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The fermentation of mixtures of D-glucose and D-xylose by *Candida shehatae*, *Pichia stipitis* or *Pachysolen tannophilus* to produce ethanol

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Abstract: The fermentation of mixtures of D-glucose and D-xylose by three non-traditional yeasts: *Candida shehatae* (ATCC 34887), *Pachysolen tannophilus* (ATCC 32691) and *Pichia stipitis* (ATCC 58376) have been studied to determine the optimal strain and initial culture conditions for the efficient production of ethanol. The comparison was made on the basis of maximum specific growth rate (μ_m), biomass productivity, the specific rates of total substrate consumption (q_s) and ethanol production (q_E) and the overall yields of ethanol and xylitol. All the experiments were performed in stirred-tank batch reactors at a temperature of 30 °C. The initial pH of the culture medium was 4.5. The highest values of μ_m (above 0.5 h^{-1}) were obtained with *P stipitis* in cultures containing high concentrations of D-xylose. All three yeasts consumed the two monosaccharides in sequence, beginning with D-glucose. The values of q_s diminished during the course of each experiment with all of the yeasts. The highest values of the specific rates of total substrate consumption and ethanol production were obtained with *C shehatae* (for $t = 10$ h, q_s and q_E were above 5 $g g^{-1} h^{-1}$ and 2 $g g^{-1} h^{-1}$, respectively), although the highest overall ethanol yields were fairly similar with all three yeasts, at around 0.4 $g g^{-1}$.

Keywords: D-xylose; D-glucose; *Pachysolen tannophilus*; *Candida shehatae*; *Pichia stipitis*; ethanolic fermentation

NOTATION

A_1	Parameter in eqn (2)
A_2	Parameter in eqn (3)
B_1	Parameter in eqn (2)
B_2	Parameter in eqn (3)
E	Ethanol concentration ($g dm^{-3}$)
E_T	Maximum theoretical concentration attainable of ethanol ($g dm^{-3}$)
g	Residual concentration of D-glucose ($g dm^{-3}$)
g_0	Initial concentration of D-glucose ($g dm^{-3}$)
$K_L a$	Overall volumetric mass-transfer coefficient (h^{-1})
p	Residual concentration of D-xylose ($g dm^{-3}$)
p_0	Initial concentration of D-xylose ($g dm^{-3}$)
q_E	Specific rate of ethanol production ($g g^{-1} h^{-1}$)
q_s	Specific rate of total substrate consumption ($g g^{-1} h^{-1}$)
r_x	Biomass productivity ($g dm^{-3} h^{-1}$)
s	Residual concentration of total substrate ($g dm^{-3}$)
s_0	Initial concentration of total substrate ($g dm^{-3}$)
t	Time (h)
x	Biomass concentration ($g dm^{-3}$)
x_0	Initial biomass concentration ($g dm^{-3}$)
$Y^G_{E/s}$	Overall ethanol yield ($g g^{-1}$)
$Y^G_{x/s}$	Overall biomass yield ($g g^{-1}$)
$Y^G_{Xy/s}$	Overall xylitol yield ($g g^{-1}$)
α	Parameter in eqn (1)
β	Parameter in eqn (1)
μ_m	Maximum specific growth rate (h^{-1})

1 INTRODUCTION

Acidic or enzymatic hydrolysis of lignocellulose residues results in a solution of sugars, principally D-glucose and D-xylose. To ferment these hydrolysates effectively and render the process cost-effective it is necessary to use yeasts capable of transforming both types of sugar.

One of the first yeasts recommended for the ethanolic fermentation of both substrates was *Pachysolen tannophilus*, although subsequently other species have been identified, such as *Pichia stipitis*, *Kluyveromyces marxianus*, *Candida shehatae*, and *Candida*

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tropicalis. With these yeasts, different research groups have identified difficulties involved in the process, eg inhibition by ethanol[1–3] or by-products of degradation of sugars contained in the hydrolysate (such as phenolic compounds, furan derivatives and aliphatic acids).[4] Likewise, depending on the operation conditions, some of these yeasts can direct their metabolism towards the main production of ethanol or xylitol.[5]

On the other hand, the fermentation rates and yields for D-xylose are usually less than those for D-glucose, which presents difficulties for the use of these residues. Furthermore, simultaneous fermentation of D-xylose and D-glucose presents issues absent when the sugars are fermented separately, among which is the phenomenon of inhibition in the assimilation of D-xylose in the presence of D-glucose with *P tannophilus* or *C shehatae*.[6]

This is not simply resolved by culturing yeasts that preferentially transform D-glucose simultaneously or sequentially together with those capable of transforming D-xylose, either because of the inhibitory effects which some of these yeasts exert over each other,[7,8] or because of the different culture conditions that they require.[9] Furthermore, the proportions of D-glucose and D-xylose present in any hydrolysate tend to vary greatly according to the type of lignocellulose residue from which it was obtained and the conditions of hydrolysis.

The aim of the present work was to study the fermentation of various mixtures of D-glucose and D-xylose so as to obtain the best yields of ethanol by using yeasts capable of transforming both sugars. The yeasts studied were *P tannophilus, P stipitis* and *C shehatae*.

2 MATERIALS AND METHODS

2.1 Microorganism

The yeasts, *P tannophilus* ATCC 32691, *P stipitis* ATCC 58376 and *C shehatae* ATCC 34887, were supplied by the American Type Culture Collection.

2.2 Fermentation equipment and operation

All the experiments were carried out at laboratory scale in a batch-culture reactor described elsewhere,[10] comprising three temperature-controlled, magnetically-stirred fermenters with a usable volume of 2 dm^3. The volume of culture medium used was 0.5 dm^3; the stirring speed was 500 rpm, and the stirring rod was 4 cm long and 0.8 cm in diameter. Under these conditions, the overall volumetric mass-transfer coefficient ($K_L a$), at the beginning of each experiment, was 2.9 h^{-1}. This value of $K_L a$ was calculated using a dynamic gassing out method.

2.3 Maintenance medium and inoculum preparation

The yeasts were stored between 5 and 10 °C in 100 cm^3 test tubes on a sterilised solid culture medium composed of (g dm^{-3}): yeast extract (3); malt extract (3); peptone (5); D-xylose (10); agar-agar (20). Before the start of each experiment the microorganisms were inoculated aseptically into glass test tubes containing the solid culture medium described above. These tubes were then kept in an incubator at 30 °C for 60 h in order to obtain cells at the same growth stage for every experiment. To prepare inocula, cells were resuspended with fresh medium and a appropriate volume was transferred to the bioreactor. The biomass concentration at the beginning of each experiment was, on a dry weight basis, approximately 0.01 g dm^{-3}.

2.4 Culture medium and procedure

The composition of the culture medium was (g dm^{-3}): $MgSO_4 \cdot 7H_2O$ (1); KH_2PO_4 (2); $(NH_4)_2SO_4$ (3); peptone (3.6); and yeast extract (4). The sum of initial concentrations of D-xylose and D-glucose in the mixture was always 25 g dm^{-3}. Culture conditions were as determined in previous studies:[11,12] a temperature of 30 °C and an initial pH of 4.5. The complete culture medium was sterilised using cellulose nitrate filters (Sartorius, type 11307-47-N) with a 0.2 μm pore size.

2.5 Analytical techniques

Dry weight (g dm^{-3}) calibration was determined by the absorbance of the suspension at a wavelength of 620 nm. The residual concentrations of the total substrate and of D-glucose (s and g) were calculated using Miller's reducing sugar and glucose-oxidase methods.[13,14] Ethanol and xylitol concentrations were measured using the methods described by Beutler[15] and Beutler and Becker,[16] based on the enzymes alcohol dehydrogenase and polyol dehydrogenase. All the concentrations were determined by averaging out at least two analytical data.

3 RESULTS AND DISCUSSION

3.1 Cell growth

In most of the experiments performed the lag phase was negligible; only in some experiments with *P tannophilus* and *P stipitis* were periods of adaptation detected, although these were of less than 2 h, particularly with higher concentrations of D-glucose. Subsequently there was an exponential phase, the length of which depended on the initial concentration of D-glucose in the culture. In the experiments with *P tannophilus* and *C shehatae* at high concentrations of D-glucose the exponential phase was followed by a stationary phase. With both yeast strains, however, at low concentrations of D-glucose growth continued after the exponential phase for a considerable amount of time, albeit at a much slower rate (Fig 1). Finally a stationary phase was reached when all the substrates had been used up. *P stipitis* behaved somewhat differently at high D-glucose concentrations in that after the exponential phase there was a growth period during which biomass increased linearly with time.

From the growth curves, the duration of the exponential phase was established and the maximum

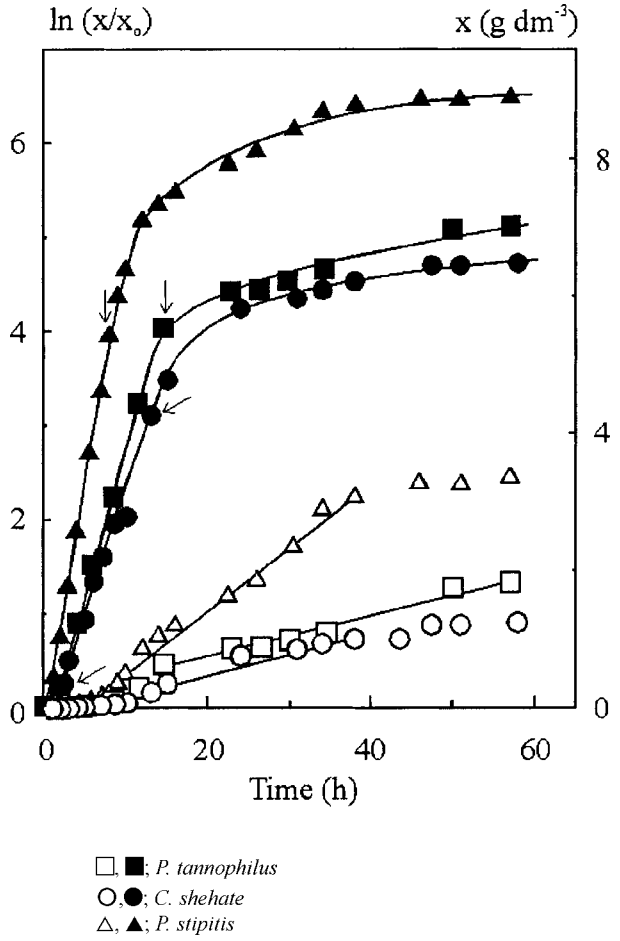

Figure 1. Growth curves (solid symbols) and biomass production (hollow symbols) for experiments with p_0=20 g dm^{-3} and g_0=5 g dm^{-3}.

specific growth rate for each of the yeasts was determined. The values of μ_m were obtained by least-squares adjustment of experimental values of $\ln(x/x_0)$ *versus* time. The results (Table 1) show that the highest values of μ_m were obtained in cultures with *P stipitis*. When the initial concentrations of both D-glucose and D-xylose in the culture medium were zero, the lowest maximum specific growth rate occurred with *C shehatae* (0.19 h^{-1}). On modifying the initial proportions of D-glucose and D-xylose, it can be seen that the highest values of μ_m obtained with *P tannophilus* were higher than when the substrate was composed entirely of D-glucose, although the concentration of D-glucose in mixed substrates seemed to have a positive effect on the growth rate with this yeast. With *P stipitis* and *C shehatae*, however, the values of μ_m obtained with mixed substrates were lower than when D-xylose alone was used.

In those cultures in which the biomass increased linearly (after the exponential phase) cell growth was quantified through biomass productivity (r_x, g dm^{-3} h^{-1}). Representations of the experimental biomass concentration *versus* time results (Fig 1) allowed the duration of this growth period to be determined together with the values of parameter r_x by least-square adjustments to the equation $x = a + r_x t$. These results, together with the values of μ_m, are set out in Table 1. The highest biomass productivity values were also obtained with *P stipitis*, reaching almost 0.28 g dm^{-3} h^{-1} in experiments with high concentrations of D-glucose in mixed substrates. With either *P tannophilus* or *C shehatae* cultures, the proportion of sugars used exerted no significant influence upon the values of r_x, which were similar for both yeasts and also to the values obtained when D-xylose alone was used as substrate. Nevertheless, in the *C shehatae* cultures no linear phase was detected with $g_0 > 15$ g dm^{-3}, whilst with *P tannophilus* this was only the case with higher values of D-glucose, $g_0 > 24$ g dm^{-3}. This post-exponential phase linear growth period detected with all three yeasts has also been reported by others[17–19] and is characteristic of processes controlled by stages of a physical nature, in this particular case the kinetic control of the bioprocess residing in the transfer of oxygen within the cell suspension.

3.2 Substrate consumption

As far as substrate consumption is concerned, all three yeasts did this sequentially, initially using up the D-glucose quite rapidly (throughout approximately the first 20 h of culture). After this there was a period during which biomass production, substrate consumption and the formation of ethanol ceased or progressed only very slowly; the duration of this second period varied according to the initial sugar concentrations in the substrate but was especially significant in those cultures using similar quantities of both. Subsequently

p_0 (g dm^{-3})	g_0 (g dm^{-3})	μ_m (h^{-1})			r_x (g dm^{-3} h^{-1})		
		Ps	*Cs*	*Pt*	*Ps*	*Cs*	*Pt*
25	0	0.55	0.33	0.26	0.074	0.024	0.023
24	1	0.46	–	0.30	0.094	–	0.028
20	5	0.51	0.27	0.31	0.090	0.030	0.033
15	10	0.52	0.28	0.30	0.090	0.025	0.018
10	15	0.39	–	0.32	0.18	–	0.011
5	20	0.41	0.28	0.31	0.23	–	0.020
1	24	0.44	–	0.31	0.23	–	–
0	25	0.49	0.27	0.31	0.28	–	–
0	0	0.33	0.19	0.26	0.023	0.013	0.005

Table 1. Maximum specific growth rates (μ_m) and biomass productivities (r_x) with *P stipitis* (*Ps*), *C shehatae* (*Cs*) and *P tannophilus* (*Pt*)

the D-xylose was consumed and further production of biomass and ethanol occurred. The present observations concur with those of Panchal *et al*[6] and du Preez *et al*,[20] who reported similar findings of a diauxic (sequential) consumption of D-glucose and D-xylose in the same order by *C shehatae* and *P stipitis* when using mixtures of these sugars in the culture medium.

To calculate the specific rate of total substrate consumption the differential method for the treatment of kinetic data was applied.[10] Among the equations tried for the three yeasts, that giving an acceptable reproducibility of the substrate concentration data over the greatest intervals of time was

$$s = s_0 \alpha^{-t^{\beta}} \qquad (1)$$

From eqn (1), by following the procedures described previously[11,21] it was possible to determine the specific rate of total substrate consumption (q_s) *versus* time for each yeast strain (see Table 2). The values obtained for the earlier times correspond to the phase during which D-glucose was utilised, while times of more than 25 h are characteristic of the stage when D-xylose was being consumed. Similarly, Laplace *et al*,[8] using *C shehatae* in a co-culture with *Saccharomyces cerevisiae*, with mixtures containing 70% (w/w) of D-glucose and 30% of D-xylose, found that 14 h after the beginning of the experiment, D-glucose had been completely consumed, while D-xylose had, in practice, not been consumed. These authors also point out that the capacity of fermentation of D-xylose by *C shehatae* is extremely low when D-glucose is present, something attributed to the fact that D-glucose strongly represses D-xylose utilisation by xylose-fermenting yeasts grown on a mixture of sugars. Furthermore, the magnitude of this repression depends on the relative proportion of both sugars.

The highest values of q_s were obtained with *C shehatae* and the lowest with *P tannophilus*. With all three yeasts the specific substrate consumption rate diminished progressively during each culture after reaching a maximum generally between 10 and 20 h into the experiment. Also, during the first hours of culture (10–15 h) the higher the initial concentration of D-glucose, the closer the specific substrate consumption rates came to those obtained in the experiment with D-glucose alone as substrate. During later stages of culture (60 h), the values of q_s were very similar to those found in experiments where D-xylose alone was used as substrate.

From graphic representations similar to that shown in Fig 2 and by following the procedure described previously,[11,21] the overall biomass yield, $Y^G_{x/s}$, for each culture was determined. The values for these yields are

p_0 (g dm^{-3})	g_0 (g dm^{-3})	$Y^G_{x/s}$ (g g^{-1})				q_s (g g^{-1} h^{-1})		
		Ps	*Cs*	*Pt*	*t (h)*	*Ps*	*Cs*	*Pt*
25	0	0.077	0.036	0.094	20.0	–	2.0	–
					30.0	0.74	1.7	–
					60.0	–	–	0.23
24	1	0.11	–	0.12	15.0	–	–	0.61
					20.0	0.77	–	–
					30.0	0.52	–	–
					60.0	–	–	0.21
20	5	0.12	0.045	0.11	15.0	–	–	0.51
					20.0	0.59	–	–
					30.0	0.36	1.2	–
					60.0	–	–	0.22
15	10	0.14	0.048	0.11	10.0	1.4	–	–
					12.0	–	–	2.2
					15.0	–	2.0	–
					30.0	0.38	0.99	–
					60.0	–	–	0.23
10	15	0.15	–	0.12	12.0	–	–	2.2
					20.0	0.34	–	–
					60.0	–	–	0.22
5	20	0.18	0.051	0.13	10.0	1.6	–	–
					13.0	–	–	2.3
					15.0	–	2.5	–
					20.0	0.39	–	–
					60.0	–	–	0.04
1	24	0.18	–	0.12	10.0	2.0	–	–
					13.0	–	–	2.8
					20.0	0.36	–	–
0	25	0.19	0.060	0.11	10.0	2.2	–	–
					15.0	–	4.8	3.6
					19.0	0.33	–	–

Table 2. Overall biomass yields ($Y^G_{x/s}$) and specific total substrate consumption rates (q_s) with *P stipitis (Ps), C shehatae (Cs)* and *P tannophilus (Pt)*

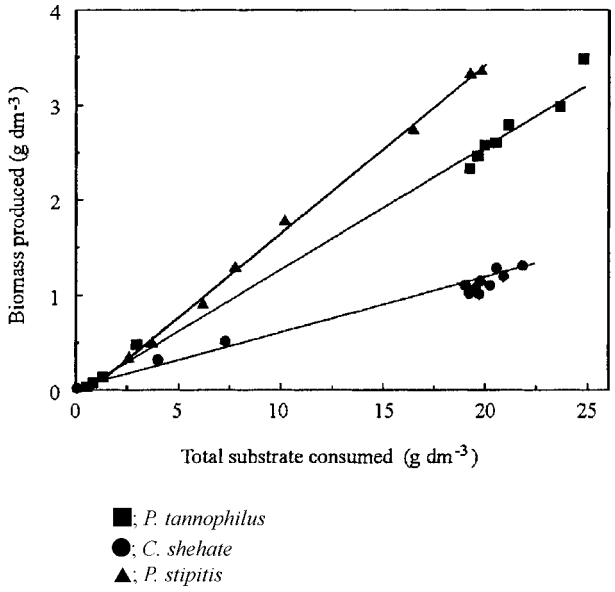

Figure 2. Biomass produced against total substrate consumed for experiments with p_0=5 g dm^{-3} and g_0=20 g dm^{-3}.

set out in Table 2, which shows that the highest values were obtained with *P stipitis* and the lowest with *C shehatae*. It can also be seen that with both yeasts the value of $Y^{G}_{x/s}$ increased concomitantly with the initial concentration of D-glucose in the mixture to reach maximum yields when this sugar alone was present as substrate. With *P tannophilus*, on the other hand, the relative concentrations of D-glucose and D-xylose did not appear to influence the overall yield in biomass, except when D-xylose alone was used as substrate, when a slight decrease in yield was observed.

3.3 Ethanol and xylitol production

The differential method for the treatment of kinetic data to calculate the values for the specific rate of ethanol production (q_E) was used.[10] Several equations were used that were able to reproduce acceptably the data representing the amounts of ethanol produced throughout wide intervals of time. Among those used that which best reproduced our experimental variations in the experiments carried out with *P tannophilus* proved to be

$$\frac{E_T}{E_T - E} = A_1^{t^{B_1}} \qquad (2)$$

and in the experiments with *C shehatae* and *P stipitis*

$$\frac{E}{E_T} = A_2^{t^{B_2}} \qquad (3)$$

In these equations, E_T represents the maximum concentration attainable if the yield of a transformation of the D-glucose/D-xylose mixture was the theoretical one, and A_1, A_2, B_1 and B_2 are adjustment parameters. From eqns (2) and (3), following the procedures described previously,[11,21] it was possible to determine the specific ethanol production rate *versus* time for each of the experiments.

As mentioned above, ethanol is produced during two distinct phases of the culture, which are separated by a stage during which cell growth, substrate consumption and the formation of products are either stationary or very slight. To calculate q_E either eqn (2) or (3) was applied for both ethanol formation phases, depending upon the yeast used.

In the experiments with the highest initial proportions of D-glucose, with *P stipitis* for example, the two periods of ethanol formation are clearly visible (Fig 3), and it is these which are responsible for the two values of q_E given in Table 3. The earlier values correspond to the specific rates of ethanol production from D-glucose, whilst the later ones refer to the conversion into ethanol of D-xylose; as can be seen, the former were considerably higher than the latter. When the initial concentration of D-glucose was very high it was only possible to determine q_E for the conversion of this

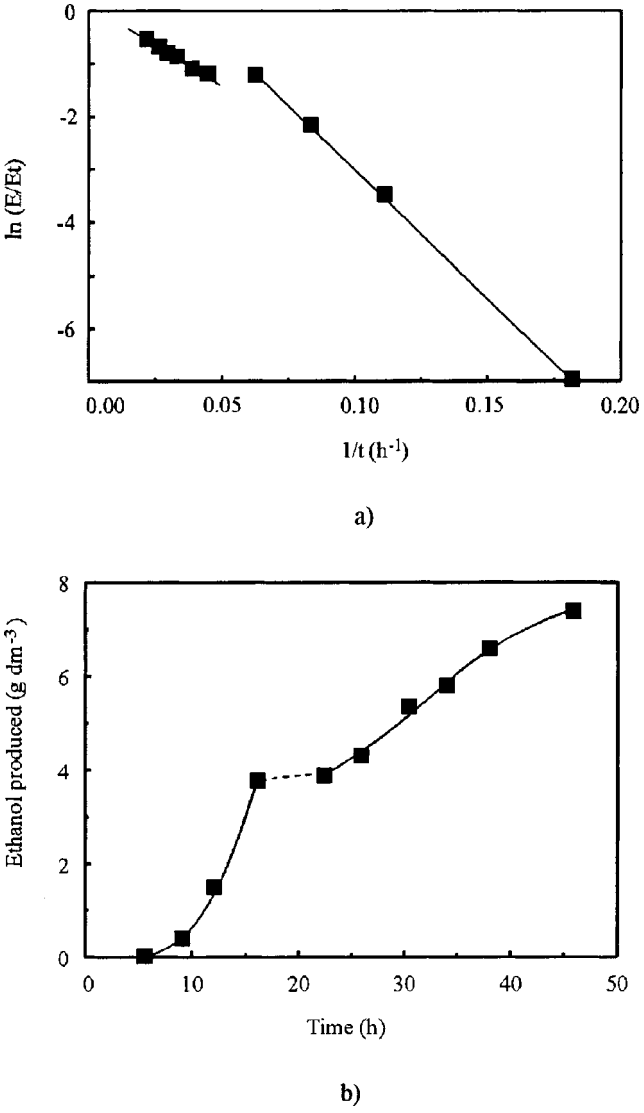

Figure 3. (a) Application of linearised form of eqn (3) for the experiment with p_0=15 g dm^{-3} and g_0=10 g dm^{-3} by *Pichia stipitis*. (b) Application of eqn (4) for the same experiment.

sugar and it would appear that the yeast is not able to adapt to the consumption of D-xylose. In the same way, the values obtained in this case were higher than those attained when D-xylose was the only component of the substrate.

As opposed to fermentation with *P tannophilus*, the specific rate of ethanol production with *C shehatae* and *P stipitis* diminished during the consumption of both substrates, and this is particularly noticeable from about 20 h onwards. This decrease, both in q_E and q_s, with the latter yeasts was caused in part by the accumulation of ethanol in the medium, which seems to inhibit their activity. This result accords with that obtained by Domínguez *et al*,[3] who found that *P stipitis* CBS 5773 is inhibited when the concentration of ethanol in the culture medium is 20 g dm^{-3}. However, in this work, the ethanol concentration in the medium was lower since the total initial concentration of the D-glucose/D-xylose mixture was only 25 g dm^{-3}. Apart from this, it can also be seen that, earlier in the fermentation, higher values of q_E were achieved with *P stipitis* and *C shehatae* than with *P tannophilus* and that higher specific rates of ethanol production were attained with these two yeasts when D-glucose was the principal component of the initial substrate, although the divergences in this parameter are considerably less than those observed with *P tannophilus*. With regard to the fermentation of mixtures of D-glucose and D-xylose with *P stipitis*, the present results concur with those obtained by Grootjen *et al*,[22] who observed that the highest specific rates of ethanol production were achieved with a substrate containing D-glucose alone.

The yields of ethanol and xylitol have been calculated in the same way as for yields in biomass. Firstly, to check whether the yields of these two components were constant, their concentrations throughout experimental time were plotted *versus* the quantity of substrate used up, always obtaining graphs similar to that shown in Fig 4. In each of the cultures, the experimental points fit a straight line reasonably well, which indicates that the yields may be considered as being constant throughout. From the slopes of these graphs, the overall yields in ethanol ($Y^G_{E/s}$) and xylitol ($Y^G_{Xy/s}$) have been calculated, all of which are set out in Table 3.

With regard to the overall yield of xylitol, it can be seen that with *C shehatae* this diminished concomitantly with an increase in D-glucose to the extent that in the experiments with a very high initial concentration of this sugar (g_o = 24 and 25 g dm^{-3}) no xylitol was produced at all, which would suggest that with this yeast xylitol appears as an intermediary product in the metabolism of D-xylose but not of D-glucose. The value of $Y^G_{Xy/s}$ obtained with *C shehatae* when the only component of the substrate was D-xylose was slightly

p_0	g_0	$Y^G_{E/s}$ (g g^{-1})			$Y^G_{Xy/s}$ (g g^{-1})				q_E (g g^{-1} h^{-1})		
(g dm^{-3})	(g dm^{-3})	*Ps*	*Cs*	*Pt*	*Ps*	*Cs*	*Pt*	*t* (h)	*Ps*	*Cs*	*Pt*
25	0	0.37	0.41	0.42	0.006	0.078	0.098	20.0	–	0.72	–
								26.0	0.21	–	–
								LP	–	–	0.067
24	1	0.40	–	0.24	0.007	–	0.16	10.0	0.46	–	–
								26.0	0.23	–	–
								LP	–	–	0.051
20	5	0.39	0.35	0.34	0.017	0.022	0.075	10.0	0.67	–	–
								11.5	–	–	0.73
								20.0	–	0.58	–
								26.0	0.19	–	–
								LP	–	–	0.061
15	10	0.32	0.39	0.36	0.023	0.026	–	10.0	0.69	2.2	–
								13.0	–	–	0.57
								20.0	–	0.55	–
								26.0	0.11	–	–
								LP	–	–	0.064
10	15	0.41	–	0.37	0.025	–	–	10.0	0.49	–	–
								12.0	–	–	0.62
								21.0	0.20	–	–
5	20	0.42	0.37	0.36	0.023	0.011	–	10.0	0.54	1.7	–
								13.0	–	–	0.76
								21.0	0.13	–	–
1	24	0.47	–	0.40	0.023	–	–	10.0	0.60	–	–
								13.0	–	–	0.72
								21.0	0.14	–	–
0	25	0.42	0.38	0.41	0.012	–	0.0047	10.0	0.62	1.6	–
								11.0	–	–	0.71
								21.0	0.19	–	–

Table 3. Overall ethanol ($Y^G_{E/s}$) and xylitol ($Y^G_{Xy/s}$) yields and specific ethanol production rates (q_E) with *P stipitis* (*Ps*), *C shehatae* (*Cs*) and *P tannophilus* (*Pt*)

LP: Linear Growth Phase.

lower than that found by Kastner *et al*[23] working under very similar experimental conditions to those used here. With *P stipitis*, on the other hand, the highest values for this parameter were obtained with similar concentrations of both sugars together. It is also worthy of note that with *P tannophilus*, values of $p_0 = 24$ and $g_o = 1\,g\,dm^{-3}$ gave a high yield in xylitol ($Y^G_{Xy/s} = 0.16\,g\,g^{-1}$), which was an anomalous occurrence but one which fits in with the lower ethanol yield ($Y^G_{E/s} = 0.24\,g\,g^{-1}$) compared with the relatively high ethanol yield compared with xylitol in the other experiments.

Finally, in some of the experiments, the values of $Y^G_{E/s}$ were more than 0.4 (g ethanol) (g substrate)$^{-1}$ with all three yeasts. With *P tannophilus* and *C shehatae*, the values for $Y^G_{E/s}$ were lower in the experiments with mixed substrates than in those where only D-glucose or D-xylose were used, particularly in the case of *P tannophilus*. With *P stipitis*, on the other hand, the highest yields were obtained with high concentrations of D-glucose and the values for $Y^G_{E/s}$ of $0.4\,g\,g^{-1}$ achieved with this yeast are similar to the maximum values reached by Delgenes *et al*,[24] using a solution of D-xylose, D-glucose and L-arabinose under similar experimental conditions to those described here. Likewise, the result obtained with *P stipitis* is similar to that observed by Chandrakant and Bisaria[25] in the simultaneous bioconversion of D-glucose and D-xylose to ethanol by *S cerevisiae* in the presence of xylose isomerase. These authors found a yield in ethanol of $0.43\,g\,g^{-1}$ and $0.30\,g\,g^{-1}$ when the initial D-glucose/D-xylose concentrations were 80/20 ($g\,dm^{-3}$) and 50/50 ($g\,dm^{-3}$); that is, the higher the initial concentration of D-glucose, the higher the value of $Y^G_{E/s}$.

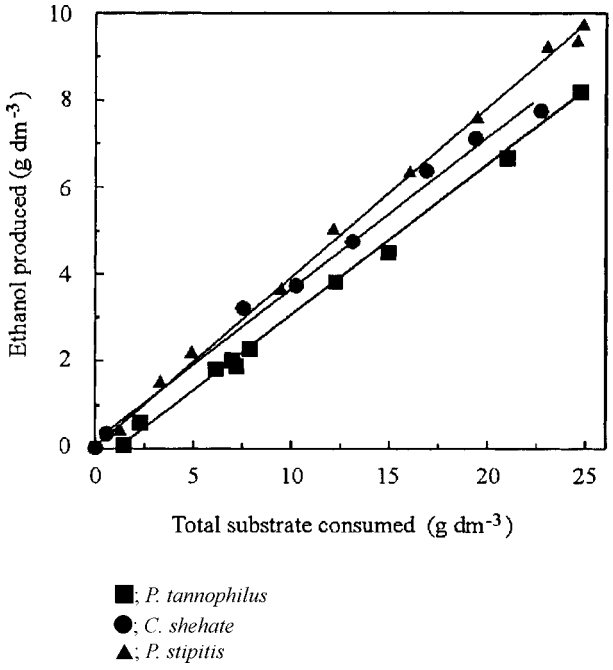

Figure 4. Ethanol concentration against substrate consumption for experiments with $p_0 = 20\,g\,dm^{-3}$ and $g_0 = 5\,g\,dm^{-3}$.

4 CONCLUSION

As far as ethanol production is concerned, based on the parameters of specific production rate and overall yield, it is concluded that of the three yeasts assayed, *P stipitis* gave the best results for the transformation of substrates consisting of mixtures of D-glucose and D-xylose. With this yeast the highest ethanol yields ($0.42\,g\,g^{-1}$ and $0.47\,g\,g^{-1}$) were obtained when the ratios between the D-glucose and D-xylose initial concentrations were 20/5 and 24/1 respectively, though the values of the specific rate of ethanol production are somewhat lower than those attained with *C shehatae*.

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