Danisco (Denmark), it accounted for about 95 % of worldwide xylitol production, amounting to 30,000 tons per year (Tran et al. 2004). The rest of production was distributed among International Sells Cerestar (Belgium), Roquette Frères (France) and Hebei Baoshuo Pipe Group Company (China), with Japan being the largest importer (about 10,000 tons per year) (Tran et al. 2004). In 1997, the consumption of xylitol in the European Union was estimated at 1,000 tons.

The scarce production of xylitol is due to its cost of production by chemical means (6.39 €/kg), which is ten times higher than those of other traditional sweeteners (51,000 tons per year) or polyols (75,000 tons per year) (Tran et al. 2004).

Xylitol can be obtained by direct extraction from fruits and vegetables as lettuce, cauliflower, yellow plums, strawberries or raspberries. However, this method is not profitable owing to the small concentrations at which it appears (less than 9 g/kg) (Hyvonen et al. 1982; Pepper and Olinger 1988). Alternatively, xylitol can be produced by chemical synthesis or by fermentation (Saha 2003; Winkelhausen and Kuzmanova 1998).

7.2.1 Chemical Synthesis

Nowadays xylitol is produced commercially by chemical synthesis from xylose. This process implies a prehydrolysis stage of lignocellulosic materials (LCM) rich in xylans (Mikkola et al. 1999; Nigam and Singh 1995). Hydrolyzates are concentrated and subject to physico-chemical treatments (ion exchange, discoloration and chromatography) to obtain solutions of pure xylose, a prerequisite for subsequent hydrogenation (Baudel et al. 2005; Winkelhausen and Kuzmanova 1998). This is carried out in the presence of catalysts such as nickel, palladium or ruthenium (Mikkola et al. 2000) at 80–140 °C and 50 atm, because the presence of other sugars generates unwanted byproducts (arabitol, ribitol, mannitol, sorbitol among others). The resulting solution is concentrated and xylitol is recovered by crystallization, yielding a product with a purity of 99.7 % and a yield of 50–60 % with respect to the initial xylose (Nigam and Singh 1995). The production costs are high due to the complexity of the purification steps needed to remove byproducts from hemicellulose hydrolyzates, low yields and difficult purification of xylitol (Choi et al. 2000).

Alternatively, xylitol can be obtained from substrates other than xylose such as 2-keto-gulonic acid, L-sorbose, glucuronic acid, L-xylonic acid, L-xylulose, L-gulonic acid and L-idonic acid, which are transformed into L-xylose or other compounds, whose hydrogenation leads to xylitol although with low yields (Heikkilä et al. 2003).

7.2.2 Fermentation

Xylitol present in fermentative media was considered for years as an unwanted byproduct obtained during D-xylose fermentation to ethanol (Perego et al. 1990); however, subsequent works showed that xylitol could be obtained with promising conversion yields and productivities (Parajó et al. 1998a, b, c). Xylitol production by fermentation of xylose has certain advantages over chemical means, as it takes

place under milder conditions of pressure and temperature and releases lower amounts of by-products (Nigam and Singh 1995; Saha 2003; Tran et al. 2004; Winkelhausen and Kuzmanova 1998). Several studies have been addressed to the selection of new microorganisms able to overproduce xylitol from commercial xylose, whereas in many others hydrolyzates of LCMs with high content of xylan were employed to produce xylitol by fermentation using bacteria, yeasts or fungi (Parajó et al. 1998a; Saha 2003; van Zyl et al. 1999).

7.2.2.1 Fermentation by Bacteria and Filamentous Fungi

Literature reports scarce information on xylitol production by bacteria, being mostly related to the use of xylose or xylulose solutions. The bacteria capable of producing xylitol are *Corynebacterium* sp. (Rangaswamy and Agblevor 2002; Yoshitake et al. 1973), *Enterobacter liquefaciens* (Yoshitake et al. 1976), *Cellulomonas cellulans*, *Corynebacterium glutamicum*, *Corynebacterium ammoniagenes*, *Serratia marcescens* (Rangaswamy and Agblevor 2002), *Bacillus coagulans* and *Mycobacterium smegmatis* (Izumori and Tuzaki 1988). Although in some cases some interesting yields were obtained, the final concentrations of xylitol were very low. The same can be said for filamentous fungi such as *Fusarium oxysporum* (Suihko 1984), *Petromyces albertensis* (Dahiya 1991), *Penicillium roqueforti*, *Penicillium crustosum*, *Penicillium brevicompactum, Penicillium crysogenum, Penicillium purpurogenum, Penicillium citrinum, Penicillium janthinellum, Penicillium griseorolsum, Penicillium expansum*, *Penicillium italicum* and *Aspergillus niger* (Sampaio et al. 2003).

Alternatively, xylitol production by genetically-modified bacteria carrying the genes responsible for xylitol production in yeasts has also been investigated (Akinterinwa and Cirino 2009; Povelainen and Myasnikov Povelainen and Miasnikov 2007); however, this process appears to be hindered by certain genetic instability and safety problems.

7.2.2.2 Fermentation by Yeasts

Yeasts exhibit a number of advantages in the production of xylitol compared to other microorganisms. Many studies have been directed towards searching for xylitol producers and identifying optimum conditions to obtain high yields ($Y_{P/S}$) and productivities (Q_P). Parajó et al. (1998b) reviewed in detail the published data on xylitol production from xylose solutions by commercial yeasts, which can be summarized as follows.

Barbosa et al. (1988) studied 44 strains of different species, selecting *Candida guillermondii* and *Candida tropicalis* as the best xylitol producers. These authors achieved concentrations of 77 g/L of xylitol from solutions containing 104 g/L of xylose. Conversely, Vandeska et al. (1995a) selected *Debaryomyces hansenii* and *Candida boidinii* as the best producers, while Sirisansaneeyakul et al. (1995)

worked with 11 different yeasts, obtaining a maximum xylitol yield of 0.62 g/g of consumed xylose with *Candida mogii*.

The largest concentrations of xylitol were achieved with different strains of *Candida* sp. (204–210 g/L from 250 g/L of xylose, $Y_{P/S} = 0.84$ g/g) (Chen and Gong 1985; Ikeuchi et al. 1999), *C. guilliermondii* (221 g/L from 300 g/L of xylose, $Y_{P/S} = 0.75$ g/g) (Meyrial et al. 1991) and *D. hansenii* (221 g/L of xylitol) (Domínguez et al. 1997), and the highest volumetric productivities (2.24–4.60 g/L h) with *D. hansenii* (Domínguez et al. 1997; Sampaio et al. 2005).

7.2.2.3 Genetically Modified Yeasts

Metabolic engineering offers opportunities to change the genetic properties of the microorganisms themselves. In the quest for a microorganism able to efficiently convert D-xylose to xylitol, strains of *Saccharomyces cerevisiae* were genetically modified (Winkelhausen and Kuzmanova 1998). Thus, the formation of xylitol by recombinant *S. cerevisiae* expressing the *XylI* gene was investigated by comparing the efficiency of different co-substrates (glucose, ethanol, acetate, and glycerol), oxygenation levels and different ratios of substrate and co-substrate. With both glucose and ethanol, the conversion yields were close to 1 g xylitol per gram of consumed D-xylose (Hallborn et al. 1994). Xylitol production was also investigated in continuous process carried out in a bench-scale packed bed reactor, where two recombinant strains of *S. cerevisiae* (H475 and S641), expressing low and high xylose reductase (XR) activities, respectively, were immobilized by gel entrapment using Ca alginate as the support (Roca et al. 1996). The effect of hydraulic residence time, substrate/co-substrate ratio, recycling ratio and aeration rate (AR) were investigated, and the highest xylitol concentration (15 g/L) was observed at a hydraulic residence time of 8.5 h. However, it still remains to be seen whether these microorganisms can remain sufficiently stable over a relatively long period of time and endure the operational conditions prevailing during the production of xylitol (Winkelhausen and Kuzmanova 1998).

7.3 Fermentation Technologies

Xylitol has been extensively produced using free cells in batch, continuous (chemostat) or fed-batch processes using either synthetic media or hydrolyzates obtained from different agroindustrial residues, although some attempts have also been made with yeasts immobilized in different supports. Some authors (Kim et al. 1999; Preziosi-Belloy et al. 1997) proposed a sequential process of utilization of the sugars present in hemicellulosic hydrolyzates, consisting in a first stage of fermentation performed under aerobic conditions, to promote glucose catabolism into biomass and prevent ethanol production, and a second stage in which the conversion of xylose to xylitol took place. This protocol was mainly developed in

batch culture (Preziosi-Belloy et al. 1997) preceding a chemostat process (Martínez et al. 2003) or during the first stage of fed-batch culture (Vandeska et al. 1996). The following section summarizes these technologies.

7.3.1 Free Cells

Most of the published works using free cells have been carried out batchwise in flasks or lab-scale stirred tank reactors, because these cultures offer versatility and easy use in preliminary tests (Winkelhausen and Kuzmanova 1998), but, comparatively, continuous or fed-batch processes allow increasing both yields and productivities.

7.3.1.1 Batch Systems

Because batch xylitol production by yeasts is strongly influenced by the operating conditions, the following sections take into consideration the variables that mostly impact on this bioprocess.

Age of Inoculum and Cell Recycling

The age of inoculum and cell recycling were shown to influence the metabolic activity as well as the viability of cells (Pfeifer et al. 1996), hence impacting on both productivities and yields. Felipe et al. (1997a) found that a 48 h-inoculum of *C. guilliermondii* resulted in a productivity 36 % smaller than a 24 h-inoculum; however, using the same microorganism, Sene et al. (1998, 2001), remarkably increased both xylitol yield and volumetric productivity using cells recycled four times, demonstrating that the more adapted the cells, the better the yeast ability to reduce xylose to xylitol in hemicellulose hydrolyzates. However, Cunha et al. (2006, 2007) found no significant differences when recycling cells immobilized in polyvinyl alcohol (PVA)-hydrogel, whereas Domínguez (1998) and Sampaio et al. (2005), working with two different strains of *D. hansenii*, observed a decrease in both xylitol yield and productivity. This behavior was justified by Rivas et al. (2003) with the tendency of the yeast, after two adaptations, to address the most significant fraction of the carbon source to respiration and production of ATP.

Initial Cell Concentration

Several studies highlighted that xylitol volumetric productivity increases linearly with the initial biomass level within a relatively wide range (Cao et al. 1994). This behavior was observed in particular with *D. hansenii* (Parajó et al. 1997; Sampaio

et al. 2008) and *Candida parapsilosis* (Oh et al. 1998), in which xylitol concentration varied in the ranges 0.042–32.4 and 8–30 g/L, respectively, while specific productivity remained almost constant or even slightly decreased. Several authors (Gírio et al. 1994; Felipe et al. 1997a; Parajó et al. 1997, 1998c) suggested that it could be the result of a reduction in the toxic effect of some inhibitors present in the culture medium. On the other hand, Domínguez et al. (1997) and Felipe et al. (1997a), working with *D. hansenii* and *C. guillermondii*, respectively, observed such a volumetric productivity increase only up to a maximum cell concentration. Rivas et al. (2003) proposed for the first yeast, that the reduction of xylitol production at high biomass level may result from an increased fraction of xylose required to sustain the large ATP requirements for cell maintenance.

pH

The optimum initial pH of cultivation is strongly species-dependent. It usually lies in the range 5.5–6.5 for *D. hansenii* (Converti and Domínguez 2001; Domínguez et al. 1996, 1997; Sampaio et al. 2006), 4–6 for *Candida* sp. (Cao et al. 1996), 4.5–5.0 for *C. parapsilosis*, 5.5–6.0 for *C. guilliermondii* (Converti et al. 2003; Felipe et al. 1997b; Nolleau et al. 1995), 2.5–6.0 for *Candida peltata* (Saha and Bothast 1999) and *C. tropicalis* (El-Batal and Khalaf 2004; Tamburini et al. 2008) and 6.0–7.5 for *Pachysolen tannophilus* (Converti et al. 1999), while *C. boidinii* (Vandeska et al. 1995a) and *Hansenula polymorpha* (Sánchez et al. 1998) have optimum pH of 7.0 and 5.5, respectively.

Converti and Domínguez (2001) and Converti et al. (2003) using *D. hansenii* and *C. guilliermondii*, respectively, explained the existence of an optimum pH for xylitol production on the basis of the fact that xylose is transported across the cell membrane by a facilitated diffusion system of the proton symport type. At pH above the optimum, this system is limited because H^+ transport must be performed against gradient, favoring respiration. On the other hand, at sub-optimal pH values, xylose transport is favored thus increasing the intracellular pH, which is likely to be restored consuming xylose by respiration to obtain ATP. As a result, both xylitol yields and productivities decrease.

Temperature

Yeasts produce xylitol in the range of 24–45 °C, but the optimal temperature is usually between 28 and 30 °C, depending on the microorganism: 28 °C for *C. guillermondii* (Converti et al. 2003), 28–35 °C for *D. hansenii* (Converti and Domínguez 2001; Domínguez et al. 1997; Sampaio et al. 2006) and 30 °C for *P. tannophilus* (Converti et al. 2001). When fermentations are carried out at temperatures different from the optimal ones, yields and productivities decrease due to reductions in xylitol dehydrogenase (XDH) activity (Winkelhausen and Kuzmanova 1998).

Nutrients of the Culture Medium

The type and concentration of the nitrogen source are key factors in the production of xylitol by fermentation, although the influence of both variables also depends on the yeast. In general, organic nitrogen sources such as urea, yeast extract, rice grain, peptone, etc. allow obtaining higher productivities and yields compared to the inorganic ones, because their presence stimulates the oxidative step of the pentose phosphate pathway in various yeasts (*C. boidinii, C. guillermondii, C. mogii*, etc.) (Barbosa et al. 1988; Kim and Moon 2003; Lu et al. 1995; Preziosi-Belloy et al. 2000; Silva and Roberto 2001; Sirisansaneeyakul et al. 1995; Vandeska et al. 1995a).

Some yeasts like *C. guilliermondii* and *P. tannophilus* require vitamins such as biotin, ascorbic acid, pyridoxine, or choline (Kim and Moon 2003; Lee et al. 1988). Similar effects have been reported for other nutrients; for example, the presence of Mg^{2+} ions can redirect the fermentation of xylose by *Pichia stipitis* towards xylitol production (Mahler and Guebel 1994), while xylitol production by *D. hansenii* can be stimulated, under phosphate-limiting conditions, by some enzyme activities like that of xylulose kinase (Tavares et al. 1999).

Initial Xylose Concentration

The initial xylose concentration has a significant impact on xylitol production by yeasts. Product yields are low when using low initial xylose concentrations, because the carbon source is mainly employed for biomass production. By increasing the concentration of xylose, a greater percentage of substrate is addressed to the production of xylitol, thereby increasing the yield (Converti et al. 2002). This behavior was demonstrated in several yeasts such as *D. hansenii* (Converti et al. 2002), *C. parapsilosis* (Oh et al. 1998) and *C. tropicalis* (Kim et al. 2002; Walther et al. 2001).

However, too high initial concentrations of xylose reduce the yield owing to excess substrate inhibition and high osmotic pressure of the medium (Kim et al. 2002; Silva et al. 1996a). Such an inhibition, which depends on the degree of aeration and the species, appears at 60 g/L of xylose in *P. tannophilus* (Thonart et al. 1987), 100–200 g/L in *C. tropicalis* (Gong et al. 1981; Silva and Afschar 1994), 200 g/L in *C. guilliermondii* (Meyrial et al. 1991; Silva et al. 1996b), 100–150 g/L in *C. boidinii* (Vandeska et al. 1995a) and 175–200 g/L in *D. hansenii* (Converti et al. 2002; Domínguez et al. 1997).

Carbon Sources Other than Xylose

Also the presence of other sugars in LCMs hemicellulose hydrolyzates impacts on xylitol production depending on their concentrations and the microorganism employed.

In some works, glucose was shown to be initially consumed by fermentation exerting an inhibitory effect on xylose uptake (Furlan and Castro 2001; Ikeuchi et al. 2000); once glucose is consumed, xylose consumption starts (Nobre et al. 1999; Preziosi-Belloy et al. 1997). In others, a stimulatory effect of glucose on xylitol production was identified in different yeasts (*C. tropicalis, C. parapsilosis, D. hansenii*) up to an optimal concentration threshold beyond which it behaved as an inhibitor (Kim et al. 1999; Sheu et al. 2004; Tavares et al. 2000; Walther et al. 2001). Other hexoses such as galactose and mannose (Preziosi-Belloy et al. 1997; Silva et al. 1996a; Walther et al. 2001) as well as ethanol (Saha and Bothast 1999; Walther et al. 2001) exert moderate inhibitory effects on the production of xylitol.

In general, other pentoses and glycerol do not exert any inhibitory effect (Saha 2003; Walther et al. 2001). Although Preziosi-Belloy et al. (1997) have suggested an inhibiting effect of arabinose on XDH activity, some yeasts showed higher affinity for xylose that was completely consumed before starting the assimilation of arabinose, with an increase in xylitol yield (Saha and Bothast 1999). The metabolism of arabinose leads to arabitol (Saha 2003), although many researchers believe it may also lead to xylitol (Gírio et al. 2000; Walther et al. 2001) or ethanol (Kim et al. 1999).

Dissolved Oxygen Concentration

The dissolved oxygen concentration is one of the most important parameters to be considered in the production of xylitol (Gírio et al. 1994), because it influences not only the operational aspects of xylitol production (du Preez 1994) but even the yeast physiology. Its effect was deeply investigated either in synthetic media (Granström et al. 2002; Nolleau et al. 1995; Vandeska et al. 1995b; Winkelhausen et al. 1996) or hemicelluloses hydrolyzates (Preziosi-Belloy et al. 2000; Roberto et al. 1999), but different methods were employed to measure it, thus making the results from different studies often not comparable (Skoog and Hahn-Hägerdal 1988).

Using yeasts with NADPH-dependent XR activity, such as *D. hansenii*, the dissolved O_2 level must be controlled at low levels to avoid xylitol consumption by respiration. Optimal values of O_2 concentration for xylitol production usually correspond to micro-aerobic conditions that allow, in addition to NAD^+ regeneration to the minimum extent necessary, producing the ATP and NADPH required to sustain cell growth and XR activity, respectively (Aguiar et al. 2002; Aranda-Barradas et al. 2000; Converti and Domínguez 2001; Faria et al. 2002a; Gírio et al. 2000; Roseiro et al. 1991).

This being so, the oxygen transfer rate (OTR), oxygen transfer coefficient (k_La) and specific rate of oxygen consumption (q_{O2}) are critical parameters to be strictly controlled during xylose-to-xylitol bioconversion (Aguiar et al. 2002; Nobre et al. 2002; Roseiro et al. 1991; Walther et al. 2001; Winkelhausen and Kuzmanova 1998). Optimal values for xylitol production in synthetic medium by *C. parasilopsis* were OTR = 65.7–211.7 mg/L h and k_La = 9.0–36.1 h^{-1}

(Aranda-Barradas et al. 2000; Faria et al. 2002a), by *C. boidinii* $k_La = 26–78\ h^{-1}$ (Winkelhausen et al. 2004) and *D. hansenii* $q_{O2} = 47.9$ mg/gMS L (Sampaio et al. 2004).

7.3.1.2 Continuous Fermentation

Contrary to batch systems, where only a small fraction of the total fermentation time corresponds to the phase of maximum rate of product formation, the use of continuous fermentation systems allows maintaining high productivities for long periods of time (Faria et al. 2002b). When compared with batch systems, the continuous ones show a number of additional advantages, the main of which being the elimination of idle time for cleaning and sterilization, greater steadiness in product synthesis and simplicity to implement automated control (Faria et al. 2002b). Consequently, several bioreactor configurations have been considered for continuous production of xylitol, which are briefly described in the following.

Continuous Stirred Tank Bioreactors

Ideally, the components of the culture broth in a Continuous Stirred Tank Bioreactor (CSTBR) are perfectly mixed, showing the same concentration as in the outflow stream. CSTBR is suitable when substrate costs are not so high and when a stable productivity is essential (Kosseva et al. 2009). This type of bioreactor was successfully employed by Cruz et al. (2000a) for the continuous production of xylitol by *D. hansenii* from barley bran hydrolyzate containing 30 g/L of xylose, varying the dilution rate from 0.008 to 0.088 h^{-1}. A maximum volumetric productivity (Q_P) of 0.60 g/L h and a product yield ($Y_{P/S}$) of 0.66 g/g were achieved at a dilution rate (D) of 0.048 h^{-1}, leaving 11 g/L of xylose in the fermented broth. These results improved those obtained in batch runs (Cruz et al. 2000b) using the same hydrolyzate and microorganism ($Q_P = 0.33$ g/L h; $Y_{P/S} = 0.61$ g/g). Martínez et al. (2003) also carried out continuous experiments using *C. guilliermondii* on a fermentation medium obtained from sugarcane bagasse hydrolyzate containing 51 g/L of xylose. Operating at $D = 0.038\ h^{-1}$, they obtained a xylitol concentration of 18.0 g/L, corresponding to $Q_P = 0.68$ g/L h and $Y_{P/S} = 0.69$ g/g.

Cross Flow Membrane Bioreactor

Increases in volumetric and specific productivities along with product yield are fundamental requisites to make the industrial xylitol bioproduction feasible (Kim et al. 2004; Oh and Kim 1998). Whereas the increase in specific productivity is in the domain of microbial genetics, such as the screening and mutant selection of a high producing strain, cell concentration can be effectively raised using cell-

recycle fermentation (Kwon et al. 2006). The Cross Flow Membrane Bioreactor (CFMBR), a combination of external membrane devices, like those employed for microfiltration or ultrafiltration, and a suspended cell bioreactor, has been proposed with the aim of improving xylitol productivity in a continuous process. Such a bioreactor configuration does in fact allow separating cells outside the bioreactor and recycling them to increase cell concentration, hence enhancing the kinetics and yields of the process as a result of reduced amount of xylose employed for microbial growth.

Silva et al. (1999) reported marked improvement of continuous xylitol production by recycling *C. guilliermondii* cells in such a system. Faria et al. (2002b) reported $Q_P = 1.14$ g/L h and $Y_{P/S} = 0.79$ g/g at $D = 0.030$ h^{-1} in a 2.6 L fermentor using *C. guilliermondii* in a synthetic medium containing 50 g/L of xylose. Q_P increased to 1.4 g/L h when the D was increased to 0.05 h^{-1}, but $Y_{P/S}$ decreased to 0.72 g/g. Likewise, Cruz et al. (2000a), using barley bran hydrolyzate containing 30 g/L of xylose and *D. hansenii* in a 2.0 L fermentor, obtained results ($D = 0.28$ h^{-1}; $Q_P = 2.53$ g/L h; $Y_{P/S} = 0.39$ g/g; residual xylose concentration of 4 g/L) much better than those obtained without membrane devices. Using the CFMBR, Choi et al. (2000) substantially improved batch xylitol production by *C. tropicalis*, obtaining results ($Q_P = 4.94$ g/L h; $Y_{P/S} = 0.82$ g/g; final xylitol concentration of 189 g/L) 1.3–2.2 times higher than without membranes.

Despite these advantages, the CFMBR have some drawbacks such as high power consumption, cell damage resulting from shear stress, and difficulty in controlling fouling (Delgado et al. 2004). In addition, the bioreactors used for cell-recycle fermentation are unsuitable for aerobic fermentation because of the lack of an air supply (Kwon et al. 2006).

7.3.1.3 Long-Term Cell Recycle by Centrifugation

Kim et al. (2004) proposed the long-term recycle of *C. tropicalis* cells by centrifugation to increase biomass concentration within the bioreactor. Using 2 L of complex or chemically defined media in a 7 L jar fermenter considerably improved the performance of batch fermentations, as a result of the reduction in the fermentation time and increase in the substrate consumption rate. After 14 rounds of fermentation, corresponding to a fermentation time of 284–333 h, the average xylitol concentration reached 105–110 g/L, and Q_P (4.4–5.4 g/L h) and $Y_{P/S}$ (0.78–0.81 g/g) were about twice and 4–7 % higher, respectively, than those of simple fermentations. However, application of centrifugation to large-scale production would not be economically viable, due to high complexity, inability to ensure long-term aseptic conditions and high cost of equipment (Kwon et al. 2006).

7.3.1.4 Submerged Membrane Bioreactor

Kwon et al. (2006) developed a new membrane-based cell recycling system, called Submerged Membrane Bioreactor (SMBR), consisting of a SMBR with suction and air sparging. According to these authors, the SMBR is more effective than the CFMBR in terms of power consumption, membrane filtration, fouling control, cell recycling and cultivation of aerobes. Operating ten recycle rounds, they achieved productivity ($Q_P = 12$ g/L h) and maximum xylitol concentration ($P = 182.0$ g/L) 3.4 and 11.0 times higher than in batch fermentation, respectively.

7.3.2 Immobilized Cells

The most common methods of cell immobilization employed in bioprocesses are adsorption, entrapment and covalent binding (Kosseva et al. 2009). According to Choi et al. (2000), calcium alginate, polyacrylamide and non-woven fabric are among the most effective entrapping materials for cell immobilization. The use of immobilized cells has several advantages compared to suspended cells, including higher cell density within the bioreactor, higher productivity, improved stability, cell reutilization, continuous operation, and no need to separate cells from substrate and products at the end of the process (Kosseva et al. 2009). However, cell immobilization techniques are characterized by lower mass transfer rate of substrates from the bulk to cells and leakage of cells to the medium (Audet et al. 1991). Immobilized cells have been studied in both batch and continuous systems. As an example, Fig. 7.1 shows a micrograph by scanning electron microscopy of the yeast *D. hansenii* immobilized in calcium alginate, while Table 7.1 summarizes the information concerning the microorganisms, immobilization supports, carbon sources and bioreactors most commonly employed.

7.3.2.1 Batch Systems

The behavior of immobilized cells can be significantly different from that of free cells. For example, conditions ensuring micro-aerobiosis to free cells may correspond to anaerobic conditions with immobilized cells due to mass transfer (diffusion) limitation. Therefore, application of low ARs in immobilized cell cultures may cause drastic reductions in productivity (Hinfray et al. 1995).

Repeated-batch fermentations were investigated by different authors to evaluate the long-term stability of immobilization with the perspective to implement the technology into a continuous process or to scale up the process from flasks to stirred tank bioreactors. According to Domínguez (1998), immobilization is a technique that allows maintaining the functionality of the fermenting microorganisms used in xylitol production processes. When cells of *D. hansenii* immobilized in calcium alginate beads were reused to ferment synthetic media in

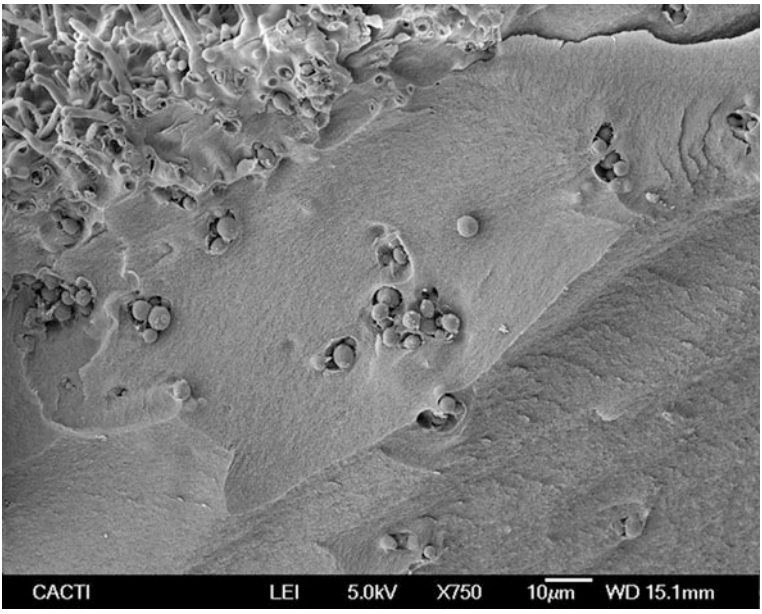

Fig. 7.1 Micrograph by scanning electron microscopy (SEM) of *Debaryomyces hansenii* cells immobilized in calcium alginate

Erlenmeyer flasks, xylitol yield increased from 0.37 g/g in the first recycling operation to 0.69 g/g after the second, and xylitol volumetric productivity from 0.31 to 2.03 g/L h after the fourth. These results improved those obtained in free cultures, where D-xylose was converted rapidly and efficiently into xylitol in the first step ($P = 106.7$ g/L of xylitol; $Q_P = 1.48$ g/L h; $Y_{P/S} = 0.84$ g/g). However, after six recycles, the bioconversion was less effective.

Cunha et al. (2006), using *C. guilliermondii* cells immobilized in 5 g of PVA-hydrogel to ferment 45 mL of sugarcane bagasse hemicellulosic hydrolyzate in 125-mL Erlenmeyer flasks, observed that the biocatalyst remained stable and exhibited a similar fermentative profile in five repeated-batch fermentations and obtained as an average $P = 35.1$ g/L, $Q_P = 0.49$ g/L h and $Y_{P/S} = 0.58$ g/g. When scaling up the process to a bench-scale stirred tank bioreactor filled with 960 mL of medium and 240 g of beads, Cunha et al. (2007) observed a small decrease in the conversion performance in the fifth cycle. The biocatalytic activity of biomass was recovered in the sixth cycle after washing the particles, and the hydrogel beads were shown to maintain their shape and size without appreciable deterioration. The best results ($P = 39.7$ g/L; $Q_P = 0.53$ g/L h; $Y_{P/S} = 0.77$ g/g) were achieved after the third cell recycling, probably because of cells adaptation to the medium.

Liaw et al. (2008) performed 52.5-day long repeated-batch fermentation in Erlenmeyer flasks, using rice straw hemicellulose hydrolyzate detoxified with activated charcoal as fermentation broth and cells of *C. subtropicalis* WF79

Table 7.1 Xylitol production in batch processes with immobilized yeasts, packed bed reactors in continuous, fluidized bed reactors and fed-batch processes

Microorganism	Carbon source	Immobilization	Reactor	Results	Reference
Batch processes with immobilized yeasts					
Candida guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Calcium alginate	Erlenmeyer flasks	$P = 11.05$ g/L $Q_P = 0.22$ g/L h $Y_{P/S} = 0.47$ g/g	Carvalho et al. (2000)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Calcium alginate	Erlenmeyer flasks	$P = 20.6$ g/L $Q_P = 0.43$ g/L h $Y_{P/S} = 0.47$ g/g	Carvalho et al. (2002[a])
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Calcium alginate	Erlenmeyer flasks	$P = 28.4$ g/L $Q_P = 0.59$ g/L h 61.5 % efficiency	Carvalho et al. (2002b)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Calcium alginate	Erlenmeyer flasks	$Q_P = 0.43$ g/L h $Y_{P/S} = 0.47$ g/g	Carvalho et al. (2002c)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Calcium alginate	Stirred tank reactor	$P = 47.5$ g/L $Q_P = 0.40$ g/L h $Y_{P/S} = 0.81$ g/g	Carvalho et al. (2005)

(continued)

Table 7.1 (continued)

Microorganism	Carbon source	Immobilization	Reactor	Results	Reference
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Calcium alginate	Stirred tank reactor	$P = 51.6$ g/L $Q_P = 0.43$ g/L h $Y_{P/S} = 0.71$ g/g	Carvalho et al. (2008)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Hydrogel based on polyvinyl alcohol (PVA)	Erlenmeyer flasks	$P = 34.2$ g/L $Q_P = 0.48$ g/L h $Y_{P/S} = 0.57$ g/g	Cunha et al. (2006)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Hydrogel based on polyvinyl alcohol (PVA)	Bench-scale bioreactor	$P = 39.7$ g/L $Q_P = 0.53$ g/L h $Y_{P/S} = 0.77$ g/g	Cunha et al. (2007)
Debaryomyces hansenii	Synthetic xylose	Calcium alginate	Erlenmeyer flasks	$P = 99.6$ g/L $Q_P = 2.08$ g/L h $Y_{P/S} = 0.79$ g/g	Domínguez (1998)
Candida subtropicalis	Rice straw hemicellulose hydrolyzates detoxified with activated charcoal	Polyacrylic hydrogel thin films	Erlenmeyer flasks	$Y_{P/S} = 0.73$ g/g	Liaw et al. (2008)
Candida boidinii	Synthetic xylose	Hydrogels based on u.v.-crosslinked poly(ethylene oxide)—matrices	Erlenmeyer flasks	$P = 4.2$ g/L	Winkelhausen et al. (2008)

(continued)

Table 7.1 (continued)

Microorganism	Carbon source	Immobilization	Reactor	Results	Reference
Packed bed reactors in continuous					
D. hansenii	*Eucalyptus globulus* wood hydrolyzate detoxified with charcoal	Calcium alginate	Upflow packed-bed bioreactors	$Q_P = 0.91$ g/L h	Domínguez et al. (1999)
C. guilliermondii	*E. globulus* wood hydrolyzate detoxified with charcoal	Calcium alginate	Upflow packed-bed bioreactors	$Q_P = 0.58$ g/L h	Domínguez et al. (1999)
Candida pelliculosa and *Methanobacterium* sp. HU	Synthetic	Polymer resins	Packed reactor	Improved stability during 2 weeks	Nishio et al. (1989)
Fluidized bed reactors					
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Porous glass spheres	Batchwise	$P = 34.2$ g/L $Q_P = 0.48$ g/L h $Y_{P/S} = 0.57$ g/g	Santos et al. (2003)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Porous glass spheres	Semicontinuous in different cycles	$P = 18.0$ g/L $Q_P = 0.32$ g/L h $Y_{P/S} = 0.44$ g/g	Santos et al. (2005a)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Porous glass spheres	Semicontinuous in different cycles	$Q_P = 0.44$ g/L h $Y_{P/S} = 0.25$ g/g	Santos et al. (2005b)

(continued)

Table 7.1 (continued)

Microorganism	Carbon source	Immobilization	Reactor	Results	Reference
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzate	Zeolite	Batchwise	$P = 38.5$ g/L $Q_P = 0.32$ g/L h $Y_{P/S} = 0.72$ g/g	Santos et al. (2005c)
Candida tropicalis	Synthetic	Porous glass	Fluidized bed reactor in continuous	$P = 90$–95 g/L $Q_P = 1.35$ g/L h	Silva and Afschar, (1994)
C. guilliermondii	Synthetic xylose	Porous glass spheres	Semicontinuous in different cycles	$Q_P = 0.52$–0.60 g/L h $Y_{P/S} = 0.79$–0.57 g/g	Silva et al. (2003)
Fed-batch processes					
Candida parapsilosis	Synthetic sugars	Free cells	2-L Setric Set 2 reactor	$Q_P = 0.46$ g/L h $Y_{P/S} = 0.72$ g/g	Furlan et al. (1997)
C. parapsilosis	Synthetic sugars	Free cells	20 L laboratory reactor	$Q_P = 0.05$ g/L h $Y_{P/S} = 0.38$ g/g	Furlan and Castro (2001)
C. guilliermondii	Synthetic sugars	Free cells	1.5 L fermenter	$P = 44.0$ g/L $Q_P = 1.06$ g/L h $Y_{P/S} = 0.76$ g/g	Godoy De Andrade Rodrigues et al. (2002)

(continued)

Table 7.1 (continued)

Microorganism	Carbon source	Immobilization	Reactor	Results	Reference
C. tropicalis	Synthetic sugars	Free cells	3.5 L fermenter	$P = 187.0$ g/L $Q_P = 0.75$ g/L h $Y_{P/S} = 3.90$ g/g	Kim et al. (2002)
C. tropicalis	Synthetic sugars	Free cells	Fermenter	$P = 237.0$ g/L $Q_P = 2.0$ g/L h $Y_{P/S} = 0.89$ g/g	Kim and Oh (2003)
C. tropicalis	Corn cob hemicellulosic hydrolyzates	Free cells	Fermenter	$P = 96.5$ g/L $Q_P = 1.01$ g/L h $Y_{P/S} = 0.83$ g/g	Li et al. (2012)
Candidu magnoliae	Synthetic sugars	Free cells	Fermentor	$P = 356.0$ g/dm^3 $Y_{P/S} = 0.75$ g/g	Nakano et al. (2000)
C. tropicalis	Synthetic sugars	Free cells	Fermentor	$P = 240.0$ g/L $Q_P = 5.58$ g/L h $Y_{P/S} = 0.80$ g/g	Oh and Kim (1997)

(continued)

Table 7.1 (continued)

Microorganism	Carbon source	Immobilization	Reactor	Results	Reference
C. tropicalis	Synthetic sugars	Free cells	5 L Fermentor	$P = 251.0$ g/L $Q_P = 4.56$ g/L h $Y_{P/S} = 0.93$ g/g	Oh and Kim (1998)
C. guilliermondii	Synthetic sugars	Free cells	5 L Fermentor	$Q_P = 0.64$ g/L h $Y_{P/S} = 0.84$ g/g	Rodrigues et al. (1999)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzates	Free cells	5 L Fermentor	$Q_P = 0.62$ g/L h $Y_{P/S} = 0.78$ g/g	Rodrigues et al. (1999)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzates	Free cells	Erlenmeyer flasks	$P = 44.0$ g/L $Q_P = 0.62$ g/L h $Y_{P/S} = 0.78$ g/g	Rodrigues et al. (1998a)
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzates	Free cells	Fermenter	$P = 26.3$ g/L $Q_P = 0.55$ g/L h $Y_{P/S} = 0.68$ g/g	Rodrigues et al. (2002)

(continued)

Table 7.1 (continued)

Microorganism	Carbon source	Immobilization	Reactor	Results	Reference
C. guilliermondii	Sugarcane bagasse hemicellulosic hydrolyzates	Ca-alginate	2 L Fermenter	$P = 28.9$ g/L $Q_P = 0.40$ g/L h $Y_{P/S} = 0.58$ g/g	Sarrouh et al. (2007)
C. boidinii	Synthetic sugars	Free cells	2 L bench top fermenter	$P = 59.3$ g/L $Q_P = 0.46$ g/L h $Y_{P/S} = 0.68$ g/g	Vandeska et al. (1996)
C. tropicalis	D-xylose and feeding D-glucose (50 g/l day)	Non-woven fabric	–	$P = 87.0$ g/L	Yahashi et al. (1996)

P maximum xylitol concentration, Q_P volumetric xylitol productivity, $Y_{P/S}$ xylitol yield

immobilized in polyacrylic hydrogel as biocatalyst. The cell immobilization procedure started with suspending yeast cells in a mixture of 2-hydroxyethyl methacrylate (HEMA, hydrophilic monomer), polyethylene glycol diacrylate (PEG-DA, crosslinking agent), and benzoin isopropyl ether (photoinitiator). The mixture was then allowed to form thin polyacrylic hydrogel films with 200 μm thickness, between two pieces of glass sheets, by UV-initiated photopolymerization. The maximum yield was 0.73 g/g, but after 40 days the fermentation activity of immobilized cells declined, and the yield was only 0.57 g/g at the end of run.

Using sugarcane bagasse hydrolyzates and *C. guilliermondii* cells entrapped in Ca-alginate beads, Carvalho et al. (2000, 2002a, b, c) studied the effects of immobilization conditions on bead chemical stability and xylitol production during repeated-batch fermentations in Erlenmeryer flasks, and successively in a 2.4 L stirred tank reactor (Carvalho et al. 2005, 2008). In bioreactor experiments, xylitol concentration increased up to 47.5 g/L within 120 h of fermentation, resulting in $Q_P = 0.40$ g/L h and $Y_{P/S} = 0.81$ g/g after one fermentation cycle (Carvalho et al. 2005). However, after five successive batches in a stirred tank reactor, the average volume of the Ca-alginate beads decreased by about 30 % after 600 h, thus demonstrating physical instability under the conditions employed in the reactor (Carvalho et al. 2008). In spite of this, almost steady xylitol production parameters were observed, with average values of xylitol concentration, overall volumetric productivity and product yield of 51.6 g/L, 0.43 g/L h and 0.71 g/g, respectively.

7.3.2.2 Continuous Fermentation

Immobilization has been employed in xylose-to-xylitol bioconversion to keep high concentration of cells as biocatalyst. Packed bed bioreactors (PBB) and fluidized bed bioreactors (FBB) are the most common bioreactors employed.

Packed Bed Bioreactors

According to Kosseva et al. (2009), PBBs have the advantage of simplicity of operation, high mass transfer rates and high reaction rates, whereas their main drawbacks are limited oxygen transfer during scale-up and periodic fluctuation in viable cell population due to nutrient depletion along the reactor length. Their long-term stability during continuous operation is one of the most important challenges.

Nishio et al. (1989) co-immobilized *Candida pelliculosa* and *Methanobacterium* sp. HU cells with glutaraldehyde and hexamethylenediamine to enhance the stability of an immobilized-cell system for the continuous production of xylitol in packed column. Using Ca-alginate beads for the same purpose, Domínguez et al. (1999) reported on the need of high sodium alginate concentrations during the immobilization step to increase the chemical stability of cell-gel beads to be employed in xylitol production from *Eucalyptus globulus* wood hydrolyzate

detoxified with charcoal. The use of immobilized cells of *D. hansenii* and *C. guilliermondii* in an upflow packed-bed bioreactor ensured volumetric productivities of 0.91 and 0.58 g/L·h, respectively.

Fluidized Bed Bioreactors

FBBs offer the advantage of good solid–fluid mixing and minimal pressure drops (Kosseva et al. 2009). The fluidization behavior is very strongly dependent on the physical properties of both liquid and solid phases (liquid viscosity, density, surface tension and solid density) (Béjar et al. 1992). Different parameters have been studied to optimize the process including the AR, the durability of the immobilization after several cycles and the influence of different hydrodynamic variables.

The influence of AR on batch xylitol production from sugarcane bagasse hemicellulose hydrolyzate in FBB was studied by Santos et al. (2003) using cells of *C. guilliermondii* immobilized onto porous glass spheres. The highest xylitol concentration (17.0 g/L) was obtained at an intermediate AR of 70 mL/min, although the specific xylitol productivity and xylitol yield were 43 and 22 % lower than the corresponding values obtained at the lowest air AR (25 mL/min), respectively.

Other authors have focused their research efforts on the number of cycles. Silva et al. (2003) employed *C. guilliermondii* FTI 20037 cells immobilized on porous glass to study the fed-batch bioconversion of xylose in a 1.4 L-fluidized bed reactor over 672 h. The fermentation was performed in seven cycles and always using fresh medium. The highest values of xylitol yield (0.79 and 0.57 g/g) and volumetric productivity (0.52 and 0.60 g/L h) were attained in the first and second cycles, respectively. Contrary to what was expected from a progressive adaptation of cells to the medium, a further increase in the number of cycles did not improve the performance of the system, due to cell wash-out that caused the cell-particles to become unsaturated, a part of xylose being consumed for the production of biomass instead of xylitol. Conversely, under similar conditions, but using sugarcane bagasse hemicellulosic hydrolyzates as carbon source, Santos et al. (2005a) obtained the lowest xylitol concentration (12 g/L) in the first cycle, and the best results ($P = 18.0$ g/L; $Q_P = 0.32$ g/L h; $Y_{P/S} = 0.44$ g/g) after five successive cycles of 72 h, because of cells adaptation to the medium. The influence of AR and carrier concentration (Cs) was evaluated by Santos et al. (2005b) during seven batch fermentation runs. The results showed that Cs had a negative influence on $Y_{P/S}$ and Q_P, whereas AR impacted positively on Q_P and negatively on $Y_{P/S}$. With AR = 0.093 min^{-1} and Cs = 62.5 g/L, $Y_{P/S}$ was low (0.25 g/g), but Q_P was the highest (0.44 g/L h), probably because cell metabolism was faster when more oxygen was available to the yeast.

Santos et al. (2005c) used *C. guilliermondii* cells immobilized on zeolite to investigate the effects of different conditions of air flowrate (0.0125–0.0375 vvm), zeolite mass (100–200 g), initial pH (4–6) and xylose concentration (40–60 g/L).

A metabolic deviation from product to biomass formation took place when raising air flowrate, while increases in pH or xylose concentration enhanced Q_P and $Y_{P/S}$ or xylitol concentration, respectively. The best results ($P = 38.5$ g/L; $Q_P = 0.32$ g/L h; $Y_{P/S} = 0.72$ g/g) were obtained using an air flowrate of 0.0125 vvm, 100 g of zeolite, pH = 6 and xylose concentration of 60 g/L.

Sarrouh and Silva (2008) evaluated the hydrodynamic characteristics (total particle density, terminal velocity, particles drag force, minimum fluidization velocity and bed porosity) and fermentation parameters (AR and fermentation time) of a bench-scale three-phase fluidized bed reactor with *C. guilliermondii* cells immobilized in calcium alginate beads for xylitol production. The reactor was shown to operate similarly to a fixed-bed bioreactor at bed porosity <0.5 and a FBB at bed porosity >0.5. The maximum flowrate needed to obtain maximum bed fluidization in the reactor was equal to the terminal velocity of the immobilized cell particles. Regarding the fermentation parameters, the best results ($P = 28.9$ g/L; $Q_P = 0.41$ g/L h; $Y_{P/S} = 0.58$ g/g) were obtained at a high AR (600 mL/min) after 70 h of fermentation, pointing out that in this system high ARs enhance oxygen transfer into the immobilized cells.

Finally, Silva and Afschar (1994) utilized a FBB for the continuous xylose-to-xylitol bioconversion by *C. tropicalis* cells immobilized on porous glass. The system exhibited a xylitol productivity of 1.35 g/L h, a value much higher than that obtained in batch culture with free cells (0.37 g/L h), thus showing the feasibility of these systems.

7.3.2.3 Fed-Batch Systems

Continuous processes can be successfully replaced by the fed-batch ones (Roberto et al. 1991) everywhere high product or substrate levels can be inhibitory to the system. In these processes, substrate concentration can be maintained at a suitable level throughout the entire course of fermentation, that is, a level sufficient to induce xylitol formation but not to inhibit yeast growth. In addition, they generally operate with high initial cell density, thereby increasing volumetric productivity (Winkelhausen and Kuzmanova 1998).

Thus, Furlan et al. (1997), using *C. parasilopsis* in synthetic medium, obtained a 40 % productivity increase compared with batch cultures, while Oh and Kim (1997), using *C. tropicalis* and maintaining high dissolved oxygen levels during the growth phase and oxygen-limited conditions during the production one, were able to increase xylitol concentration from 88 (starting with 100 g xylose/L) to 240 g/L (from 300 g xylose/L) passing from a batch to a fed-batch culture, corresponding to $Q_P = 5.58$ g/L h and $Y_{P/S} = 0.80$ g/g. Similarly, the overall performance of fed-batch cultivations with exponential feeding rate of *C. guilliermondii* on sugarcane bagasse hemicellulosic hydrolyzate was remarkably higher ($P = 44.0$ g/L; $Q_P = 0.62$ g/L h; $Y_{P/S} = 0.78$ g/g) (Rodrigues et al. 1998a) than those of batch experiments ($Q_P = 0.29$ g/L h; $Y_{P/S} = 0.50$ g/g) (Rodrigues et al. 1998b). More recently, Li et al. (2012) obtained 96.5 g xylitol/L cultivating *C. tropicalis* on corn

cob hemicellulose hydrolysate by a two-stage fed-batch fermentation process, corresponding to yield (0.83 g/g) and productivity (1.01 g/L h) about 12 and 66 % higher than those of batch fermentation, respectively, and related these improvements to a reduction of the negative effects on xylitol formation exerted by aeration and inhibitory compounds.

In fed-batch processes substrate can be added continuously or intermittently. The concentration and type of substrate as well as the optimal feeding rate were investigated by different authors. Vandeska et al. (1996), using free cells of *C. boidinii* initially in batch fermentation at high aeration levels to favor biomass production and then in three repeated fed-batch runs, observed the fastest cell growth using a mixture of glucose and xylose as a substrate. The best results were obtained performing the first fed-batch run at the highest initial xylose concentration (100 g/L) and the lowest level of aeration and using a feed rate of 0.8 g/L of xylose. Under these conditions a xylitol yield of 0.68 g/g (75 % of the theoretical yield, compared to 53 % in batch culture), a volumetric productivity of 0.46 g/L h (twice as high as the highest obtained in batch culture) and a final concentration of 59.3 g/L of xylitol were obtained. Oh and Kim (1998) using *C. tropicalis* obtained the best results ($P = 251.0$ g/L; $Q_P = 4.56$ g/L h; $Y_{P/S} = 0.93$ g/g) from 270 g xylose/L at a glucose/xylose feeding ratio of 15 % within 55 h. These results were substantially higher than those obtained in batch experiments ($P = 131.0$ g/L) starting with 150 g xylose/L after 45 h.

Although most of the efforts were directed towards the use of free cells in fed-batch processes, a few articles report on the use of cells immobilized in different supports. For example, to increase xylitol concentration up to 87 g/L by feeding 50 g D-glucose/L day, Yahashi et al. (1996) immobilized *C. tropicalis* cells in non-woven fabrics, which could be used five times in repeated fed-batch cultivations. More recently, Sarrouh et al. (2007) employed cells of *C. guilliermondii* entrapped in Ca-alginate beads to produce xylitol from concentrated hemicellulose hydrolyzate of sugarcane bagasse, in a three-phase FBB. The best results ($P = 28.9$ g/L; $Q_P = 0.40$ g/L h; $Y_{P/S} = 0.58$ g/g) were obtained at an AR as high as 600 mL/min, indicating that high ARs are required in these systems to ensure suitable oxygen transfer into the inside of the beads.

7.4 Conclusions and Future Recommendations

The microbial production of xylitol has been widely investigated in the last two decades either in synthetic media or lignocellulosic hydrolyzates, and the most suitable microorganisms and operating conditions were selected to maximize both productivity and yield. The traditional batch processes have been mostly replaced by fed-batch or continuous systems, in most of the cases using yeast cells immobilized in different matrices, where aeration plays a crucial role. Nowadays, the trends seem to be focused on the production of compounds with homogeneous composition to be obtained from cheap carbon and nutrients sources or even

wastes. In order to achieve this goal more research is needed to develop novel bioreactors for xylitol production able to keep the oxygen and energy requirements as low as possible.

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