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Production of Bakers' Yeast in Cheese Whey Ultrafiltrate†

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A process for the production of bakers' yeast in whey ultrafiltrate (WU) is described. Lactose in WU was converted to lactic acid and galactose by fermentation. *Streptococcus thermophilus* was selected for this purpose. Preculturing of *S. thermophilus* in skim milk considerably reduced its lag. Lactic fermentation in 2.3×-concentrated WU was delayed compared with that in unconcentrated whey, and fermentation could not be completed within 60 h. The growth rate of bakers' yeast in fermented WU differed among strains. The rate of galactose utilization was similar for all strains, but differences in lactic acid utilization occurred. Optimal pH ranges for galactose and lactic acid utilization were 5.5 to 6.0 and 5.0 to 5.5, respectively. The addition of 4 g of corn steep liquor per liter to fermented WU increased cell yields. Two sources of nitrogen were available for growth of *Saccharomyces cerevisiae*: amino acids (corn steep liquor) and ammonium (added during the lactic acid fermentation). Ammonium was mostly assimilated during growth on lactic acid. This process could permit the substitution of molasses by WU for the industrial production of bakers' yeast.

The comprehensive review of Moulin and Galzy (22) on the use of whey as a potential substrate for biotechnology described over 10 processes in which yeasts are used. Although most of these processes aimed at producing a protein-enriched product to be used as food or feed yeasts, there has been a growing interest in the past 20 years in producing active microbial biomasses such as lactic acid starters (29) and bakers' yeast (9). Thus, whey utilization has been studied by many research teams, and industrial productions have been attempted (38).

Since *Saccharomyces cerevisiae* cannot utilize lactose as a carbon source (4), various approaches have been suggested: conversion of lactose to glucose and galactose by free (24) and immobilized (20) enzymes or by chemical hydrolysis (22), conversion of lactose to lactic acid (9), and genetic manipulation of *S. cerevisiae* to permit it to directly use lactose as a carbon substrate (18).

Of all these approaches, lactose conversion to lactic acid was used since it presents the highest industrial potential. Indeed, it requires a single prefermentation step with organisms (lactic acid bacteria) that are widely used in the food industry, which is not yet the case for genetically manipulated yeasts (39). Moreover, lactic acid fermentations are low-cost technologies that have been well mastered by the dairy industry; this is not the case for free enzymes, which are still more expensive than lactic acid bacteria, or for immobilized enzyme systems, which require relatively large investments.

S. cerevisiae can grow well on lactic acid as the sole carbon source (11). However, high concentrations of this substrate are likely to retard or inhibit growth (9, 16). Moreover, yeasts that are obtained after growth on nonfermentable substrates such as ethanol (28) or lactic acid (11), which are obtained from the fermentation of streptococci or lactobacilli, showed inferior fermentative activity compared with that of yeasts grown on glucose. This can be prevented by selecting a lactic acid culture that does not ferment galactose. Lactic acid fermentation from whey has been widely studied (21). However, in all these studies, lactose was completely converted to lactic acid.

The purpose of this study was to convert the lactose of cheese whey ultrafiltrate (WU) to lactic acid and galactose for the production of bakers' yeast and to evaluate the growth and physiology of yeasts cultured on this fermented WU (FWU).

MATERIALS AND METHODS

Microorganisms. *Streptococcus thermophilus* VVR-81 was obtained from P. Dion (Department of Plant Science, Université Laval), while *S. thermophilus* FLY-4 was isolated from a commercial yogurt inoculum (Yalacta, France). Both microorganisms were propagated on APT agar (Difco Laboratories, Detroit, Mich.) at 37°C for 24 h, stored at 4°C, and transferred monthly.

Lactobacillus helveticus 2217 (formerly *Lactobacillus bulgaricus* 2217) was obtained from the Department of Microbiology and Public Health, Michigan State University (East Lansing, Mich.). The culture was propagated in sterile (121°C, 10 min) skim milk medium (SM), stored at 4°C, and transferred every second week. *L. bulgaricus* R#21, R#26, and R#46 were provided by Rosell Institute (Montreal, Quebec, Canada).

Four strains of *S. cerevisiae* were investigated: $CB_2$47 and $CB_2$329 were obtained from Fleischmann (Ville LaSalle, Quebec, Canada), L-1 was from a commercial sample of fresh yeasts (Lallemand Inc., Montreal, Quebec, Canada) and VVR-215 was from the culture collection of the Food Science Department of Université Laval. These cultures were transferred monthly on potato dextrose agar (Difco) and kept at 4°C.

Media. (i) SM. SM was prepared by dissolving 10 g of skim milk powder into 100 ml of distilled water and autoclaving it at 121°C for 10 min.

(ii) Cheese whey. WU was prepared by ultrafiltering fresh cheddar cheese whey through Romicon cartridges (HF15-43-PM10) and was frozen at −35°C until use.

(iii) Concentrated whey. WU was lyophilized and rehydrated in a reduced volume of distilled water in order to

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obtain a 10% (wt/vol) lactose concentration. This corresponded to a 2.3× concentration factor.

(iv) Corn steep liquor. Corn steep liquor (CSL) concentrate (St. Lawrence Starch, Montreal, Quebec, Canada) was diluted to 20% solids and clarified by batch centrifugation (20,000 × *g*, 10 min) before it was mixed with WU. Thus, only soluble and colloidal CSL solids were used in this study.

Inoculum preparation. Lactic acid cultures were prepared either in SM or WU supplemented with 0.5% (vol/vol) CSL (WU-CSL). The latter was adjusted to pH 6.4 (3 N NH_4OH) prior to membrane filtration (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.). SM and WU-CSL were inoculated at a rate of 2% (vol/vol) with test organisms and incubated for 24 h at 37°C prior to fermentations.

In biomass production studies, WU that was prefermented with *S. thermophilus* (FWU) was used. Yeast inocula were prepared in shaken flasks (300 rpm, gyratory shaker) at 30°C. The first incubation lasted 96 h, and then 1 ml of the culture was transferred to 99 ml of fresh FWU. This second culture was incubated for 48 h under the same conditions.

For respiratory studies, cells were prepared in a WU medium composed of 70 ml of WU, 2.5 ml of 20% DL-lactic acid solution, 3.66 g of $(NH_4)_2SO_4$, and 22.5 ml of distilled water. In some cases the inoculum was prepared in supplemented WU which consisted of 68 ml of WU, 2 ml of a 20% solution of CSL, 2.5 ml of a 20% lactic acid solution, 3.66 g of $(NH_4)_2SO_4$, and 22.5 ml of distilled water. These media were adjusted to pH 4.5 with concentrated H_2SO_4 and filter sterilized (pore size, 0.22 μm; Millipore) prior to the fermentation. Cells were incubated at 24°C for 72 h (but for only 48 h when CSL was added) in 500-ml bottom-baffled flasks (Bellco Glass, Inc., Vineland, N.J.) on an agitator (300 rpm; G-10; New Brunswick Scientific Co., Inc., Edison, N.J.). Cells were then centrifuged at 5,000 × *g* (10 min) and suspended in sterile saline water (0.86%). This suspension was incubated for 16 h in the absence of substrate (starvation) under the same conditions described above (24°C, 300 rpm).

Fermentations. In lactic fermentations, 20 ml of inoculum was added to 380 ml of WU-CSL. Fermentations were conducted in a fermentor (C-30 Bio-Flo; New Brunswick Scientific Co.). Depending on the strain that was used, the temperature was maintained at 40 or 44°C; the pH was automatically controlled at 5.5 (model TTT-11; Radiometer, Copenhagen, Denmark) by adding 3 N NH_4OH; mixing (100 rpm) was maintained throughout the fermentation without air injection. Samples for analysis were withdrawn periodically.

In yeast biomass production, a 30-ml inoculum was added to 370 ml of FWU. Fermentations were conducted in a reactor (C-30 Bio-Flo; New Brunswick Scientific Co.) at 30°C, 700 rpm, and 660 cm^3 of air per min. The pH was maintained at set values (between 5.5 and 6.0) by the continuous addition of 3 N NaOH or 3 N H_2SO_4 by using a pH controller (Radiometer model TTT-11). Samples were withdrawn periodically and frozen (−10°C) for further analyses.

Respiration studies. For respiration studies, cells were suspended into two types of media. Medium A contained WU supplemented with 5.2% $(NH_4)_2SO_4$, and medium B was medium A with 0.5% CSL. Concentrated H_2SO_4 was used to adjust the pH to 4.5.

Some assays were conducted in a 0.15 M K_2HPO_4 buffer (pH 4.5) containing 2.5% lactic acid or 1.8% galactose.

Manometric studies were performed in a respirometer (Gilson) by the procedure described by Umbreit et al. (41). The temperature was maintained at 29 ± 1°C, and flasks were agitated at 120 rpm (4-cm displacement). We allowed 30 min for temperature stabilization, once the flasks were immersed, and 5 min for CO_2 absorption (0.2 ml of a 10% KOH solution was in the central well). The cell suspension located in the side arm of the flask was then added to the substrate.

Analyses. Total carbohydrates were estimated colorimetrically by the anthrone method described by Dion et al. (9) or by the method of Teles et al. (37). Lactose and galactose were estimated enzymatically (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Lactic acid was measured by the method of Lawrence (17).

The dry weight was determined with fresh samples of biomass that were washed twice with distilled water and dried at 85°C for 24 h. Ammonia was determined on diluted samples (ca. 2,000-fold) with a continuous flow analytical system (Autoanalyzer II; Technicon Instruments Corp., Tarrytown, N.Y.) by method 98-70 W/A.

RESULTS AND DISCUSSION

Lactic acid fermentation. Various growth stimulants may be added to cheese whey and WU in order to improve their nutritional value for lactic acid bacteria. CSL is well suited for this purpose, and the recommendation of Dion et al. (9) of 0.5% CSL supplementation was used in this study. The addition of up to 0.5% CSL stimulated the growth of *S. thermophilus* FLY-4. However, higher concentrations failed to improve growth or acid production (data not shown).

The composition of CSL is described in detail in Table 1. Its beneficial effect on growth is most likely related to its amino acid (1), vitamin (32), and trace element (26) composition and content. On the other hand, some minerals (26) and amino acids (23) can be inhibitory to *S. thermophilus*, which could partially explain why additions of CSL at more than 0.5% failed to improve growth.

Acid production rates in WU-CSL varied considerably from strain to strain (Fig. 1). Strain FLY-4 was the fastest acid-producing strain. Strains VVR-81, R#21, and 2217 produced acid at similar rates, while strains R#26 and R#43 were the slowest acid producers. Major fermentation products were also strain dependent. Lactose was completely converted to lactic acid by *S. thermophilus* VVR-81 and *L. helveticus* 2217, while *S. thermophilus* FLY-4 fermented WU-CSL and had a reduced lactate content (2.5%) but a relatively high galactose content (1.8%). The ability of *S. thermophilus* to ferment lactose to lactic acid and galactose has been reported previously (25).

The medium that was used for propagating the culture had a considerable influence on the acid production by the strains when they were transferred to WU-CSL (Fig. 2). Preculturing of *S. thermophilus* FLY-4 in SM considerably reduced its lag phase in WU-CSL. However, neither its rate of acid production nor its final yield differed. Since the proteolytic activity of *S. thermophilus* is rather limited (21), protein utilization alone cannot explain this behavior. Therefore, a reduced lag time for SM-produced inocula is likely to be related to the buffering capacity of milk proteins. The buffering slows the drop in pH, thus allowing prolonged growth and a larger microbial cell population. Better growth performance of lactic bacteria in buffered medium has been reported by Somkuti and Gyuricsek-Damert (33). FLY-4 inocula, when propagated under pH control in WU-CSL, also showed a reduced lag phase in agitated fermentors.

TABLE 1. Minor constituents of CSL[a]

Constituent	Amt
Amino acids[b] % of CSL (dry wt basis)	
Glycine	2.1
Valine	2.2
Leucine	3.9
Isoleucine	1.3
Methionine	1.0
Cysteine	1.5
Tyrosine	1.0
Aspartic acid	2.7
Glutamic acid	6.5
Lysine	1.6
Histidine	1.3
Vitamins and trace minerals ppm (dry wt basis)[c]	
Biotin	0.1
Choline	5,600
Folic acid	0.5
Inositol	5,000
Niacin	160
Pantothenic acid	25
Pyridoxine	20
Riboflavin	10
Thiamine	5
Boron	30
Copper	25
Iron	300
Molybdenum	2
Selenium	0.7
Zinc	175

[a] Data were adapted from Corn Products North America (5).
[b] Crude protein content of CSL is 47% (dry weight basis).
[c] The metric equivalent of parts per million is micrograms per gram.

Indeed, when FLY-4 was propagated under pH control in WU-CSL, the addition of NH_4OH began after only 3 h of fermentation compared with 5 h in a fermentation in which the inoculum was not prepared under pH control. Similar behavior has been reported by Gilliland (13) with lactic streptococci, which gave much higher cell densities (~15 times) with pH control than without it.

Both the acid production rate and the final acidity were increased by preculturing *L. helveticus* 2217 inocula in SM

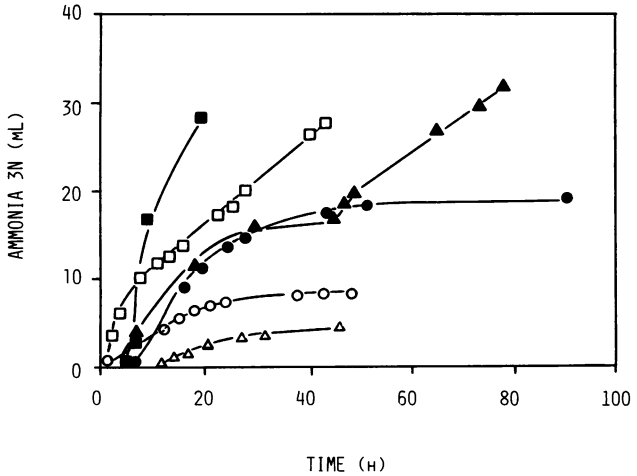

FIG. 1. Comparison of acidification rates of *L. bulgaricus* R#43 (○), R#26 (△), and R#21 (●); *L. helveticus* 2217 (□); and *S. thermophilus* VVR-81 (▲) and FLY-4 (■) in WU-CSL at 40°C.

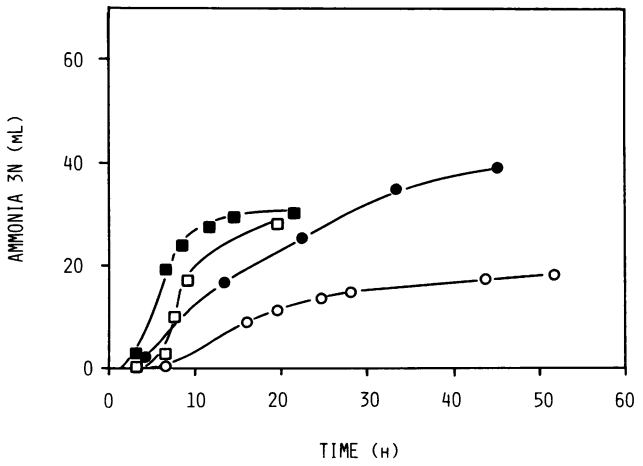

FIG. 2. Effect of media used for inoculum production on subsequent fermentation activity of *L. helveticus* 2217 and *S. thermophilus* FLY-4 in WU-CSL at 40°C. Symbols: ○, *L. helveticus* inoculum prepared on WU-CSL; ●, *L. helveticus* inoculum prepared on skim milk; □, *S. thermophilus* inoculum prepared on WU-CSL; ■, *S. thermophilus* inoculum prepared on skim milk.

(Fig. 2). The buffering capacity of milk proteins is also likely to be involved in this phenomenon.

WU contains approximately 6% solids. Yeasts can grow well on substrates with much higher substrate levels. Concentrated wheys in which lactose was brought to a 10% level were used for lactic fermentations. Acid production by *S. thermophilus* FLY-4 in 2.3×-concentrated whey was delayed by 2 h compared with that by *S. thermophilus* FLY-4 in nonconcentrated whey. Moreover, fermentation could not be completed within 60 h in this medium. The use of concentrated whey resulted in an increased lag time and reduced end product yield, although the rate of acid production remained practically unchanged. Stieber et al. (35) have reported that utilization of concentrated whey can increase the fermentation rate of *L. helveticus*. The stimulation of lactate dehydrogenase under high lactose concentrations (8, 12) could have been negated by the inhibitory effect of the high phosphate content (14) of the concentrated WU or high lactate level resulting from fermentation (27).

The lactic acid yield in WU-CSL was 95% for *L. helveticus*, which compared favorably with the data from Roy et al. (30). The yields of galactose and lactic acid with *S. thermophilus* FLY-4 were slightly inferior (92%) to those with *L. helveticus* but were higher than those reported by Dion et al. (9).

In converting lactose into suitable substrates for the possible production of bakers' yeast, *S. thermophilus* appears to be the organism of choice for many reasons. Its rate of lactose conversion is similar to that of lactobacilli, its fermentation products are essentially lactic acid and galactose, and it produces less lactic acid from the same initial concentration of lactose than do lactobacilli. The last two aspects are particularly important for bakers' yeast production, since lactic acid is somewhat inhibitory to yeast respiration at high concentrations (Fig. 3) and galactose should help maintain an active glycolytic pathway for rapid dough fermentation in bread-making. Indeed, it has been shown that cells that are cultured on nonfermentable substrates, such as lactic acid, have reduced levels of glycolytic enzymes, and when grown on galactose they can multiply these levels by 100 times (19). Therefore, *S. thermophilus* FLY-4

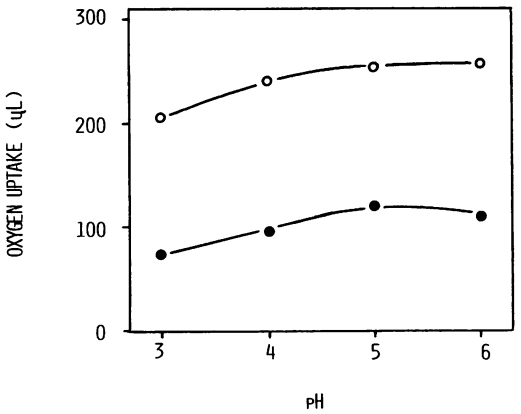

FIG. 3. Effect of pH and lactic acid concentration on respiratory activity of *S. cerevisiae* $CB_2$47 in medium A [WU + 5.2% $(NH_4)_2SO_4$]. Symbols: ○, 0.5% lactic acid added to medium A; ●, 5.0% lactic acid added to medium A.

was chosen to preferment the WU-CSL for biomass production of bakers' yeast.

Influence of bakers' yeast strain. The growth performance of bakers' yeast on FWU varied from strain to strain (Fig. 4). Very little difference in growth rates was noticed during the first 10 h of fermentation; this corresponded to galactose utilization (Fig. 5). The variation in growth mainly occurred in the later stage of the fermentation. Stains $CB_2$47 and VVR-215 showed the fastest growth rates, while strain L-1 performed about half as well after incubation for 30 h (Fig. 4). Since no major differences in growth occurred initially (0 to 10 h), it can be assumed that nutritional deficiencies were not responsible for the observed variations in the growth profiles of the various strains. The different growth ratios observed for various strains could be related to substrate utilization. Thus, while galactose respiration was similar for all strains tested, lactic acid was metabolized at a slightly lower rate (−10%) by three strains and at a much lower rate (−50%) by one industrial strain (Table 2). These results explain why growth on galactose was faster than that on lactic acid and why strain-related variations in growth rates were found.

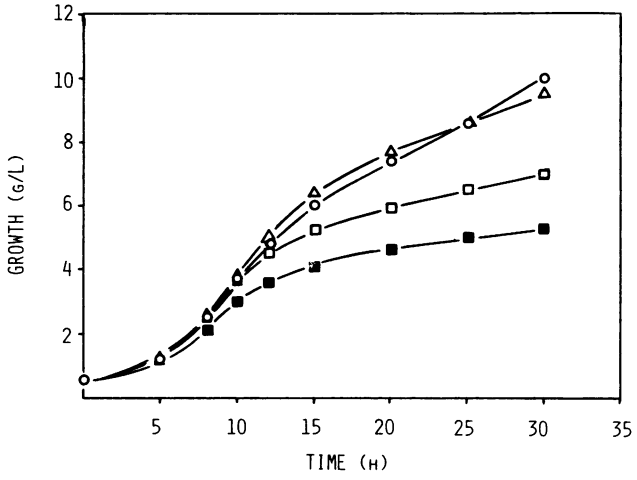

FIG. 4. Growth curves for $CB_2$47 (○), VVR-215 (△), $CB_2$329 (□), and L-1 (■) strains of bakers' yeast in FWU.

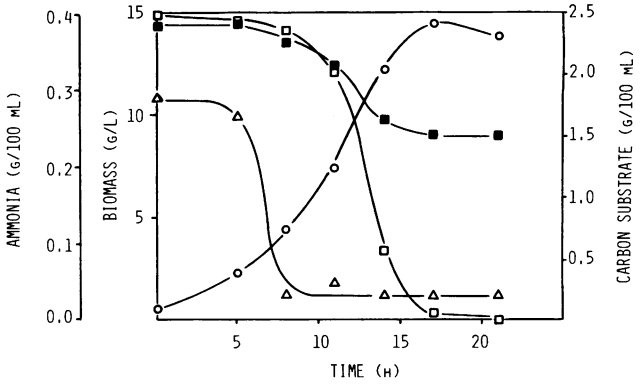

FIG. 5. Growth (○) and substrate utilization curves for galactose (△), lactic acid (□), and ammonia (■) for bakers' yeast strain VVR-215 in FWU.

Although *S. cerevisiae* was cultivated in the presence of two substrates, diauxic growth was not observed (Fig. 5). Galactose was assimilated much more rapidly than lactate was; however, lactate utilization was concomitant with galactose utilization. Therefore, total repression of lactic acid assimilation did not occur in the presence of galactose. Under such conditions, complete fermentation was obtained within 18 h with strain VVR-215.

Effect of pH on yeast growth and respiration. Lactic acid respiration in the pH range of 5.0 to 6.0 was higher than that at lower pHs (Fig. 3). The unfavorable effect of a high lactic acid concentration was amplified by low pHs. Variations in pH modified the lactic acid-lactate ratio. Lactate and lactic acid are not assimilated under the same mechanisms (3, 11, 31), and pH changes modify the substrate levels. Changes in the lactate-lactic acid ratio might also influence the inhibitory effect of the organic acid on lactate dehydrogenases (11) and inositol metabolism (10, 15).

Accordingly, growth rates in FWU and, consequently, biomass yields were affected by the pH (Fig. 6). The pH level had little effect on biomass levels during the first half of the fermentation, when growth mostly occurred on galactose, but showed a strong influence in the second half, when growth mostly occurred on lactic acid.

Effect of CSL supplementation on yeast growth and respiration. The addition of CSL (at up to 4 g/liter) to FWU increased the biomass yield by 15% compared with that of unsupplemented FWU. However, further CSL supplementation (10 g/liter) was not beneficial to the yield, and higher additions (15 g/liter) were detrimental. The addition of CSL stimulated respiration, and this beneficial effect occurred only after 2 h of incubation (Fig. 7). Since the generation

TABLE 2. Galactose and lactic acid respiration by various strains of *S. cerevisiae*

Medium[a]	Respiration by the following strains[b]:			
	$CB_2$47	$CB_2$329	VVR-215	L-1
Buffer containing 2.5% lactic acid	95	87	95	52
Buffer containing 1.8% galactose	107	113	106	111

[a] The buffer was 0.15 M KH_2PO_4.

[b] Respiration is given in units of microliters of O_2 per milligram (dry weight) after 1 h of incubation.

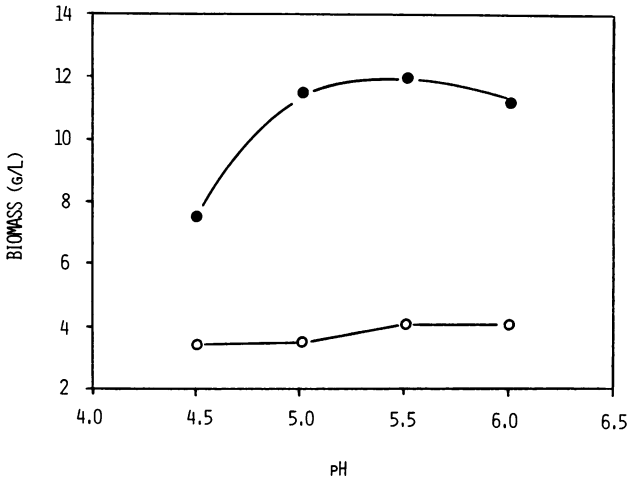

FIG. 6. Effect of pH on the production of biomass after 10 h (○) and 20 h (●) of growth in FWU.

time was about 2 h, this stimulation could also have been related to cell division. Thus, cells that have grown for one generation on CSL would have superior respiratory activity. Indeed, cells grown in the presence of CSL showed higher respiration rates. CSL contains various amino acids (Table 1) which increase Y_{ATP} (biomass yield per mole of ATP produced) (36) and, consequently, biomass (16). Although the amino acids that were found in CSL were probably responsible for the greater part of the increased biomass yield, other constituents like lactic acid (20% [wt/wt]) might also be involved. Amino acids appear to be partially responsible for the stimulatory effect of CSL, since the addition of vitaminfree Casamino Acids (Difco) stimulated respiration by all strains. Thus, the respiration rate of strain L-1 was increased by 63%, while it was increased by between 34 and 37% for the three remaining strains. Stimulation of *S. cerevisiae* respiration by amino acids has already been demonstrated by Tustanoff and Bartley (40). Heating of CSL reduced its stimulatory activity (Table 3). Therefore, substances other than amino acids are responsible for CSL stimulation of respiration. CSL contains phosphates and vitamins which can stimulate the growth rate and increase yields (2, 36).

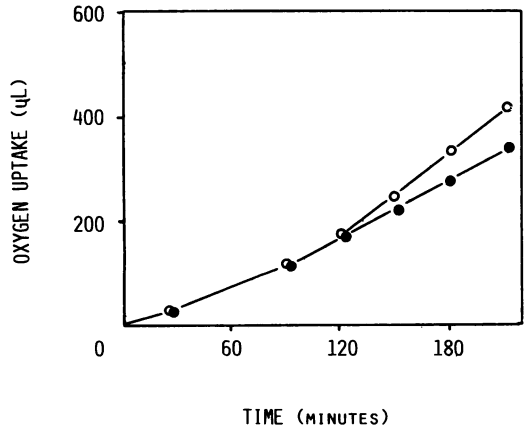

FIG. 7. Effect of supplementation of WU with 0.5% CSL on respiratory activity of *S. cerevisiae* $CB_2$47. Symbols: ●, medium A [WU + 5.2% $(NH_4)_2SO_4$]; ○, medium B (medium A + 0.5% CSL).

TABLE 3. Effect of heating CSL on its stimulation of respiration[a]

Strain	Respiration stimulation (%) by:	
	Unheated CSL	Autoclaved CSL
$CB_2$329	74	54
$CB_2$47	90	59
VVR-215	73	54
L-1	345	36

[a] Inocula were prepared on WU. Experimental conditions were 0.5 ml of cell suspension, 0.75 ml of water, and 1.75 ml of medium A or medium B.

High levels of CSL supplementation were inhibitory. The inhibitory effect of high concentrations of CSL has been reported for lactic acid bacteria (6) but has not yet been reported for bakers' yeast.

Ammonium utilization by yeasts. NH_4^+ was not completely assimilated by *S. cerevisiae* (Fig. 5). This might be related to the fact that CSL provides a good source of nitrogen. Although NH_4^+ is often preferentially assimilated, *S. cerevisiae* occasionally reduces its NH_4^+ assimilation in the presence of amino acids (2). The addition, of 4 g of CSL per liter reduced NH_4^+ assimilation, thus corroborating this hypothesis. However, greater CSL additions did not further reduce the level of NH_4^+ assimilation, suggesting that CSL does not markedly affect NH_4^+ uptake at high supplementation levels. It appears that the 0.4 N:C ratio of FWU is more than double the nitrogen needs of yeasts (7). This excess of ammonia could be reduced during FWU production by using NaOH instead of NH_4OH as a neutralizer in the final stage of the lactic acid fermentation.

Ammonium uptake occurred mostly toward the end of the fermentation. We found that very little NH_4^+ was assimilated during growth on galactose. There was, however, a direct correlation between lactic acid absorption and NH_4^+ uptake (Fig. 5). Our results suggest that *S. cerevisiae* readily utilizes amino acids during growth on galactose, but switches to NH_4^+ when it is grown on lactic acid. Cassio et al. (3) have reported that *S. cerevisiae*, when grown in lactic acid medium, transports lactate by an accumulative electroneutral proton-lactate symport with a proton lactate stoichiometry of 1:1. Our results suggest that in FWU, ammonium is assimilated in a similar manner. However, the ratio of lactic acid assimilation to that of ammonium was 1:0.4.

Conclusion. Since high levels of lactic acid inhibit bakers' yeast respiration, it is preferable not to convert lactose to lactic acid completely. Partial conversion to galactose has many advantages. (i) Galactose respiration is faster than that of lactic acid, which suggests that a faster growth rate would occur on galactose. (ii) There is much less variation in galactose respiration than in lactic acid respiration between various strains, which could translate to a more uniform growth of various industrial strains. (iii) Incremental feeding (fed batch) would not be necessary, since lactic acid inhibition would be reduced and galactose, unlike sucrose (2), would not produce a Crabtree effect (7, 34). (iv) Growth on a carbohydrate substrate would increase the level of glycolytic enzymes (19); this would prevent the low fermentative activity observed when yeast cells are grown on nonfermentable substrates such as lactic acid (11, 28).

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