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Production of Fuel Alcohol from Wheat by VHG Technology

Effect of Sugar Concentration and Fermentation Temperature

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ABSTRACT

Very high gravity (VHG) wheat mashes containing more than 300 g of dissolved solids per liter were prepared and fermented with active dry yeast at 20, 25, 30, and 35°C with and without yeast extract as nutrient supplement. At 20°C, mashes with 38% (w/v) dissolved solids end-fermented without any nutrient supplementation and maximum ethanol yields of 23.8% (v/v) were obtained. With increasing temperatures, the sugar consumption decreased. Addition of yeast extract stimulated the rate of fermentation at all temperatures, but did not increase the total amount of sugar consumed. The stimulatory effect of yeast extract on cell multiplication decreased with increasing sugar concentration, and virtually no difference in cell number was observed between yeast extract-supplemented and unsupplemented mashes at sugar concentrations above 33% (w/v). The fermentative capacity of the yeast (expressed as maximum specific rate of sugar consumption) remained the same at all sugar concentrations in unsupplemented mashes, but decreased in yeast extract-supplemented mashes at sugar concentrations below 33% (w/v). When the sugar concentration was above 33% sugar (w/v), the fermentative capacity in yeast extract-supplemented mashes was greater than that observed in unsupplemented samples.

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Index Entries: Fuel alcohol; very high gravity fermentation technology; VHG; fermentation temperature; fermentative capacity; yeast cell viability.

INTRODUCTION

Application of very high gravity (VHG) technology has considerable potential for the manufacture of fuel alcohol (*1*). VHG technology was originally defined in a brewing context to describe the use of worts that had 18 g or more of dissolved solids/100 g. In fuel alcohol production from grains such as corn and wheat, it is customary to use mashes that contain 20–22 g of dissolved solids/100 g. In this industry, this mash concentration is considered to be "normal gravity." We therefore define VHG technology for fuel alcohol production as the preparation and fermentation to completion of mashes containing 27 g or more of dissolved solids/100 g of mash. This concentration corresponds to 300 g of dissolved solids/L of mash.

In a previous communication, we reported that ethanol yields as high as 21.5% (v/v) can be obtained through fermentation of VHG wheat mashes (*2*). To obtain such high yields of ethanol within a reasonable time, wheat mashes were supplemented with yeast extract or were inoculated at very high pitching rates (cell numbers/mL) with active dry yeast and fermented at 20°C. Under industrial conditions, however, fuel alcohol is produced at temperatures in the range of 30–35°C (*3*). Temperatures in this range are preferred because the metabolic activities of the yeast are maximal, and this normally results in the completion of fermentation in a shorter time and cooling requirement may be less. High temperatures, however, have their disadvantages. The amount of carbohydrate that can be fermented to ethanol at elevated temperatures is limited because of deleterious osmotic effects coupled with an increased toxicity of the higher level of ethanol to yeast growth and metabolism (*4–7*). A large body of information is available on the relationship between temperature of fermentation and ethanol tolerance (for reviews, *see 8–10*).

Sugars in excess of 250 g/L are rarely used in industrial fermentations because of the difficulty in producing mashes containing higher concentrations of sugar and because sugars at such high concentrations cannot be fermented to completion at 30–32°C. In this article, we show that at 20°C, VHG wheat mashes containing dissolved solids as high as 38% (w/v) can be fermented to completion with a final yield of ethanol of 23.8% (v/v). However, the amount of sugar that is consumed by the yeast decreases with increasing temperature, even though 18% (v/v) ethanol can still be produced at 30°C from a wheat mash that initially contained 38 g of dissolved solids/100 mL.

MATERIALS AND METHODS

Materials

Active dry yeast, High-T™ (high-temperature α-amylase) and Allcoholase II™ (glucoamylase) were supplied by Alltech Biotechnology Center, Nicholasville, KY. Chemicals required for ethanol determinations and amino acids were purchased from Sigma Chemical Company, St. Louis, MO. All other chemicals were obtained through local suppliers and were of reagent grade. Yeast extract (type AYE 2200) was purchased from Gillette Foods Inc., Union, NJ. Wheat samples were obtained from a local supplier.

Grinding and Mashing of Wheat

Wheat was ground with a plate grinder (Type KT-30, Falling Number AB, Stockholm, Sweden) at setting number 2. Eighty-five percent of the ground wheat had a particle size distribution between 12 and 100 mesh, whereas the rest of the flour was finer than 100 mesh size. This ground wheat was gelatinized and liquefied as described previously (*11*). This mash could be saccharified without any further treatment and then pitched (inoculated) with active dry yeast, or the majority of particulate matter from the mash could be removed by straining through a stainless-steel food strainer (20 mesh) prior to saccharification. Removal of the particulate material facilitates easy stirring; this is especially important when the dissolved solid content of the mash is very high. The water-soluble portion of the mash contained 19–20 g dissolved solids (mostly sugars and dextrins)/100 mL. When the dissolved solid content of the mash was to be raised to VHG levels, freeze-dried "wheat hydrolysate" (wheat mash treated with HT™ α-amylase and clarified) (*11*) was added to the mash.

Fermentation

In these experiments, 500 g samples of wheat mash were transferred to sterile jacketed Celstir bioreactors (Wheaton Scientific, Millville, NJ) that were subsequently connected to a D3-G water bath circulator (Haake Inc., Saddle Brook, NJ) set at 30°C. To each bioreactor, 1 mL of Allcoholase II was added to saccharify the dextrins to fermentable sugars. Thirty minutes after the addition of the enzyme, the temperature of each bioreactor was shifted to the predetermined fermentation temperature. Temperatures of 15, 20, 25, 30, and 35°C were studied. Wheat mash was then inoculated with active dry yeast to give an approximate initial number of 10^6 cells/g of mash/°Plato (where 1 °Plato = 1 g of dissolved solids—measured as sucrose—per 100 g of the supernatant portion of the mash).

Determination of Dissolved Solids

The mash was centrifuged at 10,300*g* for 15 min and the specific gravity of clear supernatant was determined at 20°C with a digital density meter (DMA-45, Anton Paar KG, Graz, Austria). With the aid of appropriate tables, the specific gravities were converted to grams of dissolved solids (expressed as grams of sucrose)/100 mL. In some cases, dissolved solids were determined directly by drying aliquots (usually 5 mL) of supernatants at 105°C for 3 h. When the sugar concentration was >5 g/100 mL, supernatants were diluted with distilled water five-fold or more before drying. Total cell counts and viable cell counts were determined by the direct microscopic method described previously (*11*).

In experiments where changes in yeast cell dry weight were to be determined, wheat mashes were clarified by centrifugation (10,300*g* for 15 min) before saccharification and inoculation. For cell dry weight determination, 20-mL samples withdrawn at regular intervals were centrifuged (10,300*g* for 15 min), and the yeast pellets washed twice with ice-cold water, resuspended in distilled water, and made up to 5 mL. Four milliliters of this suspension were transferred to preweighed aluminum pans and dried at 105°C to constant weight.

Determination of Ethanol, Glycerol, and Sugars

The free amino nitrogen (FAN) in supernatant liquid was determined by the ninhydrin method of the European Brewery Convention (*12*). Ethanol was measured enzymatically by the alcohol dehydrogenase assay as reported previously (*11*). Known concentrations of ethanol were used as standards. Ethanol, glycerol, and sugars were also measured by high-performance liquid chromatography (HPLC). Clear supernatant liquids obtained by centrifugation at 10,300*g* for 15 min were diluted with distilled water, and 5 μl of this were injected into a FAM-PAK™ column (Waters Chromatographic Division, Milford, MA) maintained at 65°C. This column separates sugars, organic acids, and alcohols. These components were eluted from the column with HPLC-grade water containing orthophosphoric acid at a concentration of 1.5 m*M*. The separated components were detected and quantified with a differential refractometer (Model 410, Waters Chromatographic Division, Milford, MA). The elution rate was 1 mL/min, and methanol was used as internal standard.

RESULTS

Effect of Sugar Concentration on Fermentation

In order to study the relationship between sugar concentration and the time required to complete fermentation, a number of wheat mashes containing increasing amounts of dissolved solids were prepared and fer-

Table 1
Effect of the Initial Dissolved Solids Concentration of Wheat Mashes on Ethanolic Fermentation by *S. cerevisiae* at 20°C with and without 1% (w/w) Yeast Extract as Nutrient Supplement

Initial dissolved solids, g/100 mL	Nutrient supplement	Final dissolved solids, g/100 mL	Fermentation time, h	Ethanol yield, %, v/v	Glycerol yield, %, w/v
19.8	None	1.8 (9.0)[a]	100	14.0	0.9
20.0	YE	2.1 (10.7)	48	11.3	1.2
25.0	None	2.5 (9.8)	130	17.2	1.2
25.5	YE	2.6 (10.3)	50	16.8	0.8
30.7	None	3.8 (12.3)	168	19.7	1.0
32.0	YE	3.5 (11.1)	72	19.3	0.9
37.0	None	5.0 (13.6)	230	22.3	1.1
37.9	YE	5.1 (13.4)	130	23.8	1.4
42.6	None	20.1 (47.2)	Stuck	16.7	1.4
43.6	YE	18.8 (43.1)	Stuck	15.5	1.4

[a]Figures in parentheses represent dissolved solids remaining unused by the yeast, expressed as a precentage of dissolved solids initially present in the wheat mashes.

mented at 20°C with and without yeast extract as a nutrient supplement. The concentration of dissolved solids in these mashes ranged from 19.8 to 43.6 g/100 mL of supernatant (Table 1).

Fermentation progress was followed routinely by measuring the specific gravity of the supernatant portion of mash. This method, however, has the drawback that it progressively underestimates the dissolved solids content as the concentration of ethanol in the mash increases. Therefore, completion of fermentation was monitored by noting the cessation of alcohol production, or by determining by HPLC the complete disappearance of glucose and maltose from the mash. All the sugars from these mashes were taken up, except when the mash initially contained dissolved solids in excess of 40 g/100 mL (Table 1). In this case, the fermentation "stuck," and a large proportion of usable dissolved solids remained unused. Addition of yeast extract to this mash did not improve either the rate of fermentation or the total amount of sugar consumed. At lower sugar concentrations, yeast extract did stimulate yeast growth and allowed complete conversion of the sugar to ethanol in a much shorter time than in the unsupplemented controls.

All the dissolved solids in the wheat mash were not utilizable by the yeast. In fact 9–14 g/100 mL of dissolved solids remained after ethanol production had ceased. Some of these materials are residual solubles from the mash; and others are products of yeast metabolism. For example, a small amount of glycerol was produced (Table 1), and this would be estimated as "dissolved solid" by the dry weight method. The amounts of

glycerol produced in these fermentations are about the same as reported by Panchal and Stewart (*5*) for a brewing yeast strain fermenting under osmotic stress.

An ethanol yield of 23.8% (v/v) was obtained in 130 h from yeast extract-supplemented wheat mash, which initially contained 37.9% (w/v) dissolved solids. In contrast, 230 h were required for the complete utilization of sugars in unsupplemented mash, and the ethanol yield in this case was slightly less.

Effect of Yeast Extract on Cell Multiplication

It is clear that the extent of cell multiplication decreased as the sugar concentration (dissolved solids content) of the mash increased (Fig. 1). At dissolved solids concentrations below 32 g/100 mL, stimulation by yeast extract of fermentation appeared to be mediated through increased yeast growth. At higher sugar concentrations, yeast extract continued to stimulate the rate of fermentation, but its effect on cell growth and multiplication appeared to diminish progressively. At sugar concentrations of 37–38 g/100 mL, virtually no differences were observed in the degree of cell multiplication between mashes with and without yeast extract.

Fermentation of VHG Mashes at Different Temperatures

Wheat mashes containing 37–38% (w/v) dissolved solids were prepared and fermented at 20, 25, 30, and 35°C with and without 2% (w/w) yeast extract as a nutrient supplement. At 20°C and in the absence of added yeast extract, 92% of the dissolved solids were consumed by the yeast in 8 d (Fig. 2A). In this sample, an additional 1.5 d were required for the complete disappearance of the sugars. In the presence of yeast extract, 96% of the dissolved solids were utilized by the yeast in 6 d (Fig. 2B). At 25°C, the initial rate of fermentation was faster in yeast extract-supplemented samples, but the total time taken to complete the fermentation was not appreciably different from that observed with the unsupplemented mash. At 30 and 35°C, fermentations became sluggish and stuck. About 8–9 g of dissolved solids (determined by the specific gravity method) /100 mL remained unutilized at 30°C after 72 h, whereas at 35°C, fermentation ceased at 48 h with 11 g/100 mL unused. One interesting and important observation is that supplementing wheat mashes with yeast extract did not affect the rates of fermentation at 30 and 35°C appreciably, although it did slightly improve the final yields of ethanol. This is in contrast to the observation that yeast extract had a significant positive effect on the rate of fermentation at 20°C.

It is known that ethanolic fermentation by *Saccharomyces cerevisiae* is tightly coupled to yeast growth (*13*). A rapid fermentation resulting in the consumption of a greater amount of sugar than usual should correspond to the production of a greater amount of biomass. Since the presence

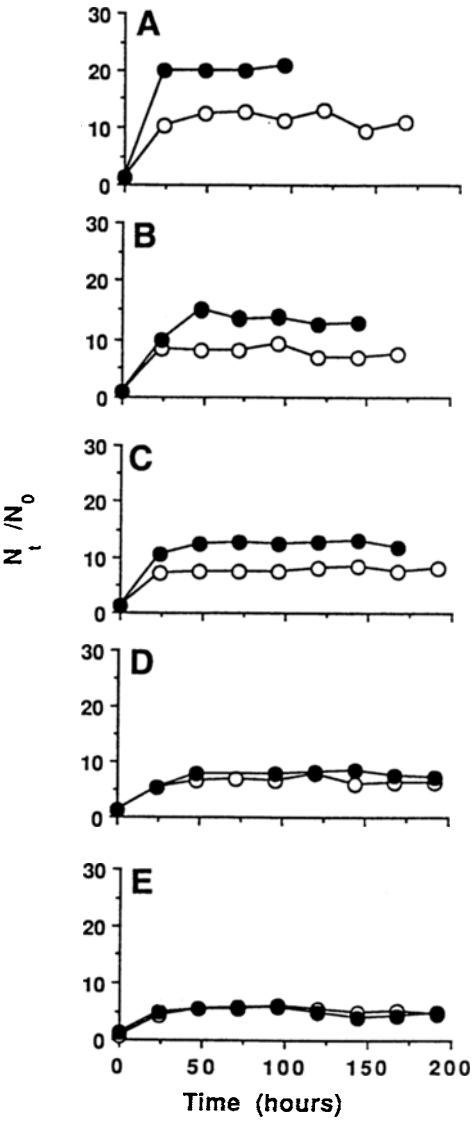

Fig. 1. Degree of yeast cell multiplication during fermentation at 20°C in unsupplemented (○) wheat mashes or following supplementation with 1% (w/w) yeast extract (●). Wheat mashes initially contained 19.8 (**A**), 25.0 (**B**), 30.7 (**C**), 37.0 (**D**), or 42.6 (**E**) g of dissolved solids/100 mL. N_t is the cell number at time t; N_0 is the cell number at zero time.

of large amounts of particulate matter in the wheat mash prevented direct determination of biomass by the dry weight method, cell number and percent viability were followed microscopically. Once again, the maximum cell numbers attained at these sugar concentrations in yeast extract-supplemented samples were not appreciably different from those observed in unsupplemented controls, although in all cases, the fermentations

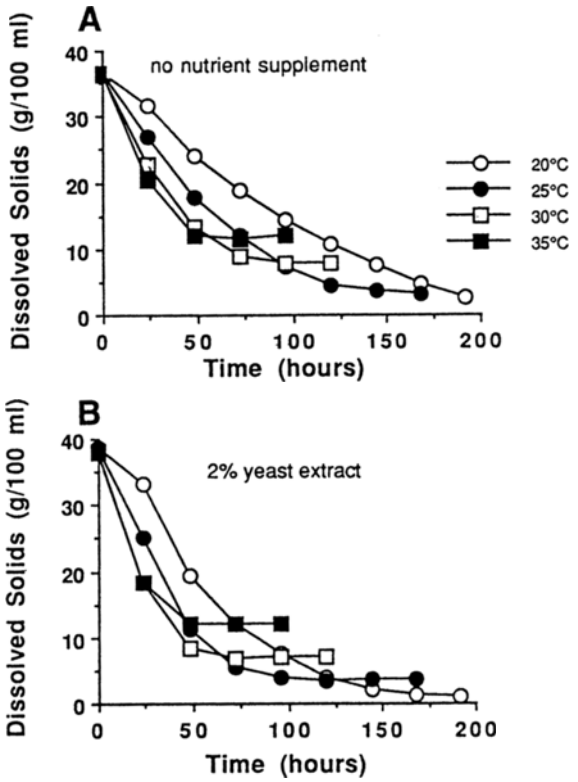

Fig. 2. Effect of temperature on the rate of dissolved solids' utilization by active dry yeast in unsupplemented VHG wheat mashes (**A**) or following supplementation with 2% (w/w) yeast extract (**B**).

completed or ceased much earlier than in the corresponding controls (Table 2, Fig. 2).

Viability of Yeast Cells

While exploring the causes for the apparent lack of effect of yeast extract on the rate of fermentation at 30 and 35°C, we observed that addition of yeast extract to the mash reduced the viability of the cell population. At all temperatures, there was a greater loss of cell viability during the later stages of fermentation in yeast extract-supplemented mashes than in the unsupplemented controls (Fig. 3). With increasing temperature, the loss of viability increased. At 30 and 35°C, nearly 99% of the yeast cells in mashes supplemented with yeast extract had lost viability by 96 h. At 25°C, the same phenomenon occurred, but required 168 h. The loss of viability at 20°C was only partial, and even after 192 h, 40% of the cells remained viable in the mash supplemented with yeast extract. In the absence of nutrient supplement, the loss of viability was less pronounced at 20°C than at the higher temperatures.

Table 2
Effect of Temperature on the Time Required
to Attain Maximum Cell Number during Fermentation
of VHG Wheat Mash in the Presence or Absence of 2% (w/w) Yeast Extract

Fermentation temperature,	Fermentation time, h		Maximum cell number, $\times 10^8$/g of mash	
°C	−YE	+YE	−YE	+YE
20	96	96	1.59	1.67
25	72	48	1.47	1.16
30	72	24	1.34	1.06
35	72	24	1.19	0.95

Change in FAN Levels

The lack of effect of added yeast extract on fermentation at 30 and 35°C may be only apparent and not real. At these temperatures, the substantial number of yeast cells that lost viability (even in the absence of added yeast extract) had lysed and released nutrients into the medium. Thus, the yeast extract-supplemented and unsupplemented mashes may have sufficient amounts of nutrients available to stimulate the fermentation. Estimation of free amino nitrogen showed that after an initial decline, there was an increase in the FAN values at 30 and 35°C (Fig. 4). At 25°C, the increase was only slight, whereas at 20°C, there was no apparent increase. At all temperatures, the amount of FAN released after the initial uptake was greater in the yeast extract-supplemented samples than in the corresponding controls.

The yield of ethanol at each fermentation temperature corresponded to the amount of dissolved solids consumed. Wheat mashes supplemented with yeast extract yielded slightly more ethanol than unsupplemented controls at 25, 30, and 35°C (Table 3). There is a possibility that the amount of sugar that can be fermented may be even greater at temperatures below 20°C. Fermentation of VHG wheat mashes at very low temperatures, however, offered some practical difficulties. For example, the viscosities of VHG mashes were considerably higher at 15°C than at 20°C, and the mashes offered great resistance to stirring. There is a possibility, however, that greater amounts of sugar can be fermented at 15°C than at 20°C. To answer this question, a number of wheat mashes (20–22 g dissolved solids/100 mL) were prepared and fermented at 15 and 20°C with and without 2% yeast extract as nutrient supplement. Use of VHG mashes was avoided for the reasons stated above.

The rates of fermentation were, as was to be expected, slower at 15°C than at 20°C (Fig. 5A). At 20°C, fermentation was complete by 120 h,

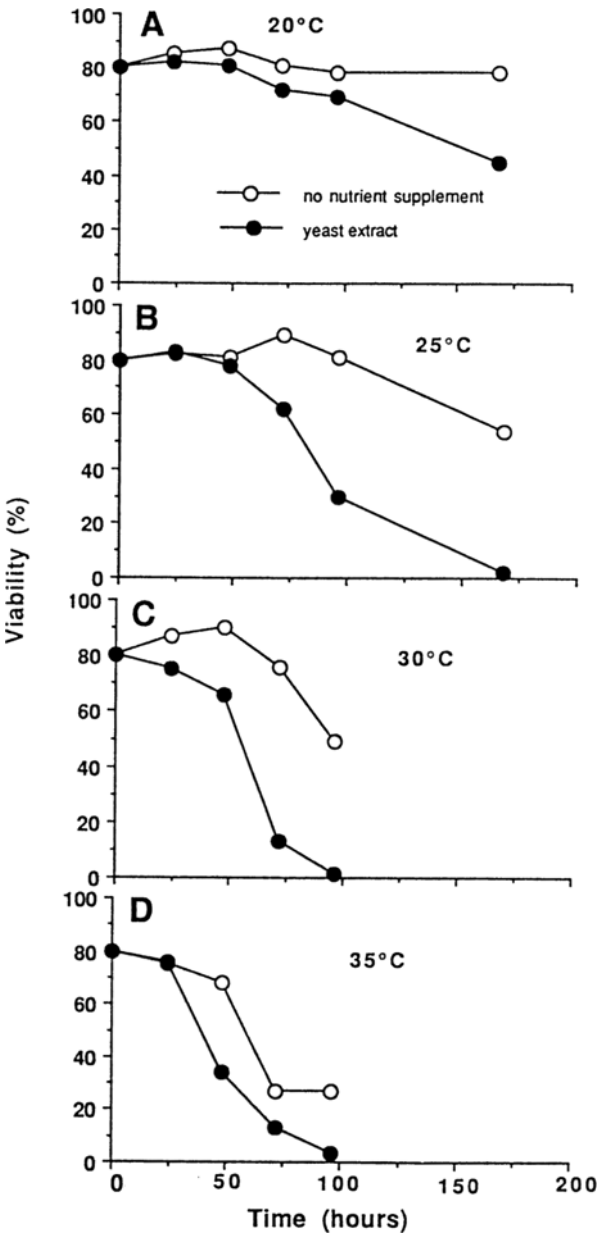

Fig. 3. Effect of temperature on viability of the yeast cell population (determined microscopically) during fermentation of unsupplemented VHG wheat mashes (○) or following supplmentation with 2% (w/w) yeast extract (●).

whereas at 15°C, 192 h were required. At both temperatures, fermentation was faster when the mash was supplemented with yeast extract—96 and 48 h at 15°C and 20°C, respectively. In unsupplemented mashes, the rate of cell multiplication was faster at 20°C than at 15°C, although the maximum cell number attained was the same at both temperatures (Fig. 5B).

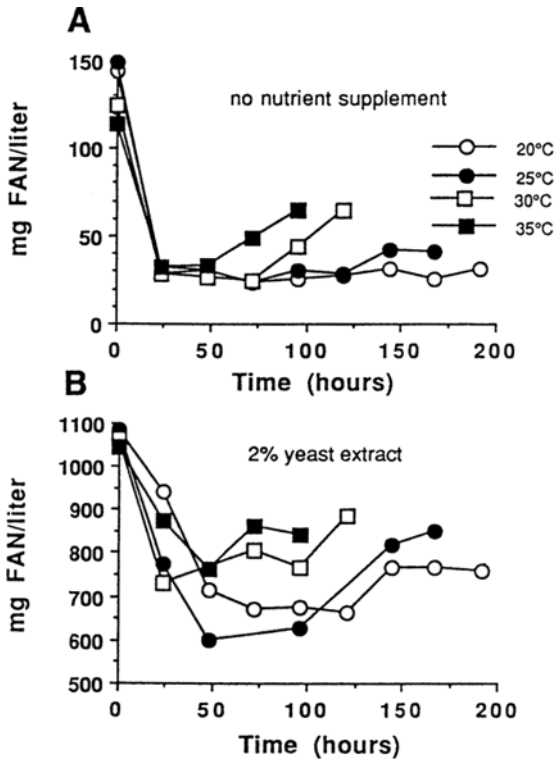

Fig. 4. Effect of temperature on the utilization of FAN during the fermentation of VHG wheat mashes remaining unsupplemented (**A**) or supplemented with 2% (w/w) yeast extract (**B**).

Table 3
Effect of Fermentation Temperature on the Carbohydrate Consumed and Ethanol Produced by *S. cerevisiae* from VHG Wheat Mash in the Presence or Absence of 2% (w/w) Yeast Extract

Fermentation temperature,	Maximum sugar consumed, %, w/v		Final ethanol yield, %, v/v	
°C	−YE	+YE	−YE	+YE
20	34.1[a]	37.5	19.7	22.4
25	33.6	34.8	19.6	20.6
30	28.6	31.2	16.7	18.2
35	24.7	25.8	14.4	15.1

[a]Results observed at 192 h. Complete removal of fermentable sugars required an additional 24–36 h.

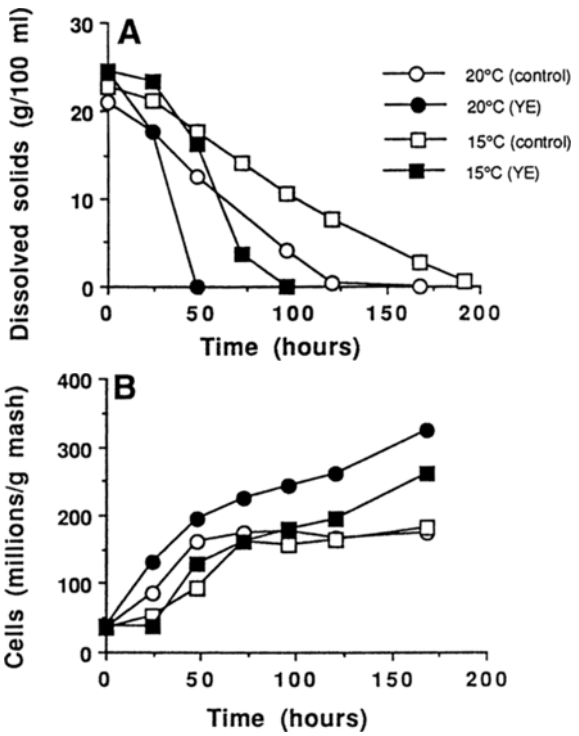

Fig. 5. Rate of fermentation (**A**) and yeast cell multiplication (**B**) during the fermentation of "normal gravity" wheat mashes at 15 and 20°C supplemented with or without 2% (w/w) yeast extract.

More cells were produced in yeast extract-supplemented samples at both temperatures than in the corresponding controls.

DISCUSSION

Ethanol yields as high as 23.8% (v/v) can be realized at 20°C from fermentation of VHG wheat mashes with *S. cerevisiae*. The maximum amount of dissolved solids that can be used at this temperature appears to be close to 38 g/100 mL. This value is considerably higher than that reported in the literature. Casey et al. (*14*), for example, reported that the highest amount of dissolved solids that could be end-fermented under brewing conditions was 31.9 g/100 mL. Panchal and Stewart (*5*) showed that only 21 g of sugar could be used by a brewing yeast at 21°C from a medium that initially contained 30% (w/v) sucrose. In the present experiments, we did not observe "stuck" fermentation until the dissolved solids concentration in the mash was raised above 40 g/100 mL.

The ethanol yields obtained in this study suggest that some of the soluble carbohydrates present in the wheat mash were di-, tri-, and higher oligosaccharides. It appears that Allcoholase II (glucoamylase), which

was added 30 min prior to inoculation, had not converted all the dextrins to monosaccharides by the time the first samples were collected from the bioreactors. This might explain why the ethanol yield in some cases exceeded the theoretical maximum if one assumed that all the sugars initially present in the mash were monosaccharides. It is known that greater amounts of ethanol can be obtained per unit weight of disaccharides and other oligosaccharides (after hydrolysis) than from an equal weight of glucose (*15*). Glucoamylase continued to catalyze the conversion of carbohydrates into fermentable sugars during the course of fermentation. Analysis of sugars by HPLC showed that all initial mash samples contained significant amounts of maltose and, in some cases, higher oligosaccharides (data not shown).

Fermentation is stimulated by the addition of yeast extract to VHG wheat mash. However, the mechanism by which this occurs appears to depend on the experimental conditions. At 20 °C and at a dissolved solids concentration that did not exceed 35 g/100 mL, stimulation by yeast extract seemed to be mediated through increased growth of the yeast. This observation is in general agreement with the previously reported effect of nutrient supplementation on ethanolic fermentation (*14,16–18*). Under these conditions, the fermentative capacity of the yeast (g of sugar consumed/g yeast/h) declined slightly compared to unsupplemented controls. This is in agreement with the report that the "fermentative power" of the yeast in unsupplemented wort was equal to or greater than that of the yeasts in supplemented worts (*14*). When the concentration of dissolved solids in the mash was raised above 35 g/100 mL, exogenously added yeast extract once again stimulated the rate of fermentation, but this stimulation was not mediated through increased biomass production, but instead by increasing the fermentative capacity of the yeast cells. This suggests that ethanol yield per cell under this condition has increased. A similar observation was made by O'Connor-Cox et al. (*15*) while studying fermentation of very high gravity wort supplemented with yeast extract. At the high sugar concentrations used in the present study, the stimulatory effect of yeast extract on the fermentative capacity of yeast cells was evident at all fermentation temperatures investigated. These results are summarized in Fig. 6. Here the maximum specific rates of sugar uptake, which in all cases occurred within the first 48 h, are plotted against initial dissolved solids concentration of the mash.

The degree of cell multiplication decreased with increasing sugar concentrations both in the presence and absence of yeast extract. This suggests that under osmotic stress, the metabolism of the yeast leading to cell division is altered or occurs at a considerably reduced rate. This is in contrast to the fact that the specific rate of sugar consumption by the yeast was unaffected with increasing sugar concentrations in unsupplemented mashes (Fig. 6). Other workers have also shown that high sugar concentrations retard the growth of *S. cerevisiae* and decrease the rate and the extent of fermentation (*4–6*).

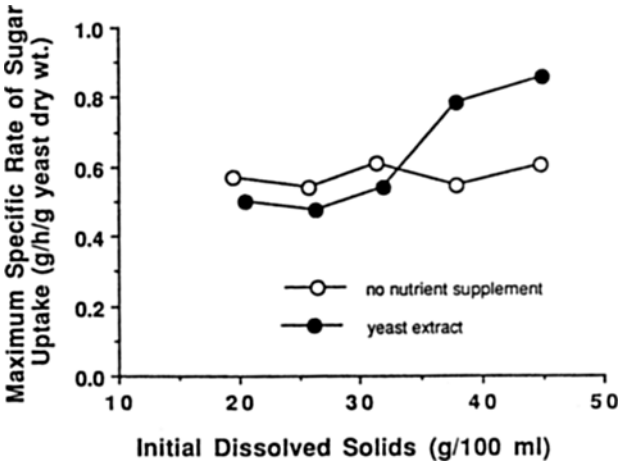

Fig. 6. Maximum specific rates of sugar consumption by active dry yeast during the fermentation of VHG wheat mashes containing 19.8–43.6 g of dissolved solids/100 mL.

The reason for the greater loss of cell viability in the presence of added yeast extract may be a combination of several things. First, the yeast cells were exposed to high concentrations of ethanol for longer periods of time, since the ethanol production in these samples proceeded at a faster rate and was completed sooner than in unsupplemented controls. This along with osmotic stress exerted by the unfermented sugars might have increased the loss of viability of yeast cells. The toxic effect of ethanol on yeast was further enhanced at higher growth temperatures. It is known that increasing the sugar concentration (osmotic stress) decreases the growth rate and increases the loss of cell viability as the amount of ethanol in the fermentation medium increases. Thus, Odumeru et al. (*19*) observed a decrease in growth rate and an increase in loss of cell viability of yeast fermenting in a 25°P wort when the ethanol concentration approached 11% by volume. The loss of viability is also known to be a function of growth temperature and ethanol concentration (*7,10,20*). The optimum temperature of growth has been shown to suffer an approximate 12°C decrease, from about 37°C in the absence of ethanol to about 25°C in the presence of 6% (w/v) ethanol (*20*). None of the above reasonings, however, fully explain the drastic decrease in cell viability observed in the yeast extract-supplemented mashes in our study. For example, despite the fact that the extent of substrate depletion and product accumulation was about the same, the loss of cell viability at 20°C was greater in yeast extract-supplemented mashes than in unsupplemented controls. It appears that the cells did not lose viability as a result of the exhaustion of the energy source, which in these mashes was fermentable carbohydrate(s). At 30 and 35°C, fermentation had ceased long before the exhaustion of carbohydrates. Apparently yeast extract stimulated the loss of

cell viability, and this stimulatory effect was further enhanced with increasing temperature. Available information also suggests that certain components of yeast extract, especially the amino acid lysine, may cause yeast cells to lose viability under some growth conditions (*21*). The greater loss of cell viability at 25, 30, and 35 °C than at 20 °C may be related to the availability of nutrients released through the lysis of cells.

In conclusion, the amount of sugar in a VHG wheat mash that can be fermented at temperatures above 20 °C appears to be independent of nutrient supplementation, although addition of yeast extract allowed completion of the process in less time. We confirmed this observation with three types of active dry yeast, all of which showed similar patterns of substrate utilization and product accumulation. However, the number of new cells formed at a given sugar concentration varied with different batches of yeast. Currently, the reasons for the excessive loss of viability of yeast cells in the presence of added yeast extract is under investigation. According to the work reported by Nagodawithana and Steinkraus (*22*), there appears to exist a relationship between the rate of fermentation and loss of viability of yeast cells in high sugar-containing media. The same authors observed that treatments, such as oxygenation of media, which reduced the loss of cell viability, also reduced the rate of fermentation.

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