Influence of Temperature and pH on Xylitol Production from Xylose by *Debaryomyces hansenii*

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Abstract: The production of xylitol from concentrated synthetic xylose solutions (S_o = 130-135 g/L) by *Debaryomyces hansenii* was investigated at different pH and temperature values. At optimum starting pH (pH_o = 5.5), T = 24°C, and relatively low starting biomass levels (0.5–0.6 g_x/L), 88% of xylose was utilized for xylitol production, the rest being preferentially fermented to ethanol (10%). Under these conditions, nearly 70% of initial carbon was recovered as xylitol, corresponding to final xylitol concentration of 91.9 g_P/L, product yield on substrate of 0.81 g_P/g_S, and maximum volumetric and specific productivities of 1.86 g_P/L · h and 1.43 g_P/g_x · h, respectively. At higher and lower pH_o values, respiration also became important, consuming up to 32% of xylose, while negligible amounts were utilized for cell growth (0.8–1.8%). The same approach extended to the effect of temperature on the metabolism of this yeast at pH_o = 5.5 and higher biomass levels (1.4–3.0 g_x/L) revealed that, at temperatures ranging from 32–37°C, xylose was nearly completely consumed to produce xylitol, reaching a maximum volumetric productivity of 4.67 g_P/L · h at 35°C. Similarly, both respiration and ethanol fermentation became significant either at higher or at lower temperatures. Finally, to elucidate the kinetic mechanisms of both xylitol production and thermal inactivation of the system, the related thermodynamic parameters were estimated from the experimental data with the Arrhenius model: activation enthalpy and entropy were 57.7 kJ/mol and –0.152 kJ/mol · K for xylitol production and 187.3 kJ/mol and 0.054 kJ/mol · K for thermal inactivation, respectively. *Biotechnol Bioeng* **75**: 39–45, 2001.

Keywords: *Debaryomyces hansenii*; xylitol production; effect of initial pH; temperature variation; thermodynamic parameters

INTRODUCTION

Xylitol, a pentitol with increasing interest owing to its dietetic and clinical properties, can be formed as a metabolic intermediary product of D-xylose fermentation: D-xylose can be converted to xylitol by aldose reductase (EC 1.1.1.21; alditol:$NADP^+$ oxidoreductase), commonly called xylose reductase (XR), which has a broad substrate specificity for aliphatic and aromatic aldehydes and for aldosugars (Kuhn et al., 1995). The halotolerant yeast *Debaryomyces hansenii* (Nobre et al., 1999) was widely studied to carry out this bioconversion (Domínguez et al., 1997; Gírio et al., 1999; Roseiro et al., 1991), for which it preferentially uses NADPH as reducing cofactor (Gírio et al., 1990). Its ability to overproduce xylitol is the result of the combination of a high NADPH-dependent XR activity and a low XDH activity. In fact, Gírio et al. (1990) reported negligible NADH- and 60 μmol min^{-1} mg^{-1} NADPH-dependent XR activities in this yeast, as well as lower affinity of XDH for NAD^+ with respect to poor xylitol producers (Gírio et al., 1996).

As demonstrated by Vandeska et al. (1995) for *Candida boidinii*, an increase in oxygen transfer rate from 0.33–1.0 g/L · h resulted in a corresponding decrease of the XR/XDH activity ratio from 2.1 to 1.1, which means that oxygen plays an important role in the conversion of xylose by yeasts. In particular, xylose can be metabolized in four different ways, whose relative significance depends on oxygen availability: reductive production of xylitol, ethanol fermentation, respiration, and cell growth.

Under anaerobic or oxygen-limited conditions (according to the species), xylitol formation can only occur in yeasts that exhibit enhanced NADH-linked aldose reductase activity, i.e., *Candida parapsilosis* (Nolleau et al., 1993, 1995), *Candida guilliermondii* (Barbosa et al., 1988; Felipe et al., 1997), *Pichia stipitis* (Bruinenberg et al., 1984; Gírio et al., 1990; Rizzi et al., 1988), *Candida shehatae* (Bruinenberg et al., 1984; du Preez et al., 1989; Gírio et al., 1989), *Candida tenuis* (Bruinenberg et al., 1984), and *Pachysolen tannophilus* (Ditzelmüller et al., 1984; Smiley and Bolen, 1982), with ethanol being in most cases the major fermentation product. Xylitol productivity may be increased in these systems by using high biomass levels (Converti et al., 1999b; Parajó et al., 1996; Roberto et al., 1996). On the contrary, xylitol production is not possible under strictly anaerobic

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conditions in some yeasts, like *Debaryomyces hansenii* (Gírio et al., 1990, 1999; Nobre et al., 1999; Roseiro et al., 1991), which show negligible NADH-dependent XR activity.

Under excess aerobic conditions, the NADH formed in the second step catalyzed by XDH can be reoxidized by the respiration chain, but xylitol is consumed for cell growth: besides, in *D. hansenii* oxygen inhibits XR activity more than XDH activity (Gírio et al., 1994), thus affecting xylitol production; to improve the yield of ATP, cell growth is then favored. But under semi-aerobic conditions (Gírio et al., 1999; Roseiro et al., 1991), oxygen availability is just sufficient to satisfy NADH regeneration for the second oxidative step; therefore, NADPH is entirely used to reduce xylose, leading to xylitol accumulation (Barbosa et al., 1988). For these reasons, semi-aerobic conditions were preferred in this study for xylose fermentation by *D. hansenii*.

Although a lot of information is available in the literature on the most important operational factors affecting the bioconversion of xylose to xylitol by yeasts, such as age and concentration of the inoculum, starting substrate level, pH, temperature, oxygen level, and aeration rate (Parajó et al., 1998b), only a few studies were performed to elucidate their influence on the metabolism of *D. hansenii* (Domínguez et al., 1996, 1997; Nobre et al., 1999; Roseiro et al., 1991).

To clarify the effects of temperature and pH on this yeast, the data of batch fermentations performed on synthetic D-xylose solutions alternatively varying these parameters were used to carry out material balances of carbon consumed through the reductive production of xylitol, ethanol fermentation, respiration, and cell growth.

The final part of this study focused on the estimation of the thermodynamic parameters (activation enthalpy and entropy) of this bioprocess in order to make clearer the phenomena involved in both xylitol production and its thermal inactivation. Among the three different approaches reported in the literature for this estimation—Arrhenius plots (Aiba et al., 1973; Sizer, 1944) and "thermodynamic" and "kinetic" approaches (Bailey and Ollis, 1986; Roels, 1983)—the first was selected because it proved the best tool for fermentation systems (Arni et al., 1999).

MATERIALS AND METHODS

Microorganism

The bioconversion of xylose into xylitol was performed with the yeast *Debaryomyces hansenii* NRRL Y-7426 (kindly provided by the Northern Regional Research Laboratory, USDA, Peoria, IL, USA). The lyophilized cells were grown for 3 days at 32°C in an incubator shaken at 200 rpm (New Brunswick, Edison, NJ, USA), in a liquid medium containing 10 g/L glucose, 10 g/L xylose, 3 g/L Bacto-yeast extract, 3 g/L Bacto-malt extract, and 5 g/L Bacto-peptone.

The cells were then maintained in agar slant tubes containing a medium formulated with the same components and concentrations as the previous one (without glucose) plus 20 g/L agar. The microorganism was finally adapted to xylose as the carbon source by carrying out four successive batch cultures using as inoculum the cells recovered by centrifugation from the previous culture.

Culture Conditions

A comparison among literature data of volumetric and specific productivities and xylitol yields obtained by different research groups (Parajó et al., 1998a) demonstrated that Erlenmeyer flasks shaken at 150–200 rpm and containing about 20–30% medium volume are able to ensure xylitol yields comparable with those of semi-aerobic tests carried out in stirred tank reactor, at values of k_La ranging from 4.8–35.4 h^{-1} and volumetric air flow/system volume ratios up to 0.29 vvm, respectively.

Thus, for simplicity, batch tests at variable starting pH (pH_o) were carried out at 24°C using a starting biomass concentration of 0.5–0.6 g_x/L, in 500-mL Erlenmeyer flasks (containing 100 mL of culture medium covered with cotton caps), placed in a rotary shaker at 180 rpm for 2–4 days. Tests at different temperatures were performed at pH_o = 5.5 and higher starting biomass level (1.4–3.0 g_x/L).

The culture media, prepared with pure xylose (S_o = 130–135 g_S/L), were supplemented with 3 g/L Bacto-yeast extract, 3 g/L Bacto-malt extract, and 3 g/L Bacto-peptone, and sterilized in autoclave.

Analytical Methods

The concentrations of xylose and xylitol were determined using a Hitachi high-performance liquid chromatographic system consisting of an AS-4000 Intelligent Auto Sampler, a Hitachi L-3350 refractive index monitor, a Hitachi L-6000 pump, and a Hitachi D-2500 chromato-integrator. Separation was achieved using an organic acid column (Aminex HPX-87 H Ion Exclusion Column 300 × 7.8 mm, Bio-Rad, Hercules, CA, USA) at 60°C with 0.01 N sulfuric acid as mobile phase at 0.8 mL/min. HPLC was also used to detect the possible formation of glycerol and other minor products of xylitol fermentation (arabinitol, ribitol, etc.).

pH determinations at the end of every test revealed that this parameter only slightly decreased (less than 0.5 pH units) as a consequence of CO_2 production.

Ethanol concentration was determined by a gas chromatograph Electrometer 200 (Carlo Erba, Milan, Italy) using a flame ionization detector and a 80/120 Carbopack B AW/5% Carbowax 20M, 6′ × ¼″ OD × 2 mm ID glass column, with nitrogen as a carrier gas at a flow rate of 20 mL/min. Temperatures of injector and detector were 200 and 150°C, respectively. Oven temperature ranged from 70–150°C. The same analysis was used to detect the possible formation of acetate as by-product of xylitol fermentation.

Biomass concentration in the fermentation broths and inocula was determined by dry weight filtering known vol-

umes of samples on 0.45 μm membrane filters. The filters were dried at 85°C until no weight change between consecutive measurements was observed (about 48 h).

CO_2 development during fermentation was followed in two preliminary blank fermentation tests carried out, under semi-aerobic conditions comparable with those of shaken flasks, in a 5-L working volume Stirred Tank Reactor, during which CO_2 concentration of the exhaust gas was monitored by a CO_2 gas analyzer (COS Co., Model GH-250E). 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 Balance

Carbon material balance was performed on the basis of the productions of xylitol, ethanol, CO_2, and biomass as well as on the consumption of xylose. The average minimal formula $CH_{1.79}\ O_{0.50}\ N_{0.20}$ proposed by Roels (1983) for yeast dry biomass was used for the calculation of carbon consumption due to biomass growth.

A parallel estimation of the amount of CO_2 produced during blank tests was done by a carbon balance, assuming that carbon is consumed by xylitol and ethanol fermentations, cell growth, and respiration. Thus, it was estimated as the difference between total starting carbon (xylose) and carbon consumed for the productions of ethanol, biomass (assuming the above yeast composition), and xylitol. For this carbon balance, we used the equation proposed by Barbosa et al. (1988) for the redox-balanced semi-aerobic production of xylitol in yeasts possessing only NADPH-dependent XR activity:

$$60\ \text{xylose} + 12\ \text{ADP} + 12\ P_i + 12\ H_2O + 3\ O_2 \rightarrow 54\ \text{xylitol} + 12\ \text{ATP} + 30\ CO_2 \quad (1)$$

which resulted from material balances for: 1) xylose reduction to xylitol by NADPH-linked XR; 2) regeneration by the respiratory chain of NADH produced in the reaction catalyzed by XDH, with ATP production; 3) partial reoxidation of xylitol to xylulose; 4) isomerization of xylulose 5-phosphate to glucose 6-phosphate; and 5) complete oxidation of glucose 6-phosphate to CO_2 via the Krebs cycle, with regeneration of NADPH.

Since the errors estimated between the experimental and calculated amounts of CO_2 produced during the blank tests did not exceed 6%, simultaneous tests at different pH_o values were carried out in Erlenmeyer flasks, being content with and trusting to the simple theoretical estimation of CO_2.

Only negligible amounts of by-products (arabinitol, glycerol, mannitol, etc.) were detected by HPLC and gas chromatographic analyses. For this reason, their formation and the relative consumption of xylose were neglected in the carbon balance. In any case, the reasonably low errors between calculated and experimental CO_2 production values seem to confirm the validity of this assumption.

RESULTS AND DISCUSSION

Carbon Material Balance

As is well known, xylose can be utilized by yeasts in four different ways: reductive production of xylose, ethanol fermentation, respiration, and cell growth.

Figure 1 shows the percentage of xylitol consumed by each of these ways by the yeast *Debaryomyces hansenii* after 48 h of batch runs. The terms "growth" and "xylitol" refer to the fractions of this carbon source consumed for the productions of biomass and xylitol. "Ethanol" and "respiration" refer, on the other hand, to the xylose fractions consumed by ethanol fermentation (with production of ethanol and CO_2) and oxidized by respiration (to CO_2 and water).

These results demonstrate that, under all conditions tested, the carbon source was preferentially consumed for xylitol production. However, the percentage of xylose consumed and that of xylose actually transformed into xylitol reached maximum values (88 and 70%, respectively) close to $pH_o = 5.5$, which coincides with the optimum reported in the literature for this yeast in synthetic medium (Gírio et al., 1999). At higher and lower pH_o values xylitol productivity decreased (Table I), and a metabolic shift to alternative xylose-consuming ways took place. Cell growth was always very poor under the conditions tested, accounting for a xylose consumption only of 0.8–1.8%.

Ethanol fermentation, which consumed only 10% of xylose at optimum pH_o, gained relative significance either at higher or lower pH_o values, managing to consume up to 23% of xylose at $pH_o = 8.0$. At extreme pH_o values, xylitol production was affected to such an extent that even the respiration became progressively more significant, being responsible for the consumption of more than 32% of the carbon source at $pH_o = 2.0$. In fact, although strongly limited by the poor oxygen availability, respiration is always possible even under semi-aerobic conditions. A similar behavior was observed for xylitol production from de-

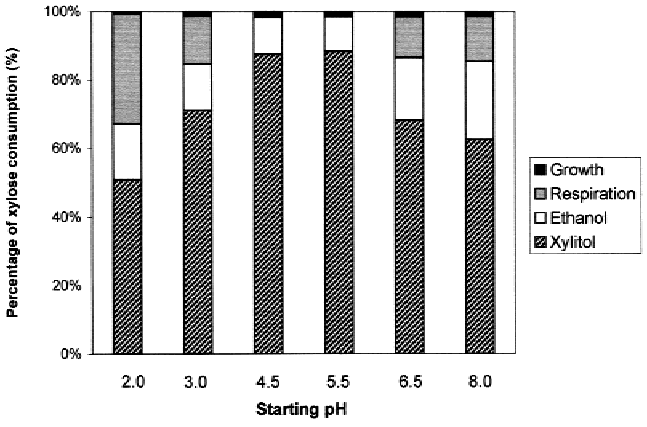

Figure 1. Percentages of xylose consumption in the metabolism of *D. hansenii* at different pH_o values. $T = 24°C$; $X_o = 0.5–0.6\ g_x/L$; $S_o = 130–135\ g_S/L$.

Table I. Kinetic results of batch productions of xylitol from synthetic xylose solutions carried out at variable pH_o with *D. hansenii*. $T = 24°C$; $X_o = 0.5 - 0.6$ g_X/L; $S_o = 130–135$ g_S/L.

pH_o	Q_p ($g_P/L \cdot h$)[a]	$Y_{P/S}$ (g_P/g_S)[b]	ν_m ($g_P/g_X \cdot h$)[c]
2.0	1.03	0.46	0.93
3.0	1.61	0.65	1.07
4.5	1.75	0.76	1.35
5.5	1.86	0.81	1.43
6.5	1.36	0.62	1.05
8.0	0.86	0.57	0.72

[a]Volumetric xylitol productivity.
[b]Yield of xylitol on consumed xylose.
[c]Maximum specific xylitol productivity.

toxified hemicellulose hydrolyzate by *Pachysolen tannophilus* (Converti et al., 1999a). In that case, however, an optimum pH_o range of 6.0–7.5 was observed, whereas about 30% of xylose was consumed for respiration at extreme pH_o values.

Additional tests were then performed to study the influence of temperature on xylitol production from D-xylose by *D. hansenii*, under conditions intentionally selected to favor xylitol production, that is, at optimum pH_o (5.5) and using a higher starting biomass level ($X_o = 1.4–3.0$ g_x/L). As expected, no appreciable cell growth took place under these conditions, so xylose was consumed only for xylitol and ethanol productions and by respiration.

Figure 2 shows the percentage of xylose consumed by *D. hansenii* after 48 h of cultivation by each of these ways at different temperatures. Between 32–37°C, more than 97% of xylose was consumed for xylitol production, which means that this process can quantitatively be carried out over a wide range of temperature; besides, when temperature was decreased to 24°C, xylitol was still the main product, its formation being responsible for more than 83% of xylose consumption. Ethanol fermentation and respiration became significant only at relatively high temperature, probably because of an increased sensitivity of xylitol fermentation to thermal inactivation. In fact, the percentage of carbon source consumed at 44°C for xylitol production decreased to 76%, whereas the percentages addressed to respiration and ethanol fermentation increased to 14% and 11%, respectively.

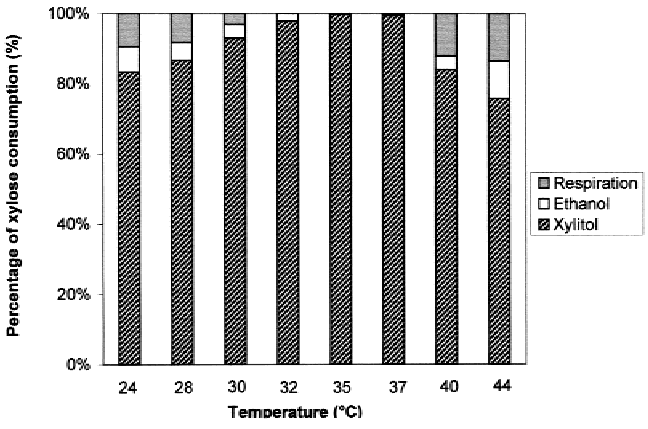

Figure 2. Percentages of xylose consumption in the metabolism of *D. hansenii* at different temperatures. $pH_o = 5.5$; $X_o = 1.4–3.0$ g_x/L; $S_o = 130–133$ g_S/L.

Effect of Starting pH on Xylitol Production

The results of Figure 1 demonstrate that pH variations strongly affected *D. hansenii* metabolism and stimulated the investigation of the possible causes of this effect. In particular, at relatively constant and high starting substrate level (130–135 g_S/L), xylitol concentration after 48 h of fermentation gradually increased from 53.3 to 91.9 g_P/L with increasing pH_o from 2.0 to 5.5, whereas an evident drop of xylitol production took place beyond this threshold (results not shown). From the kinetic results of these tests listed in Table I, some hypotheses can be made to explain these observations, taking in account that xylitol formation in *D. hansenii* is a bioreduction which involves: 1) transport of xylose into the cell, 2) reduction of xylose to xylitol under the consumption of NADPH, 3) regeneration of NADH by the respiratory chain, and 4) transport of xylitol out of the cell.

The first hypothesis is based on the recent evidence of a xylose high-affinity transport system in *D. hansenii*, which acts as a proton symport induced by substrate and controlled by salt (Nobre et al., 1999). An increase in external pH (from 5.5–8.0) could affect, in the first instance, the activity of this transporter, making xylose transport from the bulk to inside the cell the phenomenon limiting the formation of xylitol. In this case, xylose transformation should take place at the same rate as xylose transport through the cell membrane and the increased incidence of respiration and alcohol fermentation could be due to their greater ATP yield. From the productivity values listed in Table I one can estimate, according to the balance of Eq. [1], maximum volumetric and specific xylose transport rates to sustain xylitol formation of 2.04 $g_S/L \cdot h$ and 1.56 $g_S/g_X \cdot h$ at $pH_o = 5.5$ and minimum values of 0.94 $g_S/L \cdot h$ and 0.79 $g_S/g_X \cdot h$ at $pH_o = 8.0$, respectively. From these figures it is possible to calculate an oxygen transfer rate of at least 0.022 $g/L \cdot h$, which is necessary at pH_o 5.5 to avoid redox unbalance due to oxygen shortage for NADH regeneration. Since the oxygen requirements for yeasts provided only with NADPH-dependent XR activity (like *D. hansenii*) should be at least 2.1-fold higher than for yeasts which utilize also NADH as XR cofactor (Barbosa et al., 1988), our results are in satisfactory agreement with the OTR value (0.013 $g/L \cdot h$) reported by Nolleau et al. (1995) for *Candida parapsilosis*, a yeast with an NADH/NADPH activity ratio of 0.405 (Nolleau et al., 1993). Further comparison with other systems (Parajó et al., 1998a) confirms the semi-aerobic nature of the conditions selected in this study for *D. hansenii*, thus excluding possible limitations of oxygen transfer.

On the other hand, since low pH influences the maintenance requirement of the cell, the productivity decrease ob-

served with decreasing pH_o from 5.5 to 2.0 could be the result of the pH incidence on the redox balance of this bioreduction.

Another possibility is that the internal yeast homeostasis could not be as effective in counterbalancing the remarkable external pH variations investigated in this study; thus, the extracellular pH_o shift could lead to slight but significant changes of intracellular pH. As a consequence, the rate of the enzymatic step controlling the formation of xylitol could also strongly depend on pH_o. In this case, xylose uptake by the cells would occur at a higher rate than its transformation into xylitol; therefore, an increasing fraction of carbon source would be consumed by alternative means.

Finally, as shown by Sánchez et al. (1997), pH_o variations may affect yeast cell permeability and may cause some micronutrient to precipitate, thus becoming unavailable for assimilation.

Thermodynamics of Xylitol Production

A previous study clearly demonstrated that both volumetric and specific productivities can be used, likewise enzymatic systems, to estimate the thermodynamic parameters of bioprocesses, provided that a sufficient number of data at different temperatures is available (Converti et al., 1996). In this way, the thermodynamic parameters of different systems were estimated, with concern not only for the bioprocess under consideration but also for its thermal inactivation at high temperature.

Figure 3 shows that maximum xylitol productivity actually increased with temperature up to a threshold value (at 35°C), after which a sharp fall took place due to thermal inactivation.

Besides the well-known empirical approach of Arrhenius (Aiba et al., 1973; Sizer, 1944), two different thermodynamic models were proposed to take into account the thermal inactivation of enzymatic systems. The so-called "thermodynamic" approach (Bailey and Ollis, 1986; Roels, 1983) was used to describe the incidence of temperature-dependent reversible inactivation on the kinetics of enzymatic systems. On the other hand, the so-called "kinetic" approach (Converti and Del Borghi, 1997; de Queiroz et al., 1996; Owusu et al., 1992; Roels, 1983) was only useful to describe the time-dependent phenomena of irreversible inactivation and thermal death (Bailey and Ollis, 1986). For these reasons, the activation enthalpy of xylitol production was graphically estimated by the Arrhenius approach (Aiba et al., 1973) extended to microbial processes (Arni et al., 1999):

$$(dP/dt)_m = AXY_{P/X} \exp(-\Delta h^*/RT) \qquad (2)$$

Also, the decreasing tract of the productivity curve (Fig. 3) can be described by an Arrhenius-type equation, by which a further activation parameter ($\Delta h^{*\prime}$) can be estimated. An empiric approach was proposed by Sizer (1944) according to which the activation enthalpy of thermal inactivation (Δh^*_D) would be given by the sum of this parameter to the activation enthalpy of the reaction (Δh^*), while the activation entropies (Δs^* and Δs^*_D) can be estimated by standard methods from the related pre-exponential factors.

The average $Y_{P/X}$ value experimentally determined in this work (56.9 g_P/g_X) is one order of magnitude higher than those which can be estimated from the literature for this yeast in synthetic xylose solutions (1.9–9.3 g_P/g_X) (Domínguez et al., 1997; Gírio et al., 1999; Roseiro et al., 1991), due to the peculiar conditions selected here to stimulate xylitol production at the expense of cell growth (high X_o and optimum pH_o). Because of the above $Y_{P/X}$ data variability, as well as the necessity of making this approach as general as possible, an average value (4.8 g_P/g_X) estimated from literature data was also used, in addition to the one experimentally determined in this work, for thermodynamic parameters estimation.

Figure 4 shows the application of the above Arrhenius-type equations to estimate enthalpies and entropies of xylitol formation and thermal inactivation, using the productiv-

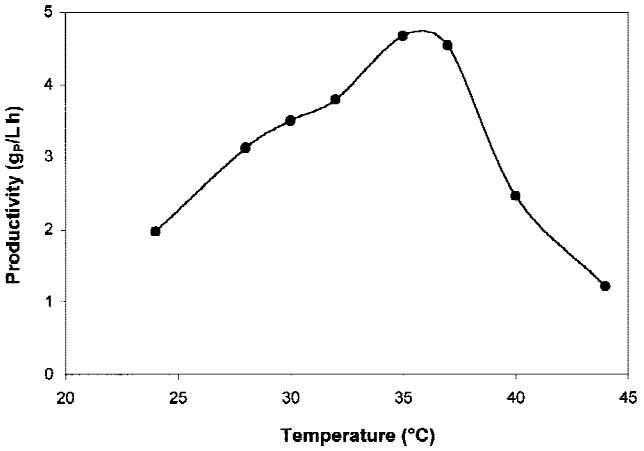

Figure 3. Maximum productivity values calculated for batch xylitol productions from synthetic xylose solutions by *D. hansenii*, carried out at different temperatures.

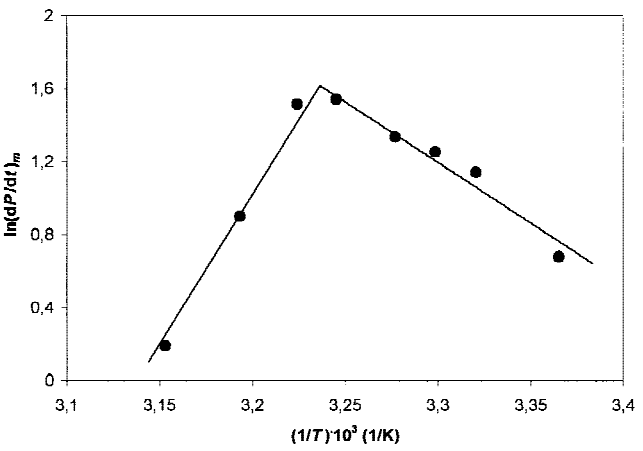

Figure 4. Graphical estimation of the thermodynamic parameters of both xylitol production and thermal inactivation according to the Arrhenius model. $Y_{P/X}$ = 56.9 g_P/g_X.

ity values plotted in Figure 3. The values of the thermodynamic parameters estimated with this model are summarized in Table II and discussed in the following.

Apart from some exceptions, activation enthalpy of xylitol formation ($\Delta h^* = 57.7$ kJ/mol) compares reasonably with those estimated for many different whole-cell bioprocesses, such as cell growth (34–70 kJ/mol) (Aiba et al., 1973; Esener et al., 1981; Saucedo-Castañeda et al., 1990), microbial respiration (56–74 kJ/mol) (Aiba et al., 1973), and alcohol fermentation (75–80 kJ/mol) (Arni et al., 1999; Converti et al., 1996), irrespective of the approach used. It also does not differ much from the activation enthalpies estimated for most free enzymatic systems (74–80 kJ/mol) (Al-Asheh and Duvniak, 1994; Roels, 1983).

The phenomenon responsible for thermal inactivation of this bioprocess is characterized by an activation enthalpy ($\Delta h^*_D = 187.3$ kJ/mol) remarkably higher than xylitol formation, which means that its rate increases much faster with temperature than product formation rate; thus, the overall productivity declines above a threshold value. As expected, Δh^*_D is quite lower than the values reported for microbial death (210–628 kJ/mol) (Bailey and Ollis, 1986; Esener et al., 1981; Saucedo-Castañeda et al., 1990) and in very good agreement with those of other bioprocesses, like alcohol fermentation (176–200 kJ/mol) (Arni et al., 1999; Converti et al., 1996). Reasonable agreement also exists with the values reported for many enzymatic systems (160–235 kJ/mol) (Converti and Del Borghi, 1997; Laidler and Bunting, 1973; Owusu et al., 1992; Roels, 1983; Sizer, 1944), whereas values from 1.5–4-fold higher (285–550 kJ/mol) characterize enzyme denaturation (Bailey and Ollis, 1986).

The above comparison would suggest that the productivity decrease observed at high temperature could be due, besides the other possible causes referred to earlier, to the reversible inactivation of an enzyme-limiting xylitol formation, but this hypothesis would need further confirmation and eventual identification of the controlling step. Nevertheless, the same hypothesis was advanced by Esener et al. (1981) to justify the dependence of the maximum specific growth rate of *Klebsiella pneumoniae* on the absolute temperature.

The activation entropy of xylitol production ($-0.152 < \Delta s^* < -0.132$ kJ/mol · K) compares with and has the same negative sign as those of enzymatic reactions (Converti and Del Borghi, 1997; Sizer, 1944) and other fermentations (Arni et al., 1999; Converti et al., 1996). As these values are consistent with the formation of a transition state with more rigid structure with respect to the reacting system, they further suggest that the phenomenon limiting xylitol productivity could be an enzymatic reaction under all conditions tested.

The activation entropy values of thermal inactivation ($0.054 < \Delta s^*_D < 0.075$ kJ/mol · K) are positive and close to zero, which indicates that this phenomenon implies a very little randomness increase during the activated state formation. In fact, these values are lower than those estimated for all the enzymatic processes considered above for comparison (0.31–0.89 kJ/mol · K) (Al-Ashieh and Duvniak, 1994; Bailey and Ollis, 1986). This result suggests a sort of protection against thermal inactivation exerted by the cell system. Finally, both activation entropies of xylitol formation and thermal inactivation only slightly varied with $Y_{P/X}$, which confirms the low sensitivity of this parameter to the operating conditions.

Table II. Thermodynamic parameters estimated by the Arrhenius approach for batch xylitol production from synthetic xylose solutions by *D. hansenii*. Reference temperature: 25°C.

	Xylitol formation	Thermal inactivation
Activation enthalpy (kJ/mol)	57.7	187.3
Activation entropy (kJ/mol · K)[a]	−0.132	0.075
Activation entropy (kJ/mol · K)[b]	−0.152	0.054
r^2	0.962	0.960

[a]Estimated with an average $Y_{P/X}$ value (4.8 g_P/g_X) calculated from literature data.

[b]Estimated with the experimental $Y_{P/X}$ value (56.9 g_P/g_X) determined in this work.

NOMENCLATURE

A	Arrhenius pre-exponential factor of xylitol formation, h^{-1}
Δh^*	activation enthalpy of xylitol formation, kJ/mol
$\Delta h^{*\prime}$	empiric thermodynamic parameter, kJ/mol
Δh^*_D	activation enthalpy of thermal inactivation, kJ/mol
k_La	volumetric oxygen transfer coefficient, h^{-1}
OTR	oxygen transfer rate, g/L · h
P	product (xylitol) concentration, g_P/L
Q_P	volumetric productivity, g_P/L · h
R	ideal gas constant, kJ/mol · K
S	substrate (xylose) concentration, g_S/L
Δs^*	activation entropy of xylitol formation, kJ/mol · K
Δs^*_D	activation entropy of thermal inactivation, kJ/mol · K
t	fermentation time, h
T	temperature, °C or K
v_m	maximum specific productivity, g_P/g_X · h
X	biomass concentration, g_X/L
XDH	xylitol dehydrogenase
XR	xylose reductase
$Y_{P/S}$	yield of product on substrate, g_P/g_S
$Y_{P/X}$	yield of product on biomass, g_P/g_X

Subscripts

m	maximum value
o	starting value
P	product
S	substrate
X	biomass

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