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Competition for Glucose between the Yeasts *Saccharomyces cerevisiae* and *Candida utilis*

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The competition between the yeasts *Saccharomyces cerevisiae* CBS 8066 and *Candida utilis* CBS 621 for glucose was studied in sugar-limited chemostat cultures. Under aerobic conditions, *C. utilis* always successfully completed against *S. cerevisiae*. Only under anaerobic conditions did *S. cerevisiae* become the dominant species. The rationale behind these observations probably is that under aerobic glucose-limited conditions, high-affinity glucose/proton symporters are present in *C. utilis*, whereas in *S. cerevisiae*, glucose transport occurs via facilitated diffusion with low-affinity carriers. Our results explain the frequent occurrence of infections by Crabtree-negative yeasts during bakers' yeast production.

Bakers' yeast is commercially produced by aerobic fed-batch cultivation under sugar limitation (23, 24). This technique is required since under aerobiosis and glucose excess *Saccharomyces cerevisiae* shifts to a metabolic pattern in which respiration and alcoholic fermentation coexist (31). The unwanted production of ethanol leads to a decreased yield in biomass.

In the commercial process, infections with other yeasts, the so-called wild yeasts, easily occur. Such contaminants, frequently *Candida* species (7, 8, 14, 24, 36), may attain such high numbers that product quality is adversely affected. Apparently, under sugar limitation these wild yeasts have a competitive advantage over the bakers' yeast. Studies on the kinetic aspects of this competition are limited. Only studies on the interactions of bakers' yeast with bacteria have been published so far (6, 26).

In previous chemostat studies with *S. cerevisiae* and *Candida utilis*, we have shown that the affinity of the glucose transport system is related to the residual substrate concentration in the culture. The yeasts appear to synthesize carriers with affinity constants that are tuned to the environmental sugar concentration. Moreover, they produce just enough of these carriers to account for the glucose flux required to sustain the growth rate (21, 22). Yeasts like *C. utilis* possess high-affinity proton/glucose symporters which enable them to effectively scavenge the sugar at low growth rates (32a). In contrast, *S. cerevisiae* transports the glucose by a low-affinity facilitated diffusion, leaving relatively high residual glucose levels in the culture (22, 32a). In this study, we show that competition between *S. cerevisiae* and *C. utilis* for glucose under aerobic conditions invariably results in the selection of *C. utilis* as the dominant species. The role of the glucose transport systems and their kinetics in this phenomenon will be discussed.

MATERIALS AND METHODS

Microorganisms and growth conditions. *S. cerevisiae* CBS 8066 and *C. utilis* CBS 621 were maintained on malt agar slopes. For continuous cultivation, the organisms were grown at 30°C in an Applikon fermentor (Applikon Dependable Instruments, Schiedam, The Netherlands) with a working volume of 1 liter, a stirrer speed of 900 rpm, and an airflow between 1 and 4 liters min^{-1}. In aerobic experiments, the dissolved-oxygen tension was maintained above 50% air saturation. In studies on oxygen-limited growth, the airflow to the fermentor was shut off. During the anaerobic experiments, the fermentor was flushed with nitrogen gas containing less than 5 ppm (5 μl $liter^{-1}$) of oxygen at a flow rate of 0.2 liters min^{-1}. The pH was automatically controlled at 5.0 by the addition of 2 M KOH. The medium was prepared as described previously (22). The glucose concentration in the reservoir was 5 g $liter^{-1}$ unless mentioned otherwise. For batch cultivation, the organisms were precultured in mineral medium with 1% (wt/vol) glucose on a rotary shaker (model G25 Incubator shaker; New Brunswick Scientific Co. Inc., Edison, N.J.) at 30°C and 250 rpm and were subsequently transferred to the fermentor. Growth conditions in these batch cultures were as described above. For determination of viable counts the same mineral medium, adjusted to pH 5.0, was used with the addition of 1.5% (wt/vol) agar (Difco Laboratories, Detroit, Mich.) and with glucose or xylose (2% [wt/vol]) as the carbon and energy source.

Determination of dry weight. For dry-weight measurements, nitrocellulose filters (pore size, 0.45 μm; Gelman Sciences, Inc., Ann Arbor, Mich.) were used. After removal of the medium by filtration, the filter was washed with demineralized water and dried to constant weight in a magnetron oven (R-7400; Sharp Inc., Osaka, Japan) for 15 min. This procedure yielded the same results as drying at 80°C on filters or in weighing flasks.

Determination of cell counts. Viable counts of *S. cerevisiae* and *C. utilis* were obtained by counting the number of colonies on glucose and xylose agar, prepared after proper dilution of the culture (50 to 400 colonies per plate). The colonies were counted as soon as they became visible (after 48 and 72 h at 30°C for glucose and xylose plates, respectively) in order to avoid confluence of separate colonies. The inability of *S. cerevisiae* to grow on xylose was used to determine the cell numbers of this organism in mixed cultures with *C. utilis*. The number of *C. utilis* cells on xylose plates was determined, and the number of *S. cerevisiae* cells could be assessed by subtracting the number of colonies on xylose plates from that on glucose plates. In all experiments, the average number on four plates was used to determine the

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viable counts. The reliability of this method was verified by mixing known amounts of pure cultures of the two organisms and determining viable counts as described above.

Total cell numbers were determined microscopically with a cell chamber (type Thoma no. 656989; Schreck, Hofheim, Federal Republic of Germany). For these measurements, more than 100 individual cells were counted per sample. Budding cells were in all cases regarded as a single cell. In none of the experiments was agglutination of cells observed.

Kinetics of glucose-dependent oxygen consumption. Oxygen consumption rates were measured with a Clark-type oxygen electrode in a Biological Oxygen Monitor at 30°C. Before the start of the experiment, cell suspensions were aerated in the vessel to allow for equilibration of oxygen at the temperature applied. The endogenous respiration was then monitored for 3 min before addition of the glucose. Oxygen uptake rates in the presence of glucose were corrected for endogenous respiration. The affinity constants for glucose oxidation and the maximal oxidation capacities were assessed from Hanes plots (9). Cell suspensions from cultures with high residual glucose concentrations were harvested by centrifugation and washed twice at room temperature with mineral medium without a carbon source. Cells from glucose-limited cultures were used without pretreatment. Washing of cells did not affect the kinetics of glucose oxidation. The maximal variation in the determination of the affinity for glucose oxidation was 25%.

Transport assays. Determination of the kinetic constants for glucose transport in the two yeasts was performed essentially as described previously (21).

Analysis of metabolites. When steady-state samples were taken, rapid sampling of culture fluid was performed as described previously (21). In all other cases, culture fluid from the fermentor was rapidly (within 3 min) centrifuged with an Eppendorf Microcentaur (2 min at 13,000 rpm; MSE, London, United Kingdom), and the supernatant was used to determine metabolites. Spectrophotometric assays were performed at 30°C with a Hitachi 100-60 spectrophotometer (Hitachi Inc., Tokyo, Japan). Ethanol was assayed by the colorimetric method of Verduyn et al. (34). Glycerol was determined with Boehringer kit no. 148270 (Boehringer GmbH, Mannheim, Federal Republic of Germany). For glucose, Boehringer hexokinase–glucose-6-phosphate-dehydrogenase kit no. 716251 or GOD-PAP kit no. 676543 was used, depending on the sensitivity required.

RESULTS

Relationship between growth rate and residual glucose concentration. In most cases, the relationship between the concentration of the growth-limiting substrate and the growth rate of a microorganism can be described by the Monod equation (19) $\mu = (\mu_{max} \times s)/K_s + s)$. This was also true for aerobic glucose-limited chemostat cultures of *C. utilis* CBS 621 (21) (Table 1). The residual glucose concentration was independent of the biomass concentration in the culture. In contrast to *C. utilis*, *S. cerevisiae* CBS 8066 did not follow Monod kinetics in aerobic glucose-limited cultures. At dilution rates below 0.39 h^{-1}, the residual substrate concentration was virtually constant at 110 μM and was independent of the biomass concentration in the chemostat (22). At dilution rates above 0.39 h^{-1}, the residual substrate concentration sharply increased and could be described by the Monod equation. There was, however, a clear influence of the reservoir glucose concentration and hence the biomass density on the K_s values obtained (22) (Table 1).

TABLE 1. Affinity constants for growth (K_s) and maximal growth rates (μ_{max}) for *C. utilis* CBS 621 and *S. cerevisiae* CBS 8066[a]

Organism	S_r (g liter^{-1})	K_s (μM)	μ_{max} (h^{-1})
C. utilis	5	15 ± 2	0.59 ± 0.01
	15	15 ± 2	0.59 ± 0.01
S. cerevisiae	5	110 ± 10[b]	0.49 ± 0.01
	15	390 ± 30[b]	0.49 ± 0.01

[a] Data were obtained from Hanes plots of residual substrate concentration and dilution rate in glucose-limited chemostat cultures at different reservoir concentrations (S_r) of glucose.
[b] Values were obtained at dilution rates above 0.39 h^{-1}.

Probably the fermentation products formed above $D = 0.39$ h^{-1} (e.g., ethanol and acetate) affect the affinity constant for growth, K_s. No effect of the reservoir glucose concentration on the maximal growth rate of the organism was observed in these chemostat cultures.

Competition of *S. cerevisiae* and *C. utilis* in aerobic glucose-limited cultures. On the basis of the affinity constants for growth of two microorganisms, the result of their competition in mixed cultures can be predicted (33). From the results presented in Table 1, it was expected that in glucose-limited chemostat cultures *C. utilis* would rapidly dominate *S. cerevisiae*. In order to verify this, an inoculum (1% dry weight) of *Candida* cells from a glucose-limited culture was added to a glucose-limited culture of *S. cerevisiae* (Fig. 1A). After only one volume change a decrease in the amount of *S. cerevisiae* cells in the culture was observed, and *S. cerevisiae* was almost completely replaced by *C. utilis* after five volume changes (Fig. 1A and B). This phenomenon was also reflected in the residual glucose concentration in the culture. During the experiment the glucose concentration dropped from 82 to 4 μM, which is the residual substrate concentration observed in a glucose-limited culture of *C. utilis* alone growing at this dilution rate (21).

Although the same biomass yields were obtained for the two yeasts (Table 2), the number of cells present in a steady-state culture was higher for *C. utilis* than for *S. cerevisiae*: $2.7 \times 10^8 \pm 0.5 \times 10^8$ and $2.0 \times 10^8 \pm 0.3 \times 10^8$ cells ml^{-1}, respectively. Thus, the total number of organisms in the culture increased during the competition experiment (Fig. 1B). The viable counts obtained with agar plates were in good agreement with the values obtained with microscopic cell counts in the separate yeast cultures, which were $3.2 \times 10^8 \pm 0.1 \times 10^8$ and $1.9 \times 10^8 \pm 0.2 \times 10^8$ cells ml^{-1} for *C. utilis* and *S. cerevisiae*, respectively.

The growth rates of the two organisms during the competition experiment in glucose-limited chemostat cultures could be assessed by plotting the natural logarithms of their cell numbers in the culture as a function of time. The growth rate of an organism in continuous culture is described by $\ln x = (\mu - D)t + \ln x_0$ (19). The growth rate of *S. cerevisiae* as calculated from the slope of the line and the dilution rate (Fig. 1B) was $-0.15 \pm 0.05 + 0.10 = -0.05 \pm 0.05$ h^{-1}. Thus, after one volume change (10 h), *S. cerevisiae* followed washout kinetics (zero growth rate). Unlike the situation with *S. cerevisiae* at $D = 0.10$ h^{-1}, the growth rate of *C. utilis* in glucose-limited chemostat cultures can be described by the Monod equation (see above). Therefore, during the competition experiment the growth rate of *C. utilis* should decrease progressively as a result of the decrease in the glucose concentration. This was indeed observed (Fig. 1B). The correlation between the growth rate of *C. utilis* deter-

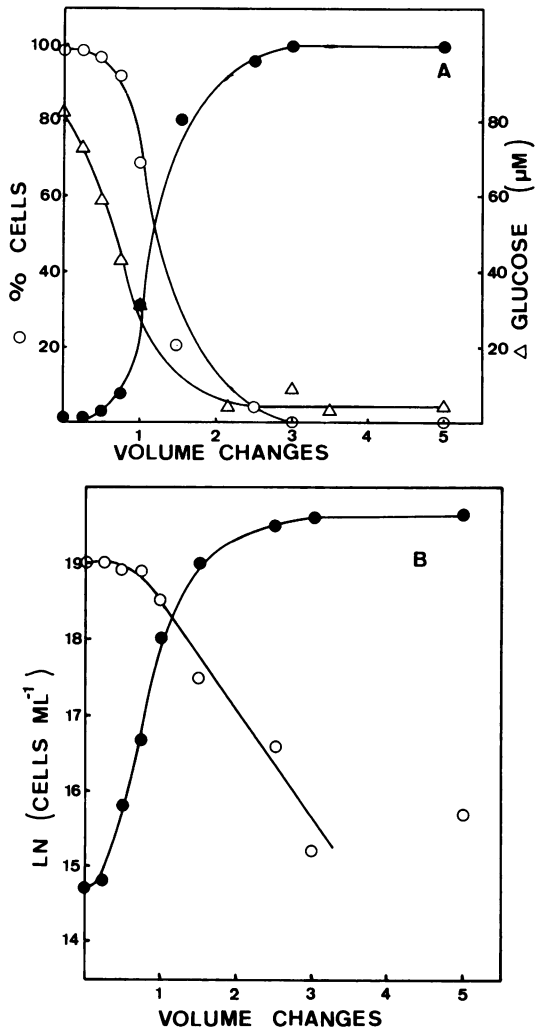

FIG. 1. (A) Competition between *S. cerevisiae* CBS 8066 (○) and *C. utilis* CBS 621 (●) in a glucose-limited chemostat culture at $D = 0.10\ h^{-1}$. At zero time, the culture was inoculated with 1% (dry weight) *Candida* cells. The number of viable cells of each yeast is expressed as the percentage of the total cell population versus the number of volume changes. Also plotted is the residual glucose concentration in the culture (△). (B) Viable counts of *C. utilis* (●) and *S. cerevisiae* (○) during the competition experiment as a function of the number of volume changes.

mined from Fig. 1B and the growth rate calculated with the kinetic constants for growth (Table 1) and the residual glucose concentration (Fig. 1A) is depicted in Fig. 2.

Competition between *S. cerevisiae* and *C. utilis* under glucose excess. Several yeast strains are known to excrete killer toxins. The formation of such products by *C. utilis* might explain the washout of *S. cerevisiae* during the competition experiment. It was therefore decided to study the behavior of a mixture of the two yeasts in batch culture. If in the mixed culture each organism would attain μ_{max}, the possibility of killer interactions would be eliminated.

For these batch experiments, the two yeasts were precultured separately in mineral medium on a rotary shaker for 24 h (exponential phase). Equal amounts (wt/vol) of both cultures were then mixed in the fermentor. In this experiment, again, the ratio of *C. utilis* to *S. cerevisiae* cells increased

TABLE 2. Cell yield, residual glucose, and ethanol and glycerol concentrations in glucose-limited chemostat cultures of *S. cerevisiae* CBS 8066 and *C. utilis* CBS 621[a]

Organism and condition	Biomass yield (g/g^{-1})	Glucose concn (μM)	Ethanol concn (mM)	Glycerol concn (mM)
S. cerevisiae				
Aerobic[b]	0.51	110[e]	0	0
Oxygen-limited[c]	0.16	90	35	0
C. utilis				
Aerobic[b]	0.51	4	0	0
Oxygen-limited[c]	0.21	40	25	0
Mixed culture				
Aerobic[b]	0.51	4	0	0
Anaerobic[d]	0.11	130	40	4

[a] Cultures were grown at $D = 0.10\ h^{-1}$ with various levels of oxygen supply. Data for mixed cultures refer to a steady-state situation obtained after five volume changes. The maximal deviations from the mean values were 25% for the glucose concentrations, 10% for the glycerol and ethanol concentrations, and 5% for the biomass yield.
[b] Dissolved-oxygen concentration higher than 50% air saturation.
[c] No air supply.
[d] Sparged with nitrogen gas.
[e] Average value; values varied between 80 and 125 μM.

with time, leading to a 9-to-1 ratio after 25 h (Fig. 3A). The growth rates of *C. utilis* and *S. cerevisiae* calculated from the slopes of the lines (exponential phase) depicted in Fig. 3B were 0.63 ± 0.05 and $0.51 \pm 0.05\ h^{-1}$, respectively. This is in good agreement with the maximal growth rates found in pure cultures in the chemostat (Table 1). The increase in viable counts after the depletion of glucose (i.e., between 10 and 25 h) must be ascribed to the growth on fermentation products (ethanol and acetate). The formation of these products in aerobic cultures is a characteristic property of *S. cerevisiae* growing in the presence of excess glucose and is known as the Crabtree effect (31, 32).

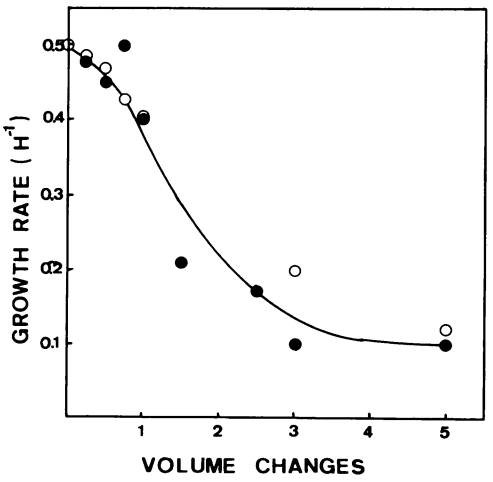

FIG. 2. Growth rate of *C. utilis* CBS 621 during glucose-limited competition with *S. cerevisiae* CBS 8066 as a function of the number of volume changes. Symbols: ●, growth rate as determined from the tangent of the line of Fig. 1B (corrected for washout); ○, growth rate calculated with the Monod equation (Table 1) and the residual substrate concentration in the fermentor (Fig. 1A).

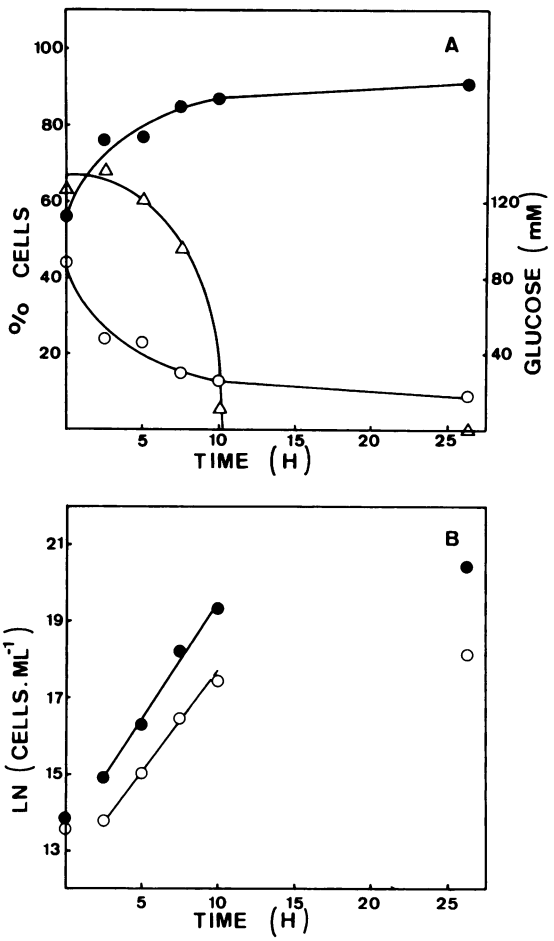

FIG. 3. (A) Competition between *C. utilis* (●) and *S. cerevisiae* (○) in pH-controlled aerobic batch cultures. The glucose concentration (△) was 23 g liter^{-1} at the start of the experiment. At zero time, equal amounts (dry weight) of cells from two exponentially growing batch cultures of the two yeasts were added to the fermentor. Viable counts of both yeasts are expressed as the percentage of the total cell population. (B) Cell numbers of *C. utilis* (●) and *S. cerevisiae* (○) during the competition experiment as a function of time.

Affinity constants of *S. cerevisiae* and *C. utilis* for glucose. As pointed out above, the low K_s of *C. utilis* may allow the organism to successfully compete with *S. cerevisiae* under conditions of glucose limitation. In order to gain further insight into the basis for this difference in K_s of the two organisms, their kinetics for glucose transport were studied. As reported previously, both *C. utilis* and *S. cerevisiae* may contain two glucose uptake systems (21, 22), the kinetic constants of which are listed in Table 3. The lower-affinity constants in *C. utilis* are in line with the low K_s of this organism for growth on glucose compared with that of *S. cerevisiae*. Also, the affinity constant for glucose oxidation, as determined with the biological oxygen monitor, was lower for *C. utilis* than for *S. cerevisiae* under glucose-limited growth conditions (Table 3). The affinity constant for glucose oxidation of *C. utilis* increased when the organism was grown under glucose excess. The affinity constant of *S. cerevisiae* was virtually independent of the conditions applied (Table 3).

Effect of oxygen on the competition between *S. cerevisiae* and *C. utilis* in glucose-limited cultures. In view of the competitive advantage of *C. utilis* over *S. cerevisiae* under both glucose limitation (Fig. 1) and glucose excess (Fig. 3), it was decided to study the effect of oxygen on the competition. Both *C. utilis* and *S. cerevisiae* are facultatively fermentative yeasts (31) that can thrive under conditions of low oxygen supply by performing alcoholic fermentation of sugars. *S. cerevisiae*, however, grows much faster under anaerobic conditions than *C. utilis* (unpublished results). Therefore, oxygen limitation could be a favorable condition for *S. cerevisiae* in its competition with *C. utilis* for glucose.

Separately, both yeasts could be grown under a dual limitation of glucose and oxygen at a dilution rate of 0.10 h^{-1}. This situation was achieved by stopping the air supply to cultures that had previously been growing aerobically at the same dilution rate. This resulted in a lower biomass yield and concurrent formation of ethanol (Table 2). When such cultures were mixed in a 1:1 ratio, *C. utilis* still dominated *S. cerevisiae* at a dilution rate of 0.10 h^{-1} (results not shown). However, when oxygen-free nitrogen gas was sparged through the culture, *S. cerevisiae* became the dominant species (Fig. 4). These anaerobic conditions also led to the formation of glycerol, which is required to maintain a closed redox balance (12, 15, 31).

DISCUSSION

Regulation of transport kinetics. In numerous studies it has been reported that yeasts adapt their sugar transport systems to the environmental sugar concentration. When a batch culture enters the stationary phase, carriers with a better affinity constant are synthesized (21, 28–30, 35). This has definite ecological implications, since cells that are better adapted to scavenge low nutrient concentrations from the environment have a competitive advantage. Crabtree-negative yeasts seem to be exemplary of organisms that survive in environments with a low nutrient supply. Their isolation is easily achieved from soil, sewage, and lakes (14, 27). In contrast, *S. cerevisiae*, a representative of the Crabtree-positive yeasts, is mainly isolated from sugar-rich environments, such as flowers and fruits (18, 25). Yeasts with a high affinity for sugars (i.e., a low K_s) are a potential threat to the production of bakers' yeast, which has a low affinity for sugars (i.e., a high K_s). It is relevant in this respect that the strain of *C. utilis* CBS 621 used in this study was originally isolated from pressed bakers' yeast (11). This type of infection has been known for a long time.

The maximal capacities (V_{max}) and affinity constants (K_m) for glucose transport of the yeasts used in this study seem to be regulated according to the growth rate and the residual glucose concentration during glucose-limited growth (21, 22). This implies that transport of glucose over the plasma membrane might be an important factor in determining the physiology of the yeast under these conditions. Relating transport characteristics to catabolism or even overall cell physiology is, however, a difficult enterprise (5). For instance, the relationships among the maximal capacities of glucose transport, glucose oxidation rate, and growth rate (Table 3) are unclear. The maximal glucose transport capacity observed with *C. utilis* is as high as 300 μmol g of dry weight^{-1} min^{-1} (21), and this would allow for a growth rate of 1.65 h^{-1} on the basis of the equation, $q_{glucose} = D \cdot Y^{-1}$ (19). This value is much higher than the observed maximal growth rate (Table 1). The same argument holds for *S. cerevisiae*. To interrelate the maximal oxidation rates of

TABLE 3. Kinetic constants for glucose utilization with respect to transport, growth, and oxidation capacity of *S. cerevisiae* CBS 8066 and *C. utilis* CBS 621 in batch and continuous cultures

Organism and culture type	Kinetic constant values for:					
	Glucose transport		Oxidation capacity		Growth	
	K_m (mM)	V_{max} (μmol g dry weight^{-1} min^{-1})	K_m (mM)	V_{max} (μmol of O_2 g dry weight^{-1} min^{-1})	K_s (mM)	μ_{max} (h^{-1})
S. cerevisiae						
Glucose limited (D = 0.10 h^{-1})	1	200	0.22	70	0.11	0.49
	20	160				
Batch culture (exponential phase)	1[a]	100[a]	0.34	30		
	20[a]	360[a]				
C. utilis						
Glucose limited (D = 0.10 h^{-1})	0.025	140	0.025	80	0.015	0.59
	0.19	90				
Batch culture (exponential phase)	2[b]	230[b]	0.68	110		

[a] Kinetic parameters determined with cells from chemostat cultures growing at D = 0.46 h^{-1}.
[b] Kinetic parameters determined with cells from chemostat cultures growing at D = 0.57 h^{-1}.

yeasts with their growth rates is also an impossible task, as yeasts can support growth by oxidation as well as by fermentation. Under fermentative conditions, several enzymes related to oxidation are repressed (3, 20, 37) (Table 3).

The transport of the sugar, however, determines to some extent the affinity constant for growth of a yeast. A comparative study with eight yeast species has revealed a correlation between the presence of proton/glucose symporters and the apparent residual substrate concentration during glucose-limited growth (32a). Proton/glucose symporters have affinity constants of 20 to 200 μM and establish residual substrate concentrations of 5 to 35 μM in the fermentor at D = 0.10 h^{-1}. In contrast, yeasts that transport glucose via carrier-mediated diffusion have transport affinity constants of 1 to 20 mM, leading to residual substrate concentrations in the fermentor of 100 to 160 μM at the same dilution rate (32a). It can therefore be postulated that yeasts with proton/glucose symporters will dominate yeasts which transport glucose by carrier-mediated diffusion under glucose-limited conditions.

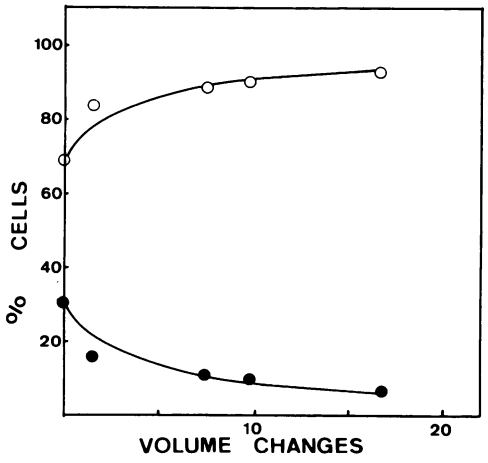

FIG. 4. Competition between *C. utilis* (●) and *S. cerevisiae* (○) under anaerobic glucose-limited conditions. At zero time, 20% (dry weight) *Candida* cells were added to the *Saccharomyces* culture. Viable counts of both yeasts are expressed as the percentage of the total cell population.

In order to investigate in more detail the relationship between sugar transport and the physiology of the yeasts, the kinetics of glucose oxidation were determined. If the kinetics of transport and catabolism are connected, the affinity constant for glucose oxidation should correlate with the affinity constant for transport. The affinity constant for glucose oxidation of *C. utilis* decreased when conditions were shifted from glucose excess to glucose limitation from a K_m of 680 ± 140 μM to a K_m of 25 ± 5 μM (Table 3). The affinity constant for glucose oxidation of *S. cerevisiae* did not vary significantly with the conditions applied. Under glucose excess, a K_m of 340 ± 70 μM was found, and under glucose limitation a K_m of 220 ± 50 μM was found. These findings are in line with the observation that induction of carriers with lower affinity constants occurs during glucose-limited conditions in *C. utilis* (Table 3). In contrast, the two glucose carriers of *S. cerevisiae* appear to be present at all growth conditions (4) (Table 3).

Competition for glucose. The results of the competition experiments (Fig. 1 and 3) are in good agreement with the kinetic parameters for growth, glucose transport, and glucose oxidation. It can be calculated that during aerobic glucose-limited competition (Fig. 1), after only 7.5 h enough *Candida* cells will be present for complete consumption of the glucose supplied to the culture. From this time onwards no glucose is left for the *Saccharomyces* cells, which, as a consequence, are washed out (Fig. 1B).

The determination of viable counts of *S. cerevisiae* during these experiments is less precise than for *C. utilis* because of the method used (see Materials and Methods). Especially when the ratio of *S. cerevisiae* to *C. utilis* cells is low, the determination of the cell number and hence of the actual growth rate of *S. cerevisiae* becomes inaccurate (Fig. 1B). The growth rate of *C. utilis* can be determined more accurately from the viable counts on the basis of direct estimation of CFU on xylose agar. By using this procedure, it was calculated that the growth rate of *C. utilis* during the competition was below its μ_{max} (Fig. 1B). This is to be

expected in view of the limiting glucose concentrations during the competition (Fig. 1A). The growth rates of *C. utilis* calculated with the Monod equation (Fig. 2) are in good agreement with the growth rates determined from viable counts, indicating that determination of viable counts may be a reliable method to assess growth rates. This is further supported by the fact that the growth rates of the two yeasts as determined on the basis of viable counts in a mixed batch culture correlated well with the values obtained via dry-weight measurements of the pure cultures.

Much attention has been paid to the production of killer toxins in yeasts (10, 16, 17). However, this phenomenon is probably irrelevant for the competition studies presented here. *C. utilis* and *S. cerevisiae* attained the same μ_{max} in mixed-batch cultures as in pure cultures. Under glucose excess in batch cultures, μ_{max} is the main determinant for the outcome of the competition as long as the Monod equation is applicable ($K_s \ll s$). However, under glucose limitation ($K_s \geq s$), the affinity constant for growth becomes decisive as well. Our results indicate that under the latter conditions the kinetics of the transport process are an important factor in the result of the competition.

S. cerevisiae dominated *C. utilis* only under anaerobic conditions. In our experimental setup, the entrance of oxygen into the culture could not be completely excluded because of the presence of trace amounts of oxygen in the nitrogen gas that was sparged through the culture, among other reasons. It is well known that, for growth in mineral media, yeasts require trace amounts of oxygen for synthesis of sterols and unsaturated fatty acids (1, 2, 13). *S. cerevisiae* is apparently better adapted to conditions of severe oxygen limitation than *C. utilis* (Fig. 4). Whether the outcome of the competition under these conditions was caused by differences in μ_{max}, K_s, or both has not been investigated. It is clear, however, that conditions may be defined for *S. cerevisiae* under which the organism has a competitive advantage. Unfortunately, these conditions are not compatible with its commercial production. Aerobic sugar-limited fed-batch cultivation, the common practice in the production of bakers' yeast, may be regarded as an artificial ecological situation for *S. cerevisiae*.

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