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Xylulose and Glucose Fermentation by *Saccharomyces cerevisiae* in Chemostat Culture

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Saccharomyces cerevisiae **ATCC 24860 was cultivated in chemostat culture under anoxic conditions with 111.1 mmol of glucose liter^{-1} alone or with a mixture of 66.7 mmol of xylulose liter^{-1} and 111.1 mmol of glucose liter^{-1}. The substrate consumption rate was 5.4 mmol g of cells^{-1} h^{-1} for glucose, whereas for xylulose it was 1.0 mmol g of cells^{-1} h^{-1}. The ethanol yield decreased from 0.52 carbon mole of ethanol produced per carbon mole of sugar consumed during the utilization of glucose alone to 0.49 carbon mole produced per carbon mole consumed during the simultaneous utilization of xylulose and glucose, while cell biomass was maintained at 2.04 to 2.10 g liter^{-1}. Xylulose coutilization was accompanied by a shift in product formation from ethanol to acetate and arabinitol. Xylulokinase activity was absent during glucose metabolism but detectable during simultaneous utilization of xylulose and glucose. Xylulose cometabolism resulted in increased in vitro activity of pyruvate decarboxylase and an increased concentration of the intracellular metabolite fructose 1,6-diphosphate without significant changes in the concentrations of 6-phosphogluconate and pyruvate. The results are discussed in relation to (i) altered enzyme activities and (ii) the redox flux of the cell.**

The yeast *Saccharomyces cerevisiae* has been considered for ethanol production from xylose-containing lignocellulose hydrolysates because of its (i) successful exploitation in the fermentation industry, (ii) proven ability to produce high ethanol concentrations rapidly, and (iii) high-level resistance to inhibitors found in lignocellulose hydrolysates (20, 27). Because *S. cerevisiae* cannot metabolize xylose directly, two genetic engineering approaches have been used to develop a xylose-fermenting *S. cerevisiae* strain: expression of bacterial xylose isomerase genes in *S. cerevisiae* (1, 14, 31) and expression of the xylose reductase (XR) and xylitol dehydrogenase (XDH) genes from the xylose-fermenting yeast *Pichia stipitis* in *S. cerevisiae* (17, 23, 37, 38, 44). So far, no functional xylose isomerase enzyme has been expressed even though genes from various bacterial sources have been used. On the other hand, recombinant *S. cerevisiae* strains expressing functional XR and XDH grow on xylose but ferment it poorly (13, 17, 23, 37, 38, 44). In both cases xylulose is the intracellular intermediary metabolite, which is phosphorylated to xylulose-5-phosphate and channelled via the pentose phosphate pathway (PPP) into glycolysis before being converted to ethanol.

The fermentation of xylulose by *S. cerevisiae* has been studied by using (i) xylulose purified from isomerization mixtures of xylose and xylulose (10, 12, 39, 45–47), (ii) xylose in combination with bacterial xylose isomerase (19, 20), and (iii) mixtures of xylulose and xylose (32). Substrate consumption rates for xylulose 10- to 20-fold lower than those for glucose have been observed (32, 47). When baker's yeast, *S. cerevisiae* ATCC 96581 (isolate 3), *S. cerevisiae* ATCC 24860, *S. cerevisiae* CBS 8066, *P. stipitis* CBS 6054, and *Candida shehatae* NJ 23 were compared in anoxic batch cultures with purified xylulose as the sole carbon source, *S. cerevisiae* ATCC 24860 and *S. cerevisiae* CBS 8066 were found to be the best xylulose-fermenting yeasts (47). In the present investigation *S. cerevisiae* ATCC 24860 was grown in chemostat culture under anoxic conditions on glucose alone and on a mixture of xylulose and glucose to investigate how xylulose metabolism influences product formation. The aim was to understand the lack of ethanol formation in recombinant *S. cerevisiae*. Xylulose is commercially available only in small quantities and at an extremely high price. Investigations of the xylulose metabolism, therefore, require in-house production of highly pure xylulose (28, 47).

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MATERIALS AND METHODS

Yeast strains. *S. cerevisiae* ATCC 24860 was maintained at 4°C on agar slants containing the following (per liter): 20 g of glucose, 5 g of peptone, and 3 g of yeast extract.

Preparation of xylulose. Xylulose was prepared by xylose isomerization followed by ion-exchange chromatographic separation (28) with modifications as earlier described (47).

Chemostat culture. A mineral medium supplemented with vitamins and trace elements (42) was used for inoculum preparation and chemostat cultivation with the following modifications. The mineral medium contained the following (per liter): EDTA, 30 mg; $ZnSO_4 \cdot 7H_2O$, 9 mg; $CoCl_2 \cdot 6H_2O$, 0.6 mg; $MnCl_2 \cdot 4H_2O$, 2 mg; $CuSO_4 \cdot 5H_2O$, 0.6 mg; $CaCl_2 \cdot 2H_2O$, 9 mg; $FeSO_4 \cdot 7H_2O$, 6 mg; $NaMoO_4 \cdot 2H_2O$, 0.8 mg; H_3BO_3, 2 mg; and KI, 0.2 mg. Ergosterol and Tween-80 were also added as anaerobic growth factors (2, 3); they were dissolved in 99.5% ethanol and added to final concentrations of 0.01 and 0.40 g liter^{-1}, respectively. For inoculum preparation, 500 ml of medium containing 111.1 mmol of glucose liter^{-1} in a 500-ml flask sealed with a rubber stopper was inoculated, through a syringe, with one loopful of yeast cells suspended in 5 ml of medium. The syringe needle was left in the rubber stopper to release evolved carbon dioxide. The flask culture was incubated at 30°C with magnetic stirring for 1 day before transfer to the fermentor.

The fermentations were performed in a 1.5-liter glass vessel (Applikon BV, Schiedam, The Netherlands) controlled by an EFC 24 control system (Electrolux Fermentation, Malmö, Sweden) using a working volume of 500 ml. The pH of the culture was maintained at 5.5 by automatic addition of 1 mol of NaOH liter^{-1}, and the temperature was kept at 30°C. After inoculation the mineral medium containing 111.1 mmol of glucose liter^{-1} was fed into the culture at a dilution rate of 0.1 h^{-1}. The substrate composition was changed to 66.7 mmol of xylulose liter^{-1} and 111.1 mmol of glucose liter^{-1} at 100 h. The cultivation was conducted under anoxic conditions, i.e., no air was supplied to the culture. The cells were considered to grow anaerobically, the level of residual oxygen in the medium was estimated to be less than 0.02 mmol of oxygen g of cells^{-1} h^{-1}, and the produced carbon dioxide prevented air from diffusing into the culture

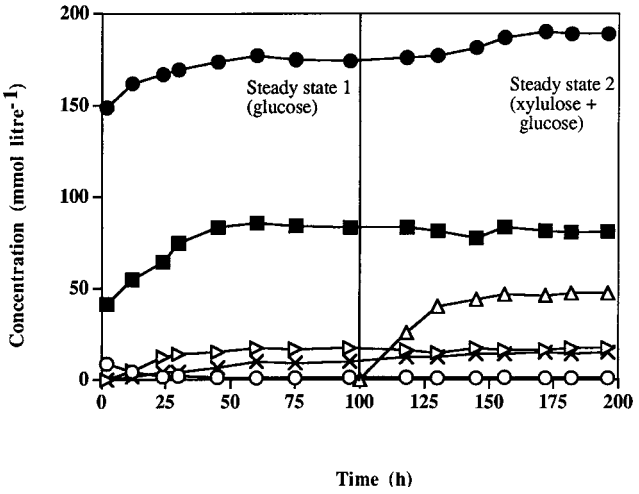

FIG. 1. Time course of substrate and product concentrations in the chemostat culture of *S. cerevisiae* ATCC 24860. The substrate composition in the medium started with 111.1 mmol of glucose liter^{-1} at 0 h and then changed to 111.1 mmol of glucose liter^{-1} and 66.7 mmol of xylulose liter^{-1} at 100 h. ○, glucose; △, xylulose; ●, ethanol; ▷, glycerol; ×, acetate; ■, cell biomass.

through the condenser. The tubings for the entire fermentor setup consisted of Norprene and Tygon tubings (Cole-Parmer Instrument Co., Niles, Ill.).

Analysis of substrates and products. Glucose, xylulose, ribitol, arabinitol, xylitol, glycerol, acetic acid, and ethanol were analyzed by liquid chromatography using a liquid chromatograph (model 5000; Varian, Palo Alto, Calif.) equipped with a refractive index detector (model 5902; Tecator, Höganäs, Sweden). Repeated analyses deviated by less than 5%. The separation was performed on two Aminex HPX-87H columns (Bio-Rad Laboratories, Richmond, Calif.) coupled in series (21). The dry cell weight was determined by filtering the sample through a 0.45-μm-pore-size Supor membrane (Gelman Sciences, Ann Arbor, Mich.), washing it five times with double-distilled water, and drying filtered cells in a microwave oven for 15 min.

Determination of intracellular enzyme activities. Cell extracts were prepared by freeze pressing as described previously (35) using PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] buffer solution (0.1 mol liter^{-1}; pH 7.0) for washing and cell suspension. Spectrophotometric analysis of enzyme activity was carried out with a Hitachi (Tokyo, Japan) U-2000 spectrophotometer at 30°C. XR was measured by a method modified from that of Smiley and Bolen (35, 36). XDH was measured by a method modified from that of Rizzi et al. (29, 35). Transaldolase, transketolase, and glucose-6-phosphate dehydrogenase were assayed as previously described (8). Pyruvate decarboxylase (PDC) levels were determined by the method of Hoppner and Doelle (15). Xylulokinase was assayed with 1 U of lactate dehydrogenase and 1 U of pyruvate (PYR) kinase in a 1-ml cuvette volume (33). The protein content in cell extract was determined by the Bradford method (7) with a commercial Coomassie brilliant blue reagent (Pierce, Rockford, Ill.) and bovine serum albumin as the standard. Specific enzyme activities in the extracts were expressed in units gram of protein^{-1}, where 1 U is equivalent to the conversion of 1 mmol of substrate per min.

Determination of intermediary metabolite concentrations. Concentrations of intracellular intermediary metabolites were determined in cell extracts obtained by a cold-methanol quenching method (11). Fructose 1,6-diphosphate (FDP), PYR (22), and 6-phosphogluconate (6PG) (4) levels were determined by enzymic assays in which the consumption or formation of NADPH or NADH was spectrofluorometrically determined. The emission was measured at 450 nm after excitation at 350 nm with a Hitachi F-3000 fluorescence spectrophotometer. The concentration of intermediary metabolites was expressed in nmol mg of dry cells^{-1}. All chemicals and enzymes used for the determination of enzyme activities and intermediary metabolite concentrations were from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

Substrate and product patterns in fermentations of glucose and a mixture of xylulose and glucose. Two steady states in the chemostat cultivation of *S. cerevisiae* ATCC 24860 were set up, one with only glucose as a carbon source and one with xylulose and glucose as carbon sources (Fig. 1). The fermentor and the inlet flow of the glucose-containing medium were started at 0 h and maintained at a dilution rate of 0.1 h^{-1}. The first steady state was reached after 50 h with a glucose feed of 111.1 mmol liter^{-1}. At 100 h the medium composition was changed to 66.7 mmol of xylulose liter^{-1} and 111.1 mmol of glucose liter^{-1}, and the second steady state was reached at 150 h. In the first steady state glucose was utilized almost completely, with a consumption rate of 5.4 mmol g of cells^{-1} h^{-1}, and the yield of ethanol was 0.52 carbon mole of ethanol produced per carbon mole of sugar consumed. The residual glucose concentration in the culture was less than 1.4 mmol liter^{-1}. The ethanol yield was 77% of the theoretical yield (0.67 carbon mole produced per carbon mole consumed), and the rate of cell biomass generation was 8.4 mmol liter^{-1} h^{-1} as determined by using the elemental composition formula $CH_{1.83}O_{0.56}N_{0.17}$ (30) to estimate the number of assimilated carbon moles converted to cell biomass. The rate of glycerol formation was 1.7 mmol liter^{-1} h^{-1}, and the rate of acetate formation was 1.0 mmol liter^{-1} h^{-1}. The cometabolism of xylulose in the second steady state did not affect the rate of glucose utilization (Fig. 1). Almost 30% of the xylulose was consumed at a rate of 1.0 mmol g of cells^{-1} h^{-1}. The ethanol concentration increased by 8%, from 174.4 mmol liter^{-1} to 189.2 mmol liter^{-1}, whereas the ethanol yield, 0.49 carbon mole produced per carbon mole consumed was reduced. Acetate formation increased, and low levels of arabinitol were formed (Table 1).

Carbon balances. The carbon mass balances based on numbers of carbon moles for the two steady states of the chemostat culture are summarized in Table 1. The carbon dioxide produced in the culture was not measured. It was assumed that 424 mmol of carbon dioxide was generated for 4,000 mmol of cell biomass (100 g [dry weight]) (9) and that 1 mol of carbon dioxide was formed for every mole of ethanol or acetate produced. The consumed substrates in the two steady states were quantitatively accounted for in the determined products within 3% (Table 1).

Redox balances. The reduction and oxidation of cofactors in the metabolism of glucose and the mixture of xylulose and glucose are summarized in Table 2. The calculations of the fluxes of the two redox systems, NADPH/$NADP^+$ and NADH/NAD^+, are based on the assumptions that 931 mmol of NADPH was consumed and 1,349 mmol of NADH was produced for the biosynthesis of 4,000 mmol (100 g) of yeast cells (9). The rate of NADPH production in the oxidation of glucose-6-phosphate was not included in the calculations, since

TABLE 1. Carbon balance in the chemostat culture of *S. cerevisiae* ATCC 24860[a]

Steady state (carbon source[s])	Amt of carbon consumed (mmol liter^{-1})			Amt of carbon produced (mmol liter^{-1})							C recovery (%)
	Glucose	Xylulose	Total	Arabinitol	Glycerol	Acetic acid	Ethanol	CO_2	Cell mass	Total	
1 (glucose)	676.7		676.7		52.2	20.0	348.7	193.3	83.5	697.7	103.1
2 (xylulose + glucose)	680.0	96.7	776.7	1.6	52.2	30.0	378.3	212.8	81.1	756.0	97.3

[a] See the text and the legend to Fig. 1 for descriptions of the steady states.

TABLE 2. Specific rates of NADPH and NADH production and consumption in the steady states of the chemostat culture of *S. cerevisiae* ATCC 24860

Reaction	Rate (mmol g of cells^{-1} h^{-1}) of production or consumption in[a]:			
	Steady state 1[b]		Steady state 2[c]	
	NADPH	NADH	NADPH	NADH
Glucose-6-phosphate oxidation	NC[d]		NC	
Arabinitol formation				−0.02
Glycerol formation		−0.83		−0.85
Acetic acid formation	+0.48	+0.48	+0.74	+0.74
Cell biomass formation	−0.93	+1.35	−0.93	+1.35
Total	−0.45	+1.00	−0.19	+1.22

[a] Positive values represent production rates, and negative values represent consumption.
[b] Carbon source, glucose.
[c] Carbon sources, xylulose and glucose.
[d] NC, not calculated.

TABLE 3. Specific activities of enzymes in the chemostat culture of *S. cerevisiae* ATCC 24860[a]

Enzyme[b]	Sp act (U g of protein^{-1}) at[c]:	
	Steady state 1[d]	Steady state 2[e]
XR (NADPH)	0.018	0.016
XR (NADH)	ND[f]	ND
XDH (NAD$^+$)	ND	ND
XK	ND	0.016
TAL	0.16	0.17
TKL	0.056	0.067
G6PDH	0.36	0.42
PDC	0.51	0.64

[a] Samples were taken at the two steady states.
[b] XK, xylulokinase; TAL, transaldolase; TKL, transketolase; G6PDH, glucose-6-phosphate dehydrogenase.
[c] The values represent the means of three to five replicate determinations. Standard errors were <15% of the mean values.
[d] Carbon source, glucose.
[e] Carbon sources, xylulose and glucose.
[f] ND, not detectable.

the calculated amount of carbon dioxide formed as a consequence of biomass formation (9) does not discriminate carbon dioxide formed in the oxidative part of the PPP. Alcoholic fermentation does not influence the final balance of pyridine nucleotides, because the NADH formed in the glyceraldehyde 3-phosphate dehydrogenase reaction is reoxidized in the alcohol dehydrogenase (ADH) reaction. The formation of acetate by the oxidation of acetaldehyde can be catalyzed by either the NADP$^+$-dependent isoenzyme or the NAD$^+$-dependent isoenzyme. When the reaction is catalyzed by the NADP$^+$-dependent enzyme, 1 mol of NADH and 1 mol of NADPH are produced in the formation of 1 mol of acetate, whereas 2 mol of NADH is formed when the NAD$^+$-dependent isoenzyme is catalyzing the reaction. For the calculations summarized in Table 2, the oxidation of acetaldehyde to acetate was considered to be catalyzed by the NADP$^+$-dependent enzyme. The formation of 1 mol of glycerol leads to the reoxidation of 1 mol of NADH. Arabinitol formation was considered to be an NADH-consuming reaction, and the formation of 1 mol of arabinitol leads to the reoxidation of 1 mol of NADH. For both steady states the analysis of the redox balance showed an overestimation of the NADH levels and an underestimation of the NADPH level. The underestimation of the NADPH levels was probably due to the fact that the NADPH required for biomass formation is supplied via the oxidative part of the PPP, which could not be examined in the present experimental setup. If acetate formation had been assumed to be catalyzed by the NAD$^+$-dependent enzyme the overestimation of NADH levels would have been even greater.

Specific in vitro activities of enzymes and levels of intermediary metabolites. Tables 3 and 4 summarize the in vitro intracellular activities of enzymes and concentrations of intermediary metabolites in the glucose and xylulose pathways. No XDH activity was detected, and very low levels of XR activity were measured. Xylulokinase activity was absent during glucose metabolism but detectable during simultaneous utilization of xylulose and glucose. The level of PDC activity and the concentration of FDP increased slightly during utilization of xylulose in combination with glucose compared with values obtained during utilization of glucose only. By using the *t* test for evaluation of mean values at the 5% level (24), no significant differences were observed between the activities of transketolase, transaldolase, and glucose-6-phosphate dehydrogenase and the concentrations of 6PG and PYR in the two steady states.

DISCUSSION

Unlike the metabolism of glucose, xylulose must be routed entirely through the PPP before being channelled into glycolysis. In the present chemostat study the xylulose consumption rate was 1.0 mmol g of cells^{-1} h^{-1}, which is two to four times higher than rates previously reported for *S. cerevisiae* ATCC 24860 in batch cultivations (32, 47) but still several times lower than the glucose consumption rate, i.e., 5.4 mmol g of cells^{-1} h^{-1}. The ethanol yield during the simultaneous fermentation of xylulose and glucose decreased from 0.52 carbon mole produced per carbon mole consumed for glucose alone to 0.49 carbon mole produced per carbon mole consumed because of an increase in the formation of acetate and arabinitol. The formation of acetate and arabinitol involves reduction and oxidation of cofactors and indicates an altered redox flux during xylulose fermentation. The fact that xylulose cofermentation changes the redox flux of the cell might contribute to the low ethanol yield from xylose compared with that from glucose produced by recombinant *S. cerevisiae* harboring XR and XDH genes from *P. stipitis* (13, 17, 23, 37, 38, 44). However, the recombinant *S. cerevisiae* strains also produced considerable amounts of xylitol during xylose fermentation (13, 17, 23, 37, 38, 44), which may have been caused by (i) a redox imbalance in the initial XR and XDH reactions or by (ii) a limitation in XDH or another enzyme further down in the metabolism.

TABLE 4. Concentrations of intermediary metabolites in the chemostat culture of *S. cerevisiae* ATCC 24860[a]

Intermediary metabolite	Concn (nmol mg of dry cells^{-1}) at[b]:	
	Steady state 1[c]	Steady state 2[d]
6PG	0.63	0.52
FDP	2.4	3.3
PYR	0.7	0.8

[a] Samples were taken at the two steady states.
[b] The values represent the means of three to five replicate determinations. Standard errors were <15% of the mean values.
[c] Carbon source, glucose.
[d] Carbon sources, xylulose and glucose.

TABLE 5. Intracellular metabolite concentrations

S. cerevisiae strain	Cultivation conditions	Carbon source(s)	Concn (nmol mg of dry cells^{-1}) of intracellular metabolite:			Reference
			6PG	FDP	PYR	
ATCC 24860	Batch	Glucose	NM[a]	6.50	2.36	32[b]
ATCC 24860	Batch	Xylulose	NM	0.78	1.38	32[b]
PUA6-9	Batch	Glucose	3.2	12.4	12.4	17[c]
PUA6-9	Batch	Xylose	7.1	1.7	2.8	17[c]
ATCC 24860	Chemostat	Glucose	0.63	2.4	0.7	The present study
ATCC 24860	Chemostat	Xylulose and glucose	0.52	3.3	0.8	The present study

[a] NM, not measured.
[b] Standard errors were between 4 and 17%.
[c] Standard errors ranged from <0.01 to 0.4 nmol mg of dry cells^{-1}.

There was no significant difference in the concentrations of the intermediary metabolites 6PG and PYR between the two different steady states, while there was an increase in the FDP concentration during xylulose cometabolism, in contrast to earlier reports (17, 32) (Table 5). This might be due to the fact that glucose was simultaneously utilized. There was a corresponding increase in the in vitro activity of PDC during xylulose cometabolism. Intracellular metabolites may act both at the level of induction and repression of enzyme synthesis and as effector molecules with enzymes already present in the cell. Ethanol production requires that the enzymes PDC and ADH channel PYR to acetaldehyde and ethanol. PDC synthesis and ADH synthesis are induced through a series of events involving key intermediary metabolites of both the upper and the lower parts of glycolysis (5, 6, 25). The additional FDP present during xylulose cometabolism may take part in the induction of PDC and/or indicates elevated levels of other glycolytic intermediates which in turn take part in the induction of PDC. The concentrations of the intermediary metabolites 6PG, FDP, and PYR in the present report are 2- to 14-fold lower than those in earlier reports (17, 32) (Table 5). The differences are due to the different cultivation conditions. The present study was carried out in glucose-limited cultures, whereas the previous studies were batch cultivations in which the intermediary metabolites were extracted from cells harvested under nonlimiting conditions. For *Lactococcus lactis* subsp. *lactis* it has been shown that the concentrations of FDP and PYR in carbon-limited cells are considerably lower than those in nonlimited cells (34).

The increased in vitro PDC activity in cells utilizing xylulose and glucose simultaneously compared with that in cells utilizing glucose alone could cause increased acetaldehyde formation. Acetaldehyde, once formed, can either be reduced to ethanol by ADH or be oxidized to acetate by acetaldehyde dehydrogenase (NAD^+ or $NADP^+$ dependent). Attempts to measure ADH activity failed because of deactivation during storage of cell extracts. At concentrations above 0.5 mM, acetaldehyde is a potent inhibitor of cellular functions, its toxicity being due to its reaction with cellular amino groups (for a review, see reference 16). The increased acetate formation may be (i) a way to decrease levels of surplus acetaldehyde not oxidized to ethanol because of a limitation in ADH or (ii) a response to a surplus of NAD^+ and $NADP^+$ during simultaneous xylulose and glucose utilization.

A redox balance was set up to investigate the increased acetate formation observed during simultaneous xylulose and glucose utilization compared with that observed during utilization of glucose only and the formation of arabinitol during simultaneous xylulose and glucose utilization. Even though the carbon balance was satisfactorily closed, no balanced redox metabolism was obtained. It is commonly accepted that no transhydrogenase activity is present in yeasts ($NADH + NADP^+ \leftrightarrow NAD^+ + NADPH$) (18, 40), and therefore the fluxes of the two redox couples were separated. NADPH required for biomass formation is assumed to be supplied via the oxidation of glucose-6-phosphate in the oxidative part of the PPP (18, 40). However, the acetate formation observed may indicate that the requirement for NADPH was met by the oxidation of acetaldehyde to acetate, if acetate formation was catalyzed by the $NADP^+$-dependent enzyme.

NADH generated from biomass formation could not be balanced by the formation of products (Table 2). Similar observations were made with a recombinant strain of *S. cerevisiae* harboring the *xyl1* gene for XR in fed-batch fermentations of glucose and xylose (26). The lack of closed redox balances could be due to (i) experimental errors in the determination of substrate and product concentrations insignificant for the carbon balance but with major impact on the redox balance, (ii) invalidity of the values for NADH production and NADPH consumption in biosynthesis (9) with regard to *S. cerevisiae* ATCC 24860 used in the present study, or (iii) incompleteness of the assumed metabolic pathways.

If the error in the redox balance was entirely due to an error in, for instance, the glycerol measurement (inferior liquid chromatographic analysis), the carbon recovery would change from 103 to 112% during glucose utilization and from 97 to 107% during simultaneous xylulose and glucose utilization. Since these numbers would not be considered satisfactorily closed carbon balances, the measurements are most likely correct. Oxygen is another substrate which might be erroneously estimated. The conditions in the present study were anoxic but not strictly anaerobic (43). If the medium in the substrate feed reservoir is assumed to be air saturated, an oxygen flow of 0.01 to 0.02 mmol g of cells^{-1} h^{-1} would have been fed into the chemostat culture. This would increase the NADH consumption rate by only 0.02 to 0.04 mmol g of cells^{-1} h^{-1}, since 1 mmol of oxygen can oxidize 2 mmol of NADH.

Another source of error could be Tween 80 added to the growth medium, which could serve as a source of oleic acid so that fatty acid synthesis would not contribute to the NADH formation (41). Thereby only 943 mmol of NADH would be produced for the biosynthesis of 100 g of yeast cells. With glucose the surplus NADH flux would be reduced from 1.00 to 0.59 mmol of NADH g of cells^{-1} h^{-1}, and with xylulose in combination with glucose the surplus NADH flux would be reduced from 1.22 to 0.81 mmol of NADH g of cells^{-1} h^{-1}. In view of these results metabolic pathways have to be reinvestigated in order for us to fully understand the regulation of the metabolic fluxes during anaerobic utilization of sugars metabolized through the PPP.

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