Appl Microbiol Biotechnol (2001) 57:738–743
DOI 10.1007/s002530100816

ORIGINAL PAPER

**L. Sene · A. Converti · M. Zilli · M. G. A. Felipe
S. S. Silva**

Metabolic study of the adaptation of the yeast *Candida guilliermondii* to sugarcane bagasse hydrolysate

Received: 25 June 2001 / Accepted: 17 August 2001 / Published online: 9 October 2001
© Springer-Verlag 2001

Abstract Batch xylitol production from concentrated sugarcane bagasse hydrolysate by *Candida guilliermondii* was performed by progressively adapting the cells to the medium. Samples were analyzed to monitor sugar and acetic acid consumption, xylitol, arabitol, ethanol, and carbon dioxide production, as well as cell growth. Both xylitol yield and volumetric productivity remarkably increased with the number of adaptations, demonstrating that the more adapted the cells, the better the capacity of the yeast to reduce xylose to xylitol in hemicellulose hydrolysates. Substrate and product concentrations were used in carbon material balances to study in which way the different carbon sources were utilized by this yeast under microaerobic conditions, as well as to shed light on the effect of the progressive adaptation to the medium on its fermentative activity. Such a theoretical means allowed estimation for the first time of the relative contribution of each medium component to the formation of the main products of this fermentation system.

Introduction

Xylitol, an anticariogenic sweetener also suitable for the use by diabetics, is currently manufactured by catalytic reduction of highly purified xylose solutions. The biotechnological production of xylitol by microorganisms and/or enzymes could be a cheaper alternative and could provide a natural product with superior organoleptic characteristics (Winkenhausen and Kuzmanova 1998).

A. Converti (✉) · M. Zilli
Department of Chemical and Process Engineering “G. B. Bonino”, University of Genoa, via Opera Pia 15, 16145 Genoa, Italy
e-mail: converti@unige.it
Tel.: +39-010-3532593, Fax: +39-010-3532586

L. Sene · M.G.A. Felipe · S.S. Silva
Department of Biotechnology,
Faculty of Chemical Engineering of Lorena (FAENQUIL),
Campus I, Rodovia Itajubá-Lorena km 74.5, 12600-000,
Lorena-SP, Brazil

Pachysolen tannophilus (Ditzelmüller et al. 1984), *Debaryomyces hansenii* (Gírio et al. 1999), and various species of *Candida* (Barbosa et al. 1988; Meyrial et al. 1991; Nolleau et al. 1995; Sirisansaneeyakul et al. 1995) have received great attention as the most-promising xylitol producers.

As is well known, xylose is reduced in xylose-fermenting yeasts by NADPH- and/or NADH-dependent xylose reductase (XR) to xylitol, which is then oxidized to xylulose by a NAD^+- or $NADP^+$-dependent xylitol dehydrogenase (XDH). In the next step, xylulose is phosphorylated by xylulose kinase to xylulose 5-phosphate, which can enter the pentose phosphate and consequently the glycolytic pathways.

Candida guilliermondii XR proved strictly dependent on NADPH, while its XDH demonstrated preferential activity with NAD^+ (Lee et al. 1996; Nolleau et al. 1995). According to Barbosa et al. (1988), the preference of XR for NADPH determines a partial inability of the yeast to regenerate, under oxygen-limited conditions, the NADH produced by XDH activity and the necessity of overproducing xylitol for a correct redox balance. Oxygen supply has the strongest effect on xylitol production in yeasts, such as *C. guillermondii*, which exhibits very high NADPH/NADH XR activity ratios (Nolleau et al. 1995) and no transhydrogenase activity (Winkelhausen and Kuzmanova 1998). At low O_2 transfer rates, the respiratory chain can not oxidize the excess NADH (Custer effect), thus preventing the oxidation of xylitol to xylulose, leading to xylitol accumulation.

Xylitol production is also regulated by pH, temperature, starting xylose level, presence of other sugars (du Preez et al. 1986a, b), and inoculum level (Parajó et al. 1996b). In addition, hemicellulosic hydrolysates contain toxic compounds, such as furfural, hydroxymethylfurfural, and acetic acid (Tran and Chambers 1985). Several treatments were proposed to minimize their toxicity, including acetic acid evaporation (Converti et al. 1999), pH variation and overliming (Converti et al. 1999; Mayerhoff et al. 1997), active charcoal adsorption (Parajó et al. 1996a), and solvent extraction (Parajó et al.

1997). However, most are expensive or not totally effective. Cell adaptation has also been shown to improve the efficiency of xylitol production: xylitol yield and volumetric productivity increased by 46%–50% when *C. guilliermondii* cells well adapted to the hydrolysate were cultivated in shaken flasks (Sene et al. 1998).

Nevertheless, no metabolic studies have yet been published for this microbial system on the effect of cell adaptation to the culture medium. For this reason, the experimental data of batch fermentations performed in a controlled fermenter at different adaptation levels were used in this study to estimate the carbon source splitting among the different xylose-consuming ways that are simultaneously active in *C. guilliermondii*.

Materials and methods

Preparation of sugarcane bagasse hydrolysate

The hemicellulosic hydrolysate was prepared and concentrated under vacuum as previously described (Sene et al. 1998), giving the following composition (g/l): 7.9–8.3 glucose, 62.1–64.0 xylose, 6.3–7.4 arabinose, 4.2–4.7 acetic acid, 0–0.01 furfural, and 0.18–0.20 hydroxymethylfurfural. The concentrated hydrolysates were treated with calcium oxide to pH 10.0, filtered, acidified to pH 5.5 with H_2SO_4, autoclaved at 115°C for 15 min, and finally supplemented with the following nutrients (g/l): 2.0 $(NH_4)_2SO_4$, 0.1 $CaCl_2.2H_2O$, and 20.0 raw rice bran extract.

Inocula preparation and fermentation conditions

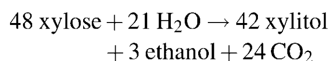
Cells of *C. guilliermondii* FTI 20037 were maintained on malt-extract agar slants at 4°C. Four different inocula, labeled as L_1, L_2, L_3, and L_4, were obtained by progressively adapting the cells from 1 up to 4 times to hydrolysates with increasing sugar levels. The inoculum cultivation was performed in 125-ml Erlenmeyer flasks containing 50 ml of each hemicellulosic hydrolysate, incubated at 30°C in a rotary shaker at 200 rpm, for 24 h. Cells were centrifuged, rinsed with sterile water, and resuspended to give an initial inoculum concentration of 0.36 g/l. The fermentations were carried out in triplicate in a 5-l Bioflo III fermenter (New Brunswick, Scientific Co) containing 2.5 l of the above medium at 30±0.2°C, agitation at 300 rpm, aeration of 0.4 vvm, corresponding to starting k_La=21/h. The operating conditions were automatically controlled through an electronic device. The pH was maintained at a selected value (5.5±0.1) by the addition of a stream of 1.0 M NaOH solution.

Analytical methods

Sugars, xylitol, arabitol, ethanol, acetic acid, furfural, and hydroxymethylfurfural concentrations were determined by high-performance liquid chromatography as previously described (Sene et al. 1998). Cell concentration was determined by absorbance at 600 nm by correlation with the dry weight obtained from a calibration curve. CO_2 development during the fermentations was followed by monitoring the exhaust gas with a CO_2 gas analyzer, and the total amount of CO_2 produced at each fermentation time was calculated by integration of the time course varying in CO_2 concentration.

Carbon material balances

Carbon material balances were based on the production of xylitol, arabitol, ethanol, CO_2, and biomass, as well as on the consumption of xylose, arabinose, glucose, and acetic acid. For this carbon balance, the equation proposed by Barbosa et al. (1988) for the redox-balanced production of xylitol under microaerobiosis:



$$48\ \text{xylose} + 21\ H_2O \rightarrow 42\ \text{xylitol} + 3\ \text{ethanol} + 24\ CO_2$$

was used to take into account the parallel metabolism necessary to obtain energy. A similar equation was assumed for arabitol production from D-arabinose. Ethanol production in excess with respect to this stoichiometry was ascribed to glucose fermentation.

Because of the catabolite repression of induction of XR activity (Lee et al. 1996) and the inactivation of the xylose transport system of the cell (Nobre et al. 1999), glucose is assimilated by pentose-fermenting yeasts better than xylose. Therefore cell growth was assumed to take place primarily at the expense of glucose, and subsequently of xylose. The average minimal formula $CH_{1.79}O_{0.50}N_{0.20}$ reported by Roels (1983) for yeast dry biomass was used to estimate the carbon consumption due to biomass growth. All the carbon fractions not consumed by these routes were supposed to be utilized for respiration, including acetic acid, which was continuously consumed during the runs. The theoretical CO_2 production, estimated as the difference between total starting carbon (from xylose, glucose, arabinose, and acetic acid) and carbon consumed for ethanol, xylitol, arabitol, and biomass production, was compared with the experimental production to check the validity of the proposed metabolic model.

Results

The experimental results of batch runs performed in controlled fermenter with cells of *C. guilliermondii* progressively adapted to hemicellulosic hydrolysates are shown in Fig. 1. Glucose consumption was very quick and not significantly influenced by the adaptation level. Its preferential consumption with respect to xylose is in agreement with the catabolite repression of induction of its XR activity (Lee et al. 1996). Glucose levels below 10% of total xylose should not affect xylitol production (Felipe et al. 1993) and should stimulate biomass growth (Parajó et al. 1998). Final biomass concentrations were similar for all tested adaptation levels, due to nearly coincident rates and fractions of glucose and xylose assumption for this purpose.

In contrast, arabinose was slowly assimilated, about 50% of its starting amount being detected in the medium at the end, regardless of the adaptation level. Its consumption, already observed in *C. guilliermondii* grown in shaken flasks (Sene et al. 1998), was mainly utilized for the production of arabitol (Meyrial et al. 1991), the final concentration of which increased according to the biomass adaptation level. Its remarkable increase observed passing from L_1 to L_4 (about 30%) can not be explained by the slight differences in the initial arabinose concentration (Fig. 1c), but can be ascribed to a progressive enhancement of D-arabinose reductive metabolism, which was qualitatively similar to that of D-xylose. This result confirms the validity of assuming for arabitol production the same stoichiometry proposed by Barbosa et al. (1988) for xylitol formation.

Arabinose reduction to arabitol took place at an appreciable rate only at the end of the runs, after xylose was nearly totally consumed during the exponential

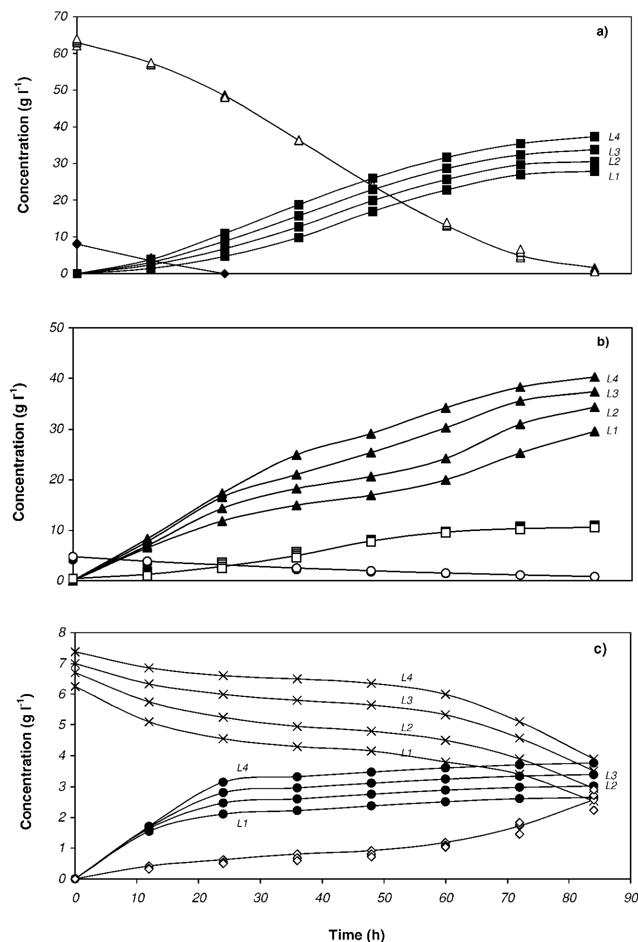


Fig. 1a–c Experimental results of fermentations of sugarcane bagasse acid hydrolysate by *Candida guilliermondii* cells progressively adapted to the medium. Adaptation levels: L_1, L_2, L_3, L_4; concentrations (g/l). **a** *White triangles* xylose; *black diamonds* glucose, *black squares* xylitol; **b** b*lack triangles* carbon dioxide, *white squares* biomass, *white circles* acetic acid; **c** crosses arabinose, *black circles* ethanol, *white diamonds* arabitol

phase, according to the typical trends of mixed substrate fermentations (Gírio et al. 1999). Since D-xylose and D-arabinose are partly assimilated by the same metabolic pathway, it is unlikely that such a surprising sequential utilization could be due to any repression mechanism. This suggests that (1) only one aldose reductase could be, at least partly, responsible for both D-xylose and D-arabinose reductions to their respective pentitols, (2) its affinity for the latter substrate could be less than for the former, and (3) its activity could be modulated by simple competitive inhibition.

Ethanol formation, which was initially very fast because of glucose fermentation, accelerated with increasing the number of adaptations (by approximately 40% when comparing L_4 with L_1). As the initial glucose assimilation was similar for all the adaptation levels, the difference in final ethanol concentration was due to the contributions of xylose and, to some extent, of arabinose reductive metabolism.

Table 1 Influence of the number of biomass adaptations (L) on xylitol yield ($Y_{P/S}$) and volumetric productivity (Q_P) in different fermentation phases

Phase	Lag	Exponential	Stationary
L_1			
$Y_{P/S}$ (g_P/g_S)	0.293	0.568	0.284
Q_P (g_P/l per hour)	0.125	0.592	0.080
L_2			
$Y_{P/S}$ (g_P/g_S)	0.423	0.575	0.290
Q_P (g_P/l per hour)	0.197	0.594	0.078
L_3			
$Y_{P/S}$ (g_P/g_S)	0.516	0.580	0.295
Q_P (g_P/l per hour)	0.260	0.597	0.116
L_4			
$Y_{P/S}$ (g_P/g_S)	0.595	0.595	0.281
Q_P (g_P/l per hour)	0.322	0.600	0.157

With regard to xylose-xylitol conversion, the highest xylitol level (37.3 g/l) was obtained with the best-adapted cells (L_4), which corresponds to a 34% increase when compared with the worst-adapted inoculum (L_1) (Fig. 1a). As shown in Table 1, both xylitol yield ($Y_{P/S}$) and volumetric productivity (Q_P) progressively increased with the degree of cell adaptation and were higher during the exponential than the lag phase. Nevertheless, the highest xylitol yield (0.59 g_P/g_S) was already reached with the best adaptation level (L_4) during the lag phase, while the highest xylitol productivity (0.60 g_P/l per hour) was observed with L_3 during the exponential phase. Ignoring the behavior during the less-significant stationary phase, these results demonstrate the expected generalized improvement of the fermentation performance with progressively adapting biomass to the medium. The marked improvement of kinetic parameters in the presence of well-adapted biomass is probably due to a combination of progressive induction of the enzymes for xylose utilization and improved cell capacity to metabolize toxic compounds present in the hydrolysate. For example, acetic acid, present in the starting medium at higher levels (4.2–4.7 g/l) than that reported by Watson et al. (1984) as totally inhibitory for the growth of *P. tannophilus* (1.45 g/l), was consumed well below this concentration, at a starting rate that appreciably increased with the level of cell adaptation.

Because of glucose fermentation, CO_2 formation was, as that of ethanol, very fast at the beginning of all the runs, but proceeded at a lower rate during the next phases, due to the poor contribution of xylose and arabinose reductive metabolism to its formation. The appreciable CO_2 production during the stationary phase, not linked to these activities, can be justified by a significant role of respiration, under conditions of carbon source depletion, to obtain the largest amount of energy as possible as ATP.

Finally, xylitol concentration (27.9–37.3 g_P/l) and product yield on consumed xylose (0.46–0.58 g_P/g_S) ob-

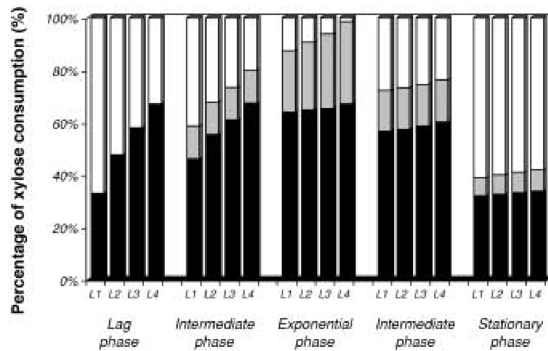


Fig. 2 Percentages of xylose consumed by different metabolic activities during different fermentation phases in *C. guilliermondii* cells progressively adapted to sugarcane bagasse acid hydrolysate. Adaptation levels: L_1, L_2, L_3, L_4. *Black bars* xylitol production, *white bars* respiration, *grey bars* biomass growth

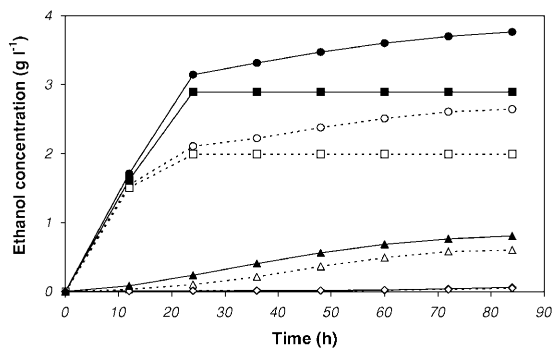


Fig. 3 Ethanol produced from total carbon by different metabolic activities in *C. guilliermondii* cells progressively adapted to sugarcane bagasse acid hydrolysate. Total ethanol: L_1 (*white circles*), L_4 (*black circles*); ethanol from glucose fermentation: L_1 (*white squares*), L_4 (*black squares*); ethanol from the parallel metabolism linked to xylitol production: L_1 (*white triangles*), L_4 (*black triangles*); ethanol from the parallel metabolism linked to arabitol production: L_1 (*white diamonds*), L_4 (*black diamonds*)

served at the end of all tested adaptation levels were higher than those obtained by Carvalho et al. (in preparation) with immobilized cells (21.3 g_P/l and 0.47 g_P/g_S, respectively), because of possible mass transfer limitations and/or inhibitions occurring inside the pellet. Since arabitol and ethanol were detected in the broths only as secondary products (about 8% and 10% of final xylitol concentration, respectively), this work suggests that four successive adaptations could be sufficient to ensure the best fermentation performance.

To study the metabolic behavior of *C. guilliermondii* under variable adaptation conditions, the results of Fig. 1 were used to estimate xylose splitting among the different metabolic activities simultaneously present under microaerobic conditions (xylitol and ethanol production, respiration, and cell growth) (Fig. 2) and the ethanol and carbon dioxide production from total carbon (Figs. 3 and 4, respectively).

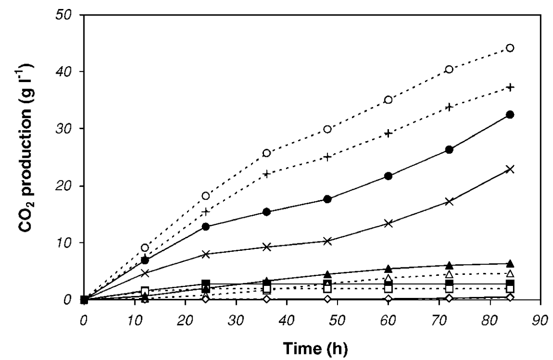


Fig. 4 CO_2 production estimated from total carbon by different metabolic activities in *C. guilliermondii* cells progressively adapted to sugarcane bagasse acid hydrolysate. Total CO_2: L_1 (*white circles*), L_4 (*black circles*); CO_2 from respiration: L_1 (+), L_4 (×); CO_2 from glucose fermentation: L_1 (*white squares*), L_4 (*black squares*); CO_2 from the parallel metabolism linked to xylitol production: L_1 (*white triangles*), L_4 (*black triangles*); CO_2 from the parallel metabolism linked to arabitol production: L_1 (*white diamonds*), L_4 (*black diamonds*)

Discussion

Figure 2 shows that ethanol production was not reliably linked to any excess xylose consumption, which means that only glucose was fermented under the selected conditions.

At all tested levels of adaptation, the percentage of xylose consumed for xylitol production reached maximum values during the exponential phase (65%–67%), after glucose exhaustion. Alternative metabolic routes seemed to be favored under suboptimal conditions. In the absence of alcohol production from xylose, the respiratory activity gained relative importance, consuming from 33% to 67% xylose during the lag phase and about 60% xylose during the stationary phase.

The most-marked effect of biomass adaptation was the relative xylose consumption by the different metabolic activities during the lag phase. While at the lowest adaptation level (L_1), the xylose fraction consumed for xylitol production progressively increased from the lag to the exponential phase, nearly the same percentages of xylose were consumed during these phases (67%) when using well-adapted cells (L_4). In other words, the progressive biomass adaptation could have induced the enzymes responsible for xylitol formation, thus shortening the lag phase and improving the productivity. Regardless of the adaptation level, the xylose fraction consumed for xylitol formation progressively decreased after the exponential phase, reaching a minimum (<40%) during the stationary phase.

According to the sequential assimilation of sugars by yeasts, it was estimated that cell growth, initially sustained by glucose, accounted for 23%–31% of xylose consumption, depending on the adaptation level, during the exponential phase. This decreased significantly during the stationary phase (up to 7%–8%). These results confirm the validity of adopting microaerobic conditions

Table 2 Influence of the number of adaptations (L) on the rates of carbon dioxide (CO_2) and ethanol (EtOH) formation (mg/l per hour) due to different metabolic activities in different fermentation phases

Phase	Lag		Exponential		Stationary	
	CO_2	EtOH	CO_2	EtOH	CO_2	EtOH
L_1						
Total productivity	759.6	128.4	348.6	13.0	312.8	3.1
Xylitol formation	20.7	2.7	97.9	12.8	13.2	1.7
Glucose fermentation	119.7	125.1	–	–	–	–
Arabitol formation	4.7	0.6	1.5	0.2	10.79	1.4
Respiration	614.5	–	249.2	–	288.9	–
L_2						
Total productivity	683.9	136.5	298.7	13.0	296.8	3.2
Xylitol formation	32.6	4.3	98.3	12.8	12.8	1.7
Glucose fermentation	125.8	131.4	–	–	–	–
Arabitol formation	5.8	0.8	1.6	0.2	11.6	1.5
Respiration	519.7	–	198.8	–	272.4	–
L_3						
Total productivity	623.3	139.4	256.1	13.1	409.7	4.2
Xylitol formation	43.0	5.6	98.7	12.9	19.2	2.5
Glucose fermentation	127.4	133.2	–	–	–	–
Arabitol formation	4.8	0.6	1.7	0.2	13.1	1.7
Respiration	448.1	–	155.7	–	377.4	–
L_4						
Total productivity	574.1	142.5	186.2	13.2	513.2	5.3
Xylitol formation	53.3	7.0	99.3	13.0	25.9	3.4
Glucose fermentation	129.0	134.9	–	–	–	–
Arabitol formation	4.4	0.6	1.8	0.2	14.7	1.9
Respiration	387.4	–	85.1	–	472.6	–

to initially stimulate cell growth and, subsequently, xylitol production.

Although total carbon was split among the different metabolic activities, as xylose (results not shown), CO_2 instead of xylitol was the main product, because of the respiration or fermentation of minor carbon sources to obtain energy.

As shown in Fig. 3, about 80% of total ethanol came from glucose fermentation within the first 12–24 h, regardless of the adaptation level. Negligible amounts were produced by the parallel metabolism linked to xylose and arabinose reductions to their respective pentitols. However, xylose was the major carbon source for ethanol production after this period, because of the complete glucose depletion. The large increases in ethanol production observed using well-adapted cells (L_4) can be explained by the acceleration of xylitol production from the lag to the exponential phase.

Analogously, Fig. 4 shows the fractions of CO_2 progressively produced by the same ways and by respiration. As for ethanol, the CO_2 release remarkably accelerated at the start of the run, because of the good oxygen availability for respiration. In addition, this activity was always responsible for the largest CO_2 production, while the fraction associated with arabitol production was negligible. In contrast, glucose fermentation was responsible for a significant proportion of CO_2 formation only at the beginning, while the contribution of xylitol production became appreciable only during the log phase.

The above effects of cell adaptation are shown more clearly in Table 2, which gives both ethanol and CO_2 productivity estimated for the different metabolic activities. Total ethanol productivity was maximal during the lag phase, because of the strong contribution of glucose fermentation to ethanol formation (94.7%–97.4%). The subsequent and progressive deceleration of ethanol production during the exponential and the stationary phases is because this product was almost totally formed by the parallel metabolism linked to xylitol formation (98.3%–98.4%). Passing from L_1 to L_4, total ethanol productivity during the lag phase slowly increased from 0.128 to 0.142 g/l per hour, the relative increase in productivity linked to xylitol formation increased from 2.1% to 4.9%, while, as expected, the relative contribution of glucose fermentation decreased from 97.4% to 94.7%.

Even more interesting was the behavior of total CO_2 formation during the lag phase. This decreased sharply from 0.760 to 0.574 g/l per hour with increasing adaptation level, because of the increasing importance of xylitol formation (from 2.7% to 9.3%) and alcohol fermentation (from 15.8% to 22.4%), at the expense of respiration (from 80.9% to only 65.7%). A similar trend can be observed for the exponential phase. As a consequence, at the poorest adaptation levels (L_1 and L_2), total productivity decreased progressively from the lag to the stationary phase, while at the best-adapted levels (L_3 and L_4), the rate of CO_2 formation increased significantly during the stationary phase to support the cell energy requirements.

Even though the errors were always less than 10%, the experimental data for CO_2 production were always less than the estimated values, which could be the result

of systematic loss of CO_2 due to the experimental procedure and/or inability of the proposed model to accurately describe the actual metabolic behavior of the system.

Finally, the results of this study confirm the energetic role of secondary carbon sources contained in the hydrolysate and the validity of carbon material balances in metabolic studies.

Acknowledgement L. Sene received a research grant from FAPESP, São Paulo, Brazil.

References

Barbosa MFS, Medeiros MB, Mancilha IM, Schneider H, Lee H (1988) Screening of yeasts for production of xylitol from D-xylose and some factors which affect xylitol yield in *Candida guilliermondii*. J Ind Microbiol 3:241–251

Converti A, Perego P, Domínguez JM (1999) Xylitol production from hardwood hemicellulose hydrolysates by *Pachysolen tannophilus*, *Debaryomyces hansenii* and *Candida guilliermondii*. Appl Biochem Biotechnol 82:141–151

Ditzelmüller G, Kubicek CP, Wöhrer W, Röhr M (1984) Xylitol metabolism in *Pachysolen tannophilus*: purification and properties of xylose reductase. Can J Microbiol 30:1330–1336

Felipe MGA, Mancilha IM, Vitolo M, Roberto IC, Silva SS, Rosa SAM (1993) Preparação de xilitol por fermentação do hidrolisado hemicelulósico de bagaço de cana-de açúcar. Arq Biol Tecnol 36:103–114

Gírio FM, Amaro C, Azinheira H, Pelica F, Amaral-Collaço MT (1999) Polyols production during single and mixed substrate fermentations in *Debaryomyces hansenii*. Biores Technol 71:245–251

Lee H, Sopher CR, Yau KFY (1996) Induction of xylose reductase and xylitol dehydrogenase activities on mixed sugars in *Candida guilliermondii.* J Chem Technol Biotechnol 66:375–379

Mayerhoff ZDVL, Roberto IC, Silva SS (1997) Xylitol production from rice straw hemicellulose hydrolysate using different yeast strains. Biotechnol Lett 19:407–409

Meyrial V, Delgenes JP, Molletta R, Navarro JM (1991) Xylitol production from D-xylose by *Candida guilliermondii*: fermentation behaviour. Biotechnol Lett 13:281–286

Nobre A, Lucas C, Leão C (1999) Transport and utilization of hexoses and pentoses in the halotollerant yeast *Debaryomyces hansenii*. Appl Environ Microbiol 65:3594–3598

Nolleau V, Preziosi-Belloy L, Navarro JM (1995) The reduction of xylose to xylitol by *Candida guilliermondii* and *Candida parapsilosis*: incidence of oxygen and pH. Biotechnol Lett 17:417–422

Parajó JC, Domínguez H, Domínguez JM (1996a) Charcoal adsorption of wood hydrolysates for improving their fermentability: influence of the operational conditions. Biores Technol 57:179–185

Parajó JC, Domínguez H, Domínguez JM (1996b) Production of xylitol from concentrated wood hydrolysates by *Debaryomyces hansenii*: effect of the initial cell concentration. Biotechnol Lett 18:593–598

Parajó JC, Domínguez H, Domínguez JM (1997) Xylitol production of from *Eucalyptus* wood hydrolysates extracted with organic solvents. Proc Biochem 32:599–604

Parajó JC, Domínguez H, Domínguez J M (1998) Biotechnological production of xylitol. II. Operation in culture media made with commercial sugars. Biores Technol 65:203–212

Preez JC du, Bosch M, Prior BA (1986a) The fermentation of hexose and pentose sugars by *Candida shehatae* and *Pichia stipitis*. Appl Microbiol Biotechnol 23:228–233

Preez JC du, Bosch M, Prior BA (1986b) Xylose fermentation by *Candida shehatae* and *Pichia stipitis*: effects of pH, temperature and substrate concentration. Enzyme Microb Technol 8:360–364

Roels JA (1983) Energetics and kinetics in biotechnology. Elsevier Biomedical, Amsterdam

Sene L, Felipe MGA, Vitolo M, Silva SS, Mancilha I M (1998) Adaptation and reutilization of *Candida guilliermondii* cells for xylitol production in sugar cane bagasse hydrolysate. J Basic Microbiol 38:61–69

Sirisansaneeyakul S, Staniszewski M, Rizzi M (1995) Screening of yeasts for xylitol from D-xylose. J Ferment Bioeng 6:565–570

Tran AV, Chambers RP (1985) Red oak wood derived inhibitors in the ethanol fermentation of xylose by *Pichia stipitis* CBS 5776. Biotechnol Lett 7:841–846

Watson NE, Prior BA, Lategan PM (1984) Factors in acid treated bagasse inhibiting ethanol production from D-xylose by *Pachysolen tannophilus*. Enzyme Microb Technol 6:451–456

Winkelhausen E, Kuzmanova S (1998) Microbial conversion of D-xylose to xylitol. J Ferment Bioeng 86:1–14