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The Effect of Carbon Dioxide on Xylose Fermentation by *Pichia stipitis*

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ABSTRACT

Carbon dioxide was found to reduce the xylose fermentation rate of two different strains of *Pichia stipitis* (CBS 5773 and CBS 5776) significantly in anaerobic batch fermentations. The maximum specific fermentation rate in a carbon dioxide atmosphere was about 45% lower than the fermentation rate in nitrogen atmosphere. Furthermore, the fermentation rate was found to be correlated to the growth rate. It is probable that the carbon dioxide influences the fermentation rate partly through decreasing the growth rate. It was also found that the fermentation rate of CBS 5773 was higher than for CBS 5776 and that the specific fermentation rate was lower at a higher cell density.

Index Entries: Anaerobic xylose fermentation; carbon dioxide; *Pichia stipitis;* factorial design; degree of reduction balance.

NOTATION

f_{red}	recovery factor for reducing equivalents
$q_{p,max}$	maximum specific fermentation rate (g ethanol/g dry wt·h)
$Y_{E/S}$	ethanol yield (g ethanol/g xylose)
$Y_{XYL/S}$	xylitol yield (g xylitol/g xylose)
$Y'_{E/S}$	ethanol yield (C-mole ethanol/C-mole xylose)
$Y'_{X/S}$	biomass yield (C-mole biomass/C-mole xylose)
$Y'_{XYL/S}$	xylitol yield (C-mole xylitol/C-mole xylose)
$Y'_{CO_2/S}$	CO_2 yield (C-mole/C-mole xylose)

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γ_E degree of reduction of ethanol
γ_S degree of reduction of xylose
γ_X degree of reduction of biomass
γ_{XYL} degree of reduction of xylitol

INTRODUCTION

The fermentation of xylose to ethanol has been studied extensively during the last decade (*1*). *Pichia stipitis* has been found to be among the most promising organisms for this fermentation (*2,3*). Considerable attention has been given the problem of the so-called oxygen effect, i.e., the fact that fermentations in the presence of small amounts of oxygen show a much higher specific fermentation rate than strictly anaerobic fermentations (*2,4–8*). Reported results on anoxic fermentation rates differ to a large extent among different investigations. One possible reason for the scattering results may be that it is difficult to obtain completely anoxic conditions. However, what may also be of importance is the dissolved carbon dioxide concentration. There are, in principle, two different approaches that have been used to obtain anoxic conditions: either to sparge the system with, e.g., nitrogen, and then seal it completely or to retain a constant flow of nitrogen through the system during the entire experiment. In the first case, any evolved carbon dioxide remains in the system, whereas in the second case, evolved carbon dioxide is transferred out of the system with the nitrogen stream.

A lot of attention is often given to the effect of oxygen, whereas the effect of carbon dioxide is seldom considered. Carbon dioxide fulfills a complicated role in microorganisms, and is involved in both the catabolic and the anabolic pathways of the cell. It is a well-known fact that the growth of a number of microorganisms can be inhibited by elevated pressures of carbon dioxide (*9*), a fact that has been used for the preservation of food. As pointed out by Jones and Greenfield (*10*), there is increasing evidence that carbon dioxide also plays a major role in both aerobic and anaerobic fermentations. Since increased ethanol productivity is always accompanied by increased carbon dioxide evolution, the study of the effect of carbon dioxide is very important when considering any potential large-scale fermentation for ethanol production.

In the present article, the effect of carbon dioxide on the fermentation of xylose by two different strains of *Pichia stipitis* was studied. Batch fermentation of xylose was carried out in a lab-scale fermentor, which was either sparged with nitrogen or kept under a carbon dioxide atmosphere. Two different cell concentrations were also used. The experiments were carried out as a 2^3 factorial design.

MATERIALS AND METHODS

Yeast Strains and Medium

The yeast strains used were *Pichia stipitis* CBS 5773 and CBS 5776 (obtained from Centraalbureau voor Schimmelcultures, Delft, the Netherlands). The cells were maintained on agar plates made from yeast extract (Serva, Heidelberg, Germany) 10 g/L, peptone from soy (Serva, Heidelberg,) 20 g/L, and agar-agar (Serva, Heidelberg) 20 g/L with D-xylose 20 g/L (Research grade, Serva, Heidelberg) as additional carbon source. Inoculum cultures were grown in 250-mL Erlenmeyer flasks on a rotary shaker at 30°C for approx 70 h. The liquid vol was 150 mL, and the shaker speed 150 rpm. The medium consisted of Yeast Nitrogen Base (DIFCO, Detroit, MI), 6.7 g/L, and D-Xylose (Research grade, Serva, Heidelberg, Germany), 10 g/L. The experimental medium in the fermentor consisted of Yeast Nitrogen Base w/o amino acids (DIFCO, Detroit, MI), 1.7 g/L, ammonium sulfate (Serva, Heidelberg, Germany), 5.0 g/L, and D-Xylose, 5.0 g/L or 10.0 g/L. A few drops of antifoam (< 10 mg/L) (antifoam 289, SIGMA Chemical, St. Louis, MO) were also added to the medium in the fermentor. The temperature in the fermentor was kept at 30°C, and the pH was controlled at 4.5 by addition of 1*M* NaOH.

Fermentation Experiments

The experiments were made in a 2.5-L lab-scale fermentor (BIOFLO III, New Brunswick Scientific, Edison, NJ), with a working vol of 1.6 L, in two stages. In the first stage, the cells were grown aerobically on either 5.0 or 10.0 g/L of xylose, depending on whether a low or a high cell concentration was desired for the fermentation stage of the experiment. Inoculation was made with 50 mL of inoculum culture. The air flow rate used was 1.6 L/min (1 vvm) and the stirrer rate was 500 rpm. The dissolved oxygen concentration was not kept constant during the aerobic batch growth. The batch growth was concluded when the pH of the broth started to increase. This increase was clearly seen, since only base was used to control the pH. Analysis of the broth showed that there was no xylose left in the broth at this point, and there was no ethanol or acetic acid found in the broth. Samples to determine cell dry weight were taken after the aerobic batch growth. In the second stage, the fermentor was deaerated for 20 min at a flow rate of about 0.3 L/min, using either nitrogen (ADR class 2, 1[a], AGA, Lidingö, Sweden) or carbon dioxide (ADR class 2, 5[a], AGA, Lidingö, Sweden), and xylose was added to the fermentor, giving a concentration of approx 20 g/L. During the experiments with nitrogen sparging, the same nitrogen flow was kept during the entire fermentation. However, during the experiments with carbon dioxide, a constant flow of

Table 1
Factorial Design of Xylose Fermentation Experiments

Level	Strain Factor A	Gas Factor B	Cell concentration Factor C
–	CBS 5773	Nitrogen	"Low"
+	CBS 5776	Carbon dioxide	"High"

carbon dioxide would have caused troubles with the pH control, which was done by base addition. Therefore, the gas flow was stopped after the deaeration, and the reactor gas outlet was put into a beaker of water to prevent pressure buildup during the experiments. An oxygen trap (oxytrap, Alltech Ass., Arlington, IL) was used to remove oxygen impurities in the nitrogen and carbon dioxide gases. The total fermentation time was 30 h, and samples were taken every 3–4 h during the fermentation stage for sugar and ethanol analyses as well as absorbance measurements.

Analytical Methods

Dry weight was determined from duplicate samples of 10 mL that were centrifuged, washed with distilled water once, and dried at 120°C for 24 h. Absorbance measurements were made at 610 nm after dilution of the samples to obtain absorbance values of < 0.5. In this range, the cell concentration was linearly related to the absorbance. Xylose, xylitol, and ethanol were quantitatively determined by liquid chromatography. Determination of concentrations from repeated samples showed an SD of $< 1\%$ for these compounds. Also, acetic acid could be detected with the used column, but not accurately quantitatively determined. A polymeric cation-exchange column (Sugarpak, Waters) at 75°C was used together with a refractive index detector (Waters 410). Pure water was used at the eluent.

Factorial Design

The experiments were conducted as a 2^3 factorial design with two repeat experiments. The studied factors were: gas (nitrogen sparging/carbon dioxide atmosphere), strain (CBS 5773/CBS 5776), and cell concentration (high/low) (Table 1). The cell concentration was varied by having different initial xylose concentrations in the aerobic batch growth (*see above*). The initial low xylose concentration (5 g/L) gave a cell concentration of approx 2.5 g dry wt/L, whereas the initial high xylose concentration (10 g/L) gave a cell concentration of approx 4 g dry wt/L. The complete scheme of experiments is shown in Table 2. The experiments were performed in random order.

Table 2
Complete Experimental Plan

Experiment	Factor A	Factor B	Factor C
(0)	−	−	−
a	+	−	−
b	−	+	−
ab	+	+	−
c	−	−	+
ac	+	−	+
bc	−	+	+
abc	+	+	+
bc*	−	+	+
abc*	+	+	+

* = Repeated experiments.

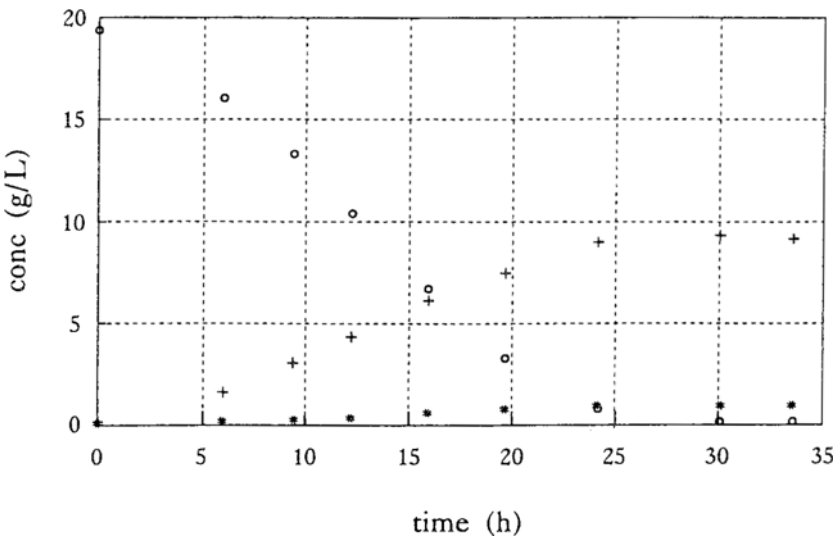

Fig. 1. Anaerobic batch fermentation of xylose by *Pichia stipitis* (CBS 5773). o = Xylose (g/L), + = ethanol (g/L), * = xylitol (g/L).

RESULTS AND DISCUSSION

Calculation of Rates and Yields

A typical fermentation is shown in Fig 1. As expected, it takes some time for the cells to reach maximum fermentation rate. The maximum specific fermentation rate, $q_{p,max}$ ($g \cdot g^{-1} \cdot h^{-1}$) was calculated from the maximum slope and the cell concentration. A slow growth of cells was observed, and a specific growth rate was calculated based on the absorbance

Table 3
Experimental Results from Anaerobic Fermentation of Xylose by *Pichia stipitis*

Run	$q_{p,max}$, $g \cdot g^{-1} \cdot h^{-1}$	$Y_{E/S}$, $g \cdot g^{-1}$	$Y_{XYL/S}$, $g \cdot g^{-1}$	μ, h^{-1}
(0)	0.181	0.47	0.08	0.0143
a	0.148	0.49	0.06	0.0149
b	0.094	0.51	0.07	0.0081
ab	0.096	0.52	0.05	0.0102
c	0.129	0.49	0.05	0.0193
ac	0.066	0.44	0.07	0.0087
bc	0.073	0.50	0.05	0.0163
abc	0.056	0.45	0.06	0.0058
bc*	0.068	0.49	0.04	0.0081
abc*	0.045	0.49	0.07	0.0039

* = Repeated experiments.

measurements. The yield of ethanol, $Y_{E/S}$ (g ethanol formed/g of xylose consumed) and the yield of xylitol, $Y_{XYL/S}$ (g xylitol formed/g of xylose consumed), were also calculated. The results are summarized in Table 3.

Mass Balances

The obtained yield of ethanol seemed on average to be slightly too high. The commonly accepted maximum theoretical yield of ethanol is 0.51 ($g \cdot g^{-1}$) (*2*). A complete mass balance for the carbon could not be made, since evolved carbon dioxide was not quantified. An approximate carbon balance is sometimes calculated by assuming 1 mol of carbon dioxide formed/mol of ethanol formed. This is, however, not quite correct as soon as other products than ethanol, such as biomass and xylitol, are formed. It is more correct to make a balance of the degree of reduction of substrates and products (*11*). If it is assumed that the composition of *Pichia stipitis* is approximately given by $CH_{1.8}O_{0.5}N_{0.2}$ and that other byproducts than xylitol can be neglected, the overall anaerobic fermentation reaction, based on 1 C-mol of xylose, is described by Eq. (1) (*12*).

$$CH_2O + Y'_{N/S}NH_3 \rightarrow Y'_{X/S}CH_{1.8}O_{0.5}N_{0.2} + Y'_{E/S}CH_3O_{0.5} + Y'_{XYL/S}CH_{2.4}O + Y'_{CO_2/S}CO_2 \quad (1)$$

where $Y'_{N/S}$ is the yield factor for ammonia in units of mole per C-mole of xylose, $Y'_{X/S}$, $Y'_{E/S}$, $Y'_{XYL/S}$, and $Y'_{CO_2/S}$ are the yield factors for biomass, ethanol, xylitol, and carbon dioxide, respectively, in units of C-mole per C-mole. The carbon balance requires that Eq. (2) be satisfied.

$$Y'_{X/S} + Y'_{E/S} + Y'_{XYL/S} + Y'_{CO_2/S} = 1 \quad (2)$$

By making an elemental balance using the concept of degrees of reduction (*11*), one can show that Eq. 3 must also be satisfied.

Table 4
Recovery of Reducing Equivalents

Experiment	$Y'_{E/S}$	$Y'_{XYL/S}$	$Y'_{X/S}$	f_{red}
(0)	0.613	0.079	0.062	1.07
a	0.639	0.059	0.053	1.08
b	0.665	0.069	0.054	1.13
ab	0.678	0.049	0.054	1.13
c	0.639	0.049	0.092	1.11
ac	0.574	0.069	0.090	1.03
bc	0.652	0.049	0.123	1.16
abc	0.587	0.059	0.081	1.03
bc*	0.639	0.039	0.093	1.10
abc*	0.639	0.069	0.043	1.08

* = Repeated experiments.

$$\gamma_X Y'_{X/S} + \gamma_E Y'_{E/S} + \gamma_{XYL} Y'_{XYL/S} = \gamma_S \quad (3)$$

where γ_X, γ_E, γ_{XYL}, and γ_S are the degrees of reduction of biomass, ethanol, xylitol, and xylose, respectively, when ammonia is used as a nitrogen source. With this nitrogen source, the degree of reduction for a compound with the composition $CH_aO_bN_c$ is given by Eq. (4).

$$\gamma = 4 + a - 2b - 3c \quad (4)$$

With values for the degrees of reduction inserted, Eq. (3) can be written:

$$4.2Y'_{X/S} + 6.0Y'_{E/S} + 4.4Y'_{XYL/S} = 4.0 \quad (5)$$

A slight rearrangement of Eq. (5) gives Eq. (6), which explicitly gives the yield of ethanol.

$$Y'_{E/S} = (4.0 - 4.2Y'_{X/S} - 4.4Y'_{XYL/S}) / 6 \quad (6)$$

The maximum yield of ethanol ($Y'_{E/S} = 0.667$ C-mole/C-mole corresponding to $Y_{E/S} = 0.51$ g·g^{-1}) is obviously obtained when no xylitol or biomass is formed.

As stated above, Eq. (2) could not be used to check the material balance. However, all quantities in Eq. (5) were measured. The ratio between the left-hand side and the right-hand side could be regarded as a recovery factor of reducing equivalents, f_{red}, and it should be unity if all substrates and products are accurately quantified. Table 4 shows the results of such an analysis. As may be expected from the rather high ethanol yield values, the recovery factor is higher than one. This indicates that either the obtained yield factors are somewhat too high, or that Eq. (1) does not accurately describe the fermentation. Since the medium used was a defined medium, it is immediately realized that the only component present in the medium in sufficient amount to influence the degree of reduction balance was ammonia (or rather ammonium ions). However, to close the balance,

ammonia would have to be oxidized to nitrogen, which is not possible. A more likely explanation, therefore, is that the obtained yield factors are slightly too high. This could be caused by either too low an initial determination of the xylose concentration, too high a determination of the ethanol concentration after fermentation, or a combination of these. The error is bigger than expected from the analysis accuracy, but the most probable explanation is that the initial xylose determination is too low, since the first measured value of the xylose concentration tended to be lower than the xylose weighed in before autoclaving. The yield values reported here are thus probably slightly too high, which must be kept in mind when comparing to other reported yields in the literature.

Factorial Design Analysis

Of particular interest in this study was the maximum specific fermentation rate, $q_{p,max}$. Plots of $q_{p,max}$ vs the different main factors indicated that the fermentation rate was influenced by all of these three factors (Fig. 2A–C). To determine which effects were indeed significant, not only did all the effects have to be calculated, but an estimate of the variance was also required. The repeat experiments were used to provide such an estimate of the variance. Appropriate *t*-values, i.e., the factor effects divided by an estimate of the variance for that effect, could thus be calculated and significance tests could be made. Since a factorial design was used in this study, it was possible to examine not only the main effects, such as if the fermentation rate was higher in a nitrogen atmosphere than in a carbon dioxide atmosphere, but also to examine the interaction effects. An interaction effect means a difference in effect of one factor at different levels of another factor. As a hypothetical example, assume that the fermentation rate would be decreased by carbon dioxide for one strain, but increased for another. This would be a strong interaction effect. These kind of effects can be examined in a factorial design (good references on factorial designs are, e.g., Box et al. [*13*] and Montgomery [*14*]).

Calculated effects as well at *t*-values for both $q_{p,max}$ and the yield of xylitol, $Y_{XYL/S}$, are presented in Table 5. For the fermentation rate, the largest effect found was that of B (gas), but also the other main effects, C (cell concentration) and A (strain), were significant at the 5% level (*t*-value >4.3). All the main effects were negative, i.e., $q_{p,max}$ was decreased by carbon dioxide, was decreased by a high cell concentration, and was lower for CBS 5776 than for CBS 5773. For the largest effect, B, it can be calculated from Table 3 that $q_{p,max}$ for the experiments with a carbon dioxide atmosphere is on average 45% lower than that obtained during nitrogen-sparged conditions. One interaction effect, AB, was found to be significant and positive. This means that the maximum specific fermentation rate was not decreased as much for CBS 5776 as for CBS 5773 by carbon dioxide. Since the fermentation rate in general was lower for CBS 5776, this is

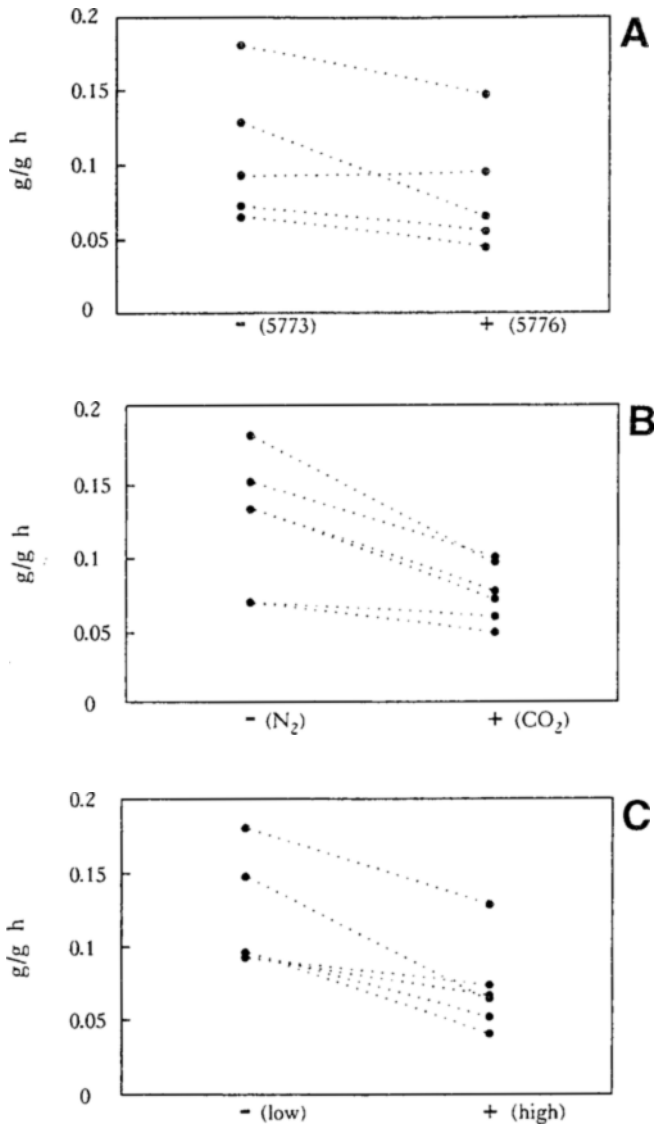

Fig. 2. A. Effect of different strains on the maximum specific fermentation rate, $q_{p,max}$. The dotted lines indicate experiments for which other factors than the strain are at the same level (0 – a, b – ab, c – ac, bc – abc). B. Effect of different gases on the maximum specific fermentation rate, $q_{p,max}$. The dotted lines indicate experiments for which other factors than the gas are at the same level (0 – b, a – ab, c – bc, ac – abc). C. Effect of different cell concentrations on the maximum specific fermentation rate, $q_{p,max}$. The dotted lines indicate experiments for which other factors than the cell concentration are at the same level (0 – c, a – ac, b – bc, ab – abc).

Table 5
Evaluation of Factor Effects

Factor	Effect on $q_{p,max}$, $g \cdot g^{-1} \cdot h^{-1}$	*t*-Value	Effect on $Y_{XYL/S}$, $g \cdot g^{-1}$	*t*-Value
Average	0.105		0.061	
A	−0.028	−6.5*	−0.0025	−0.5
B	−0.051	−12.0*	−0.0075	−1.5
C	−0.049	−11.5*	−0.0075	−1.5
AB	0.020	4.8*	−0.0025	−0.5
AC	−0.012	−2.9	0.0175	3.5
BC	0.018	4.3*	0.0025	0.5
ABC	0.003	0.7	−0.0025	−0.5

A = Effect of strain, B = effect of gas, C = effect of cell concentration. AB, AC, BC, and ABC are interaction effects. * = Significant effects ($t_{2,0.975} = 4.30$).

not really surprising. It is reasonable to believe that the carbon dioxide atmosphere decreases the fermentation rate by a certain percentage rather than by a certain absolute amount.

For the xylitol yield, no significant effects were found. Also, the effects on the ethanol yield were evaluated, but no significant effects were found.

Carbon Dioxide Equilibria

The carbon dioxide present in the liquid phase appears in several different forms. The most important reactions are given below.

$$CO_2\,(g) \rightleftharpoons CO_2\,(aq) \tag{7}$$

$$CO_2\,(aq) + H_2O \rightleftharpoons H_2CO_3\,(aq) \tag{8}$$

The carbonic acid dissociates according to:

$$H_2CO_3\,(aq) \rightleftharpoons H^+\,(aq) + HCO_3^-\,(aq) \tag{9}$$

$$HCO_3^-\,(aq) \rightleftharpoons H^+\,(aq) + CO_3^{2-}\,(aq) \tag{10}$$

The solubility of the carbon dioxide is described by Henry's law. At 30°C, the solubility of CO_2 is 1.257 g CO_2/kg of water, and most of the undissociated form is present as CO_2(aq) (*10*). In fact, the ratio between the concentration of CO_2(aq) and H_2CO_3 is about 1000. The pK_a values at 25°C for the dissociation reactions are 6.38 for the first reaction (Eq. [9]) and 10.38 for the second reaction (Eq. [10]) (*15*). The dissolved carbon dioxide is therefore almost entirely present in the undissociated form during the experiments of the present study, since the pH value in the broth was as low as 4.5. However, the situation is different inside the cell, since the intracellular pH of a yeast cell is normally between 6 and 7, and CO_2(aq) diffuses through the cell membrane (*10*). Thus, the intracellular concentration of HCO_3^-(aq) is much higher than the extracellular concentration.

Inhibitory effects on the yeast metabolism may therefore be caused by either $CO_2(aq)$ or $HCO_3^-(aq)$, even though $CO_2(aq)$ is the predominant extracellular form.

Fermentation Rate and Growth Rate

In the present study, the cell dry weight concentration was found to increase slightly during the fermentation (seen as a specific growth rate in Table 2). *Pichia stipitis* CBS 6054 has been reported not to grow under anaerobic conditions (*4*). However, for *Pichia stipitis* CBS 5773 (NRRL Y-7124), a slow growth during anaerobic conditions has been reported (*8,16*), although no growth was observed by Bruinenberg et al. (*17*). A very slow growth, leading to a doubling of the population in 6 d and then stopping, was also reported for *Pichia stipitis* NRRL Y-7124 under anaerobic conditions in a carbon dioxide atmosphere (*18*). Reported differences in anaerobic growth could be strain related, but could also result from the different experimental conditions used by the different investigators.

An estimate of the amount of oxygen diffusing into the fermentor was obtained in similar way as by Visser et al. (*19*). The fermentor, equipped with a polarographic oxygen electrode and filled with deionized water to the working volume level 1.6 L was autoclaved. The fermentor was dearated by sparging with nitrogen until the registered dissolved oxygen concentration was zero, after which the gas flow was stopped, and the gas outlet was put into a beaker of water. The stirrer rate was maintained at 500 rpm. After 20 h, the polarographic oxygen electrode showed an oxygen concentration of 3% of the air saturation value. The increase was linear, and the signal returned to the baseline, when the fermentor was again sparged with nitrogen, showing that the increase was not due to electrode drift. The amount of oxygen diffusing into the broth could thus be calculated to 12 $\mu g \cdot L^{-1} \cdot h^{-1}$. (The saturation value of dissolved oxygen at equilibrium with air at 30°C is 7.9 mg/L). As pointed out by Visser et al. (*19*), completely anoxic conditions are not possible in a real fermentor, but only in completely sealed vials. However, with the initial cell concentration used in the fermentation experiments (2.5–4 g dry wt/L), the amount of oxygen entering the fermentor must be considered to be very small.

When $q_{p,max}$ was plotted vs the specific growth rate, μ, a clear trend was seen (Fig. 3), and a regression showed a significant positive slope. This suggests that the ethanol productivity is partly growth associated, which was also found by Slininger et al. (*7*). Also for glucose fermentation by *P. stipitis*, the specific fermentation rate has been found to increase with increasing growth rate (*5*). A growth-related production of ethanol can be understood in terms of availability of ATP for growth (*11*). During anaerobic conditions, the cell will obtain the necessary ATP for growth and maintenance from the breakdown of sugar to ethanol. Hence, an increased specific growth rate should be accompanied by a larger specific

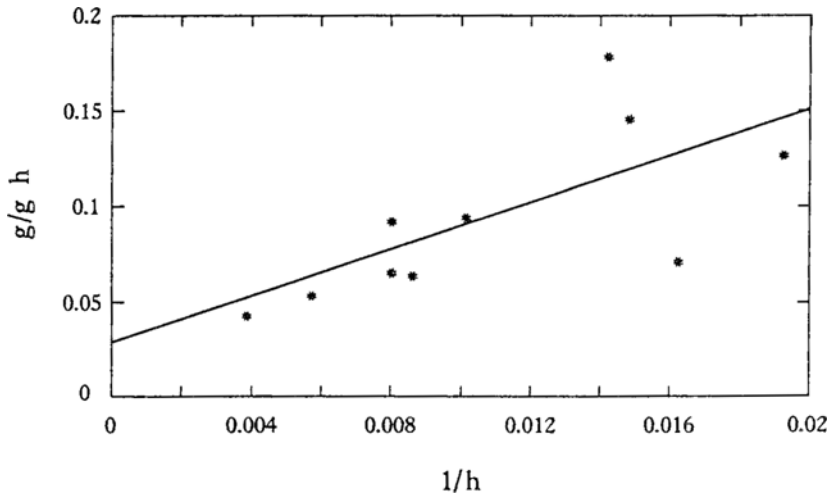

Fig. 3. Plot of maximum specific fermentation rate vs growth rate. Also, the regression line is shown in the figure.

fermentation rate. This assumes that the degree of uncoupling is low, i.e., that the amount of ATP lost in energy-spending reactions not coupled to growth or maintenance, e.g., futile cycling, is low.

In a review article (*10*), it was concluded that carbon dioxide normally does not inhibit the anaerobic glycolytic metabolism in yeasts. However, the growth rates of several yeast species, such as *Candida utilis*, *Candida scotti*, and *Saccharomyces cerevisiae*, were reported to decrease at elevated carbon dioxide levels. For *S. cerevisiae*, a complete inhibition of growth was reported at $p_{CO_2} > 2.7$ atm, but already at $p_{CO_2} > 0.5$ atm, the growth rate was decreased. Yeasts, in general, are more tolerant to increased levels of carbon dioxide than are bacteria, in particular *Pseudomonas spp.* It could also be expected that *S. cerevisiae*, being such an excellent ethanol producer, is among the most tolerant yeasts and that other yeast species are more susceptible.

Since the specific growth rate, μ, was very low in all experiments in the present study, a small error in cell concentration determination resulted in a large relative error in the determination of the growth rate (cf Table 3). A complete factorial design analysis did not show any significant effect on the growth rate by any of the factors. However, using a stepwise variable selection method (which gives more degrees of freedom for the error), two significant factors were found. The t-value for factor B, the gas, was -2.7, and the t-value for the AC interaction effect was -2.8. With six degrees of freedom for the error, this was significant at the 5% level ($t > 2.45$). Therefore, although only marginally significant, it seems reasonable to believe that the growth rate was decreased by carbon dioxide. Further insight could probably be gained by determining the maximum aerobic growth rate for different concentrations of carbon dioxide in the liquid phase.

CONCLUSIONS

The obtained results indicate that carbon dioxide is an important factor in determining the fermentation rate of xylose by *Pichia stipitis*. It was found that carbon dioxide decreases the maximum specific fermentation rate, $q_{p,max}$, by about 45%. It was further found that $q_{p,max}$ was significantly higher for CBS 5773 than for CBS 5776, and also higher for lower cell concentrations. This shows the importance of considering the ultimate fate of evolved carbon dioxide during anaerobic fermentation experiments, as well as during industrial fermentations. If carbon dioxide is not transferred out of the fermentor, but instead accumulates in the system, it may significantly influence the fermentation rate.

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