Ethanolic Fermentation of Blackstrap Molasses and Sugarcane Juice Using Very High Gravity Technology

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The fermentability of blackstrap sugarcane molasses was examined under very high gravity (VHG) conditions. Molasses fermentations were carried out over the range of 10.4–47.6% (w/v) dissolved solids. As the concentration of dissolved solids increased, the percentage of sugar actually converted to ethanol decreased. The suitability of molasses as a carbohydrate adjunct for VHG ethanolic fermentation was also studied; molasses was used to raise the dissolved solids content of both clarified wheat mash base and sugarcane juice to VHG levels. Fermentation of such mashes was 90–93% efficient. In VHG wheat mashes prepared with molasses adjunct, yeast extract accelerated the rate of fermentation but had little effect on the final ethanol concentration. Sugarcane juice was not limiting in assimilable nitrogen since yeast extract or urea failed to stimulate the rate of fermentation of cane juice/molasses worts or to increase the final ethanol concentration achieved. This is the first report of the application of VHG technology to fermentation substrates other than wheat, wort, and grape juice. It is concluded that VHG fermentation of saccharine substrates could lead to moderate increases in alcohol concentration as compared to those presently achieved in industry.

INTRODUCTION

Several renewable substrates are under investigation as feedstocks for bioconversion to fuel alcohol. In temperate climates, grain starch is the basic raw material for fermentation ethanol due to its availability and low cost. In tropical countries such as Brazil, the main sources of fermentation ethanol are sugarcane juice, molasses, and, to a certain extent, starches derived from tuberous crops such as cassava. Currently in Brazil, sugarcane worts of 16–20 °Brix are routinely fermented to produce 7.5–10.0% (v/v) ethanol (Laluce, 1991). In general, the ethanolic fermentation of these saccharine materials is much simpler than the fermentation of grain starch (Hodge and Hildebrandt, 1954; Maiorella, 1985). Consequently, few technological improvements have been forthcoming in bioethanol production from molasses and other sugar substrates (Wayman and Parekh, 1990; Laluce, 1991). Process improvements are needed, however, to increase the productivity and cost effectiveness of all ethanol production plants. Very high gravity (VHG) fermentation technology is one such process improvement. It aims to increase the final ethanol concentration and reduce production costs. Industrial implementation of this technology will allow increased concentrations of ethanol to be produced using the same physical plant, the same labor, and nearly the same services and capital equipment (Ingledew, 1993).

For fuel ethanol production, VHG fermentation technology is the preparation of media containing 300 g or more of dissolved solids/L and its fermentation to completion (Thomas *et al.*, 1993). Laboratory-scale studies with wheat mashes showed that complete fermentation of as much as 38% (w/v) dissolved solids is possible with an ethanol yield approaching 23% (v/v) (Thomas *et al.*, 1993). Thus, ethanol concentrations heretofore not considered possible by fermentation on an industrial scale (Nagodawithana, 1986) can be achieved by the application of VHG technology. Although more fuel alcohol is produced from sugarcane sources than from any other material (Wayman and Parekh, 1990), information is not available concerning the application of VHG technology to produce higher concentrations of fermentation ethanol from substrates other than wheat. In the present study, we report the application of VHG technology to the ethanolic fermentation of blackstrap molasses and to sugarcane juice or wheat mash to which molasses was added as a carbohydrate adjunct.

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MATERIALS AND METHODS

Fermentation Worts. Fermentations were conducted using wheat mash, molasses, and sugarcane juice as substrates. Clarified wheat mash containing 20-21 g of dissolved solids/100 mL was prepared as described previously (Thomas and Ingledew, 1990). When the dissolved solids content of a wheat mash was to be raised to VHG levels, freeze-dried wheat hydrolysate was added to the mash (Thomas and Ingledew, 1990). Blackstrap sugarcane molasses was obtained from Westway Trading Corp. (St. Paul, MN). It was diluted with distilled water to yield 10.4–47.6 g of dissolved solids/100 mL and fermented at 25 °C. Alternatively, it was used as an adjunct to raise the dissolved solids content of clarified wheat mash to VHG levels. Sugarcane stalks were purchased from a local store, and the juice was extracted using a Carver press (Fred S. Carver Inc., Menomonee Falls, WI). The juice was pasteurized at 71.7 °C for 15 s in an Armfield Laboratory FT 43B pasteurizer (Armfield Ltd., Hampshire, U.K.). This cane juice contained 22.4 g of dissolved solids/100 mL, and either it was fermented directly or its dissolved solids content was raised to VHG levels by supplementation with molasses or wheat hydrolysate and then fermented. Relevant analytical data for these fermentation substrates are summarized in Table 1.

Fermentation. Fermentations were conducted in 500-mL jacketed Celstir bioreactors (Wheaton Scientific, Millville, NJ). These bioreactors had a working volume of 500 mL and a headspace of about 250 mL. The bioreactors containing either 10 mL of distilled water or 10-mL solutions of various nutrient supplements were sterilized by autoclaving at 121 °C for 15 min. Urea solutions were sterilized by membrane filtration and added to the fermentors aseptically. Five hundred gram quantities of mash were transferred aseptically to each sterile fermentor and

0021-8561/94/1442-1242$04.50/0

Table 1. Comparison of Fermentation Substrates

constituent[a]	substrate: wheat mash	molasses	cane juice
total dissolved solids	21.2	76.6	22.4
fermentable sugars	18.0	57.0	22.4
sucrose	0.34	33.4	21.9
fructose	ND[b]	13.0	0.29
glucose	17.1	10.0	0.20
maltose	0.043	0.11	0.029
maltotriose	0.46	0.43	ND
isomaltose	0.016	0.020	ND
total nitrogen	0.072	0.36	0.12
free amino nitrogen	0.0079	0.10	0.034
ash	0.29	9.9	0.21
minerals			
sodium	150	1600	<1
phosphorus	270	600	72
potassium	1030	27200	647
sulfur	76	3800	123
calcium	16	10600	200
magnesium	130	4200	120
copper	0.2	17	<0.1
iron	<1	1170	2.1
manganese	0.2	53	2.5
zinc	<0.1	19	0.1
pH	6.2	5.4	5.1

[a] Percent (w/w), except for minerals (mg L^{-1}) and pH. [b] ND, not detected.

treated with 0.02% (v/w) diethyl pyrocarbonate (Sigma Chemical Co., St. Louis, MO), a chemical sterilant, and stored at 4 °C for 64 h. On storage, diethyl pyrocarbonate in wort or mash decomposes to ethanol and carbon dioxide and is no longer detrimental to the viability of yeast cells. The bioreactors were then connected to a 30 °C circulating water bath, and their contents were mixed with a magnetic stirrer. Wheat mash dextrins were then saccharified at 30 °C to fermentable sugars by adding 0.006 mL of glucoamylase (Allcoholase II; Alltech Biotechnology Center, Nicholasville, KY) per gram of dissolved solids present in the fermentor. After 30 min, the temperature was adjusted to that desired for fermentation. Active dry yeast (Alltech) was preconditioned by incubation in prewarmed (38 °C) sterile 0.1% peptone for 20 min and immediately used to inoculate the fermentation worts at the recommended rate of 1.0×10^6 viable cells mL^{-1} per degree Plato (Casey and Ingledew, 1985). This inoculation level equates to 10^8 cells/g of dissolved solids present in the fermentor. Fermentations were monitored at chosen intervals for yeast cell counts, total dissolved solids, free amino nitrogen (FAN) levels, and ethanol concentrations.

Analyses. Yeast cells were enumerated by the methylene blue technique (Thomas and Ingledew, 1990), while percentage viability was estimated by determining microcolony formation. For this, appropriately diluted samples were spread on yeast extract–peptone–dextrose agar solidified on microscope slides. The slides were incubated in humidity chambers at 28 °C for 4–6 h. Viable cells formed microcolonies which were counted using a microscope at a magnification of 400; cells that did not form microcolonies following incubation were considered nonviable.

Mash samples were collected and centrifuged (10300*g*; 15 min) to yield clear supernatant for analysis. Total dissolved solids were then estimated by measuring specific gravity at 20 °C with a digital density meter (DMA-45, Anton Paar KG, Graz, Austria). Specific gravity was converted into grams of dissolved solids (expressed as sucrose) per 100 mL using appropriate tables. Total solids were also determined by drying 5-mL aliquots of clear supernatants at 105 °C for 3 h. Free amino nitrogen (FAN) was measured colorimetrically using the ninhydrin method (European Brewery Convention, 1987) with glycine as the standard. Ethanol was measured enzymatically using alcohol dehydrogenase (Sigma Technical Bulletin 331-UV) with known concentrations of ethanol as standards. Clarified wheat mash, molasses, and sugarcane juice were also analyzed for total nitrogen by the Kjeldahl method (Bremner, 1960), for ash content (American Society of Brewing Chemists, 1976), and for fermentable carbohydrates by high-performance liquid chromatography (Low and Wudrich, 1993). Mineral analysis was conducted by the Saskatchewan Feed Testing Laboratory (University of Saskatchewan, Saskatoon, SK).

Table 2. Effect of Initial Dissolved Solids Concentration on Ethanol Yield from Fermentation of Diluted Molasses at 25 °C

initial dissolved solids (g/100 mL)	available fermentable sugar (g/100 mL)	dissolved solids consumed[a] (g/100 mL)	ethanol[b] (% v/v)	ethanol yield[c] (% of theoretical)
10.4	5.9	7.0 ± 0.21	4.0 ± 0.05	101
21.5	12.2	13.7 ± 0.43	7.7 ± 0.16	94
33.8	19.2	20.9 ± 0.90	11.5 ± 0.32	90
47.6	27.1	26.6 ± 1.20	13.3 ± 0.62	74

[a] Average of duplicate fermentations with standard deviation. [b] Average ethanol values with standard deviations. Ethanol was determined in duplicate on samples obtained from duplicate fermentations. [c] For this calculation, it is assumed that none of the sugars taken up is used for growth or for the production of nonethanolic end products. The following theoretical ethanol yield values have been used for the calculation (g of ethanol/100 g of sugar): sucrose, 53.8; maltose, 53.8; glucose, 51.1; fructose, 51.1; maltotriose, 54.8. Theoretical yield from undiluted molasses = 29.8 g/100 g of molasses (37.8 mL/100 g).

RESULTS

VHG Fermentation of Blackstrap Molasses. The composition of molasses varies widely depending on its origin and processing history (Baker, 1987). Mineral analysis of the blackstrap sugarcane molasses used in this study revealed high levels of potassium and calcium but low levels of phosphorus (Table 1). The total nitrogen content of the molasses was relatively high (3.6 g/L) with a free amino nitrogen (FAN) level at 1.0 g/L (45% of which was of low enough molecular weight to be utilized by yeast—see Discussion and Conclusions).

In this study, molasses (76.6% w/w total solids) was diluted with distilled water to 10.4–47.6% (w/v) and fermented with active dry yeast (*Saccharomyces cerevisiae*) at 25 °C with and without nutrient supplementation. With the exception of the 47.6% (w/v) molasses concentration, all of the available fermentable sugars were consumed by the yeast at all dissolved solids concentrations (Table 2). While the rate of ethanol production correlated with the rate of dissolved solids utilization, the ethanol yield did not correlate with the amount of dissolved solids consumed from the fermenting wort. For example, fermentation of 47.6% (w/v) molasses produced 13.3% (v/v) ethanol, which was only 74% of the theoretical maximum (based on utilized sugars) (Table 2). With decreasing initial dissolved solids content the percentage of sugars converted to ethanol increased, and at 10.4% (w/v) molasses, the ethanol yield was approximately 100% of theoretical. In yeast extract-supplemented 33.8% (w/v) molasses, fermentable sugars were completely utilized within 48 h, while about 72 h was required for the unsupplemented controls. Despite increasing the rate of fermentation, nutrient supplementation of 33.8% (w/v) molasses did not increase the amount of fermentable sugar being channeled to ethanol production, as 11.5% (v/v) ethanol was realized in both the presence and absence of yeast extract. Yeast extract supplementation of 47.6% (w/v) molasses actually reduced the ethanol yield from 13.3% (v/v) to 12.1% (v/v).

Mineral analysis (Table 1) indicated that the slow and incomplete fermentation of molasses may be due to phosphate deficiency. The addition of 20 mM diammonium phosphate to 47.6% (w/v) molasses increased the ethanol yield compared to both the unsupplemented control and the yeast extract-supplemented molasses. In

Table 3. Effect of Temperature on the Fermentation of VHG Molasses (47.7 g/100 mL) Supplemented with Diammonium Phosphate

temp (°C)	fermentation time (h)	dissolved solids consumed[a] (%)	ethanol[b] (% v/v)
15	>240	44 ± 1.8[c]	11.1 ± 0.31[c]
20	198	78 ± 2.0	14.8 ± 0.28
25	144	76 ± 2.1	14.1 ± 0.21
30	96	48 ± 3.0	12.9 ± 0.50
35	72	38 ± 3.2	10.2 ± 0.62

[a] Average of duplicate fermentations with standard deviations. [b] Average ethanol values with standard deviations. Ethanol was determined in duplicate on samples obtained from duplicate fermentations. n [c] Fermentations at 15 °C were severely protracted and were not complete by 240 h.

the presence of 20 mM diammonium phosphate, the ethanol yield was 14.6% (v/v) compared to 13.3% (v/v) in its absence, indicating that 82% of the fermentable sugar was converted to ethanol. The rate of ethanol production, however, was unaffected. This is the first demonstrable evidence in this laboratory that a nutrient other than a source of usable nitrogen is required for maximum ethanol yield.

The extent of cell multiplication decreased with the increasing dissolved solids content of the molasses medium. Despite supplementation with yeast extract, only 0.9–1.0 × 10^8 cells/mL were produced. The low maximum cell number attained may be related to the inhibitory compounds of molasses and its phosphate deficiency. Furthermore, a rapid loss of cell viability was noted in the above fermentations (data not shown). Addition of diammonium phosphate to 47.6% (w/v) molasses increased the ethanol yield by improving the efficiency of conversion of sugars to ethanol and by maintaining the viability of the yeast cell population.

Effect of Fermentation Temperature. While all fermentations reported above were conducted at 25 °C, we also studied the effect of temperature on fermentation rates and alcohol production with VHG concentrations of molasses. Molasses at 47.6 g/100 mL was supplemented with 20 mM diammonium phosphate and fermented at 15, 20, 25, 30, and 35 °C. At 15 °C, fermentation was very slow with only 44% of the dissolved solids utilized by 240 h (Table 3). In contrast, at 35 °C the fermentation ceased within 72 h but the amount of the dissolved solids used was even less (38%). Maximum sugar utilization, and therefore ethanol yield, occurred at 20 and 25 °C (Table 3).

VHG Fermentation with Molasses as an Adjunct. In an attempt to determine the suitability of molasses as an adjunct in VHG fermentation, normal gravity clarified wheat mash (21.2% w/v dissolved solids) was supplemented with molasses to raise the sugar concentration of the mixture to VHG levels. It was then fermented at 20 °C with and without nutrient supplementation.

As shown in Table 4, wheat mash fortified with molasses and without any nutrient supplementation end-fermented in 192 h. In the presence of 1.5% (w/w) yeast extract, the duration of fermentation was reduced to 96 h. Approximately 28% of the dissolved solids initially present in these media remained unused. Regardless of nutrient supplementation, fermentation of VHG wheat mash containing molasses adjunct was 91–93% efficient, yielding approximately 13.5% (v/v) ethanol (Table 4). Of the 267 mg of FAN/L originally present in the unsupplemented wort, only 53% was assimilated by the yeast (Table 4).

For comparison, freeze-dried wheat hydrolysate was used to raise the dissolved solids content of wheat mash to VHG levels. Such preparations are fermentable to completion and produce high levels of ethanol (Thomas and Ingledew, 1990, 1992). VHG wheat mash (34.0 g/100 mL) contains more fermentable sugar than the corresponding wheat mash fortified to 34 g/100 mL with molasses. An additional 24 h, however, was required to end-ferment the former, and 19.3–19.9% (v/v) ethanol was produced (Table 4). This yield, calculated on the basis of dissolved solids utilized, was close to 100% of the theoretical maximum.

Yeast cell multiplication and the time required to attain maximum cell numbers in fermentation varied with the adjuncts used (data not shown). In general, yeast cell populations were higher in the presence of yeast extract than in its absence. There was a further increase in maximum cell number when wheat hydrolysate was the carbohydrate adjunct. Wheat hydrolysate supplies readily utilizable, balanced nitrogenous nutrients and contains, we believe, none of the inhibitory substances found in molasses. Yeast cell viability remained high (>80%) during fermentation, but, regardless of the medium, once the fermentation was complete, viability rapidly decreased.

VHG Fermentation of Sugarcane Juice. Sugarcane is the basis of the large Brazilian fuel ethanol industry, and as such it is of interest to apply VHG fermentation technology to the ethanolic fermentation of cane juice. In this study we raised the gravity of sugarcane juice to VHG levels by adding molasses or wheat hydrolysate. The resulting VHG preparations were fermented at 30 °C with or without yeast extract or urea as nutrient supplements. A fermentation temperature of 30 °C was chosen to minimize cooling requirements. This is of practical importance in tropical climates (Wayman and Parekh, 1990; Laluce, 1991).

Table 5 summarizes the characteristics of VHG fermentation of sugarcane juice. Fermentation of unsupplemented cane juice (22.4 g of dissolved solids/100 mL) was complete within 48 h at 30 °C and produced 14.0% (v/v) ethanol. Fermentation of cane juice fortified with molasses was also completed within 48 h, but in this case the ethanol yield (15.1% v/v) was only marginally higher. Similarly, 48 h was required to ferment VHG cane juice fortified with wheat hydrolysate; an ethanol yield of 16.2% (v/v) ethanol was realized. Supplementation with 1.5% (w/w) yeast extract did not appreciably enhance the rate of fermentation in these media (Table 5). Complete conversion of the fermentable sugar to ethanol was observed within 48 h when molasses was used as the adjunct. The ethanol yield was close to 100% of the theoretical maximum calculated on the basis of the fermentable sugar content of the media.

Cane juice contains 1200 mg of N/L with 340 mg/L present as FAN (Table 1). This is far in excess of the 68–80 mg of N/L required for complete fermentation of wheat mash of equivalent sugar content (Thomas and Ingledew, 1990). Since sugarcane juice was fermentable to completion in the absence of a nutrient supplement, it can be safely concluded that this medium is not deficient in assimilable nitrogen. The yeast was able to use approximately 65% of the available FAN during fermentation of cane juice. The FAN concentrations of fortified cane juices increased by 52 and 12 mg of N/L, respectively, when molasses and wheat hydrolysate were used as adjuncts. The yeast utilized about 50% of FAN from these fortified mashes (Table 5).

When molasses was used as an adjunct for sugarcane juice, ethanol concentration increased by only 1–2%. Interestingly, at this fermentation temperature neither

Table 4. Characteristics of VHG Fermentation at 20 °C of 21.2% (w/v) Wheat Mash Fortified to Approximately 34% (w/v) with Freeze-Dried Wheat Hydrolysate or Molasses Adjuncts

adjunct	yeast extract[a]	dissolved solids (% w/v) initial	dissolved solids (% w/v) final	time (h)	ethanol (% v/v)	pH initial	pH final	FAN utilized (mg/L)
wheat hydrolysate	-	33.7	5.4 (16)[b]	216	19.3[c]	5.8	4.5	69 (58)[d]
	+	34.1	4.1 (12)	72	19.9	5.1	4.5	501 (79)
molasses	-	34.4	9.7 (28)	192	13.8	5.4	5.2	141 (53)
	+	33.0	9.4 (28)	96	13.3	5.2	5.1	259 (48)

[a] A nutrient supplement provided at 1.5% (w/w). [b] Figures in parentheses represent dissolved solids remaining unused by the yeast, expressed as a percentage of that initially present in the fermentation medium. [c] In the absence of an adjunct, 21.2 g/100 mL clarified wheat mash is fermented to yield 11.9% (v/v) ethanol. [d] Figures in parentheses represent FAN utilized by the yeast, expressed as a percentage of that initially present in the fermentation medium.

Table 5. Characteristics of VHG Fermentation at 30 °C of 22.4% (w/v) Sugarcane Juice Fortified to 34–35% (w/v) with Freeze-Dried Wheat Hydrolysate or Molasses Adjuncts

adjunct	yeast extract[a]	dissolved solids (% w/v) initial	dissolved solids (% w/v) final	time (h)	ethanol (% v/v)	pH initial	pH final	FAN utilized (mg/mL)
wheat hydrolysate	-	36.5	8.8 (24)[b]	48	16.2[c]	5.3	4.7	185 (52)[d]
	+	35.9	9.5 (27)	48	15.6	5.5	4.8	184 (20)
molasses	-	34.2	10.3 (30)	48	15.1	5.3	5.1	191 (48)
	+	34.0	9.9 (29)	48	15.8	5.4	5.1	229 (23)

[a] A nutrient supplement provided at 1.5% (w/w). [b] Figures in parentheses represent dissolved solids remaining unused by the yeast, expressed as a percentage of that initially present in the fermentation medium. [c] In the absence of an adjunct, 22.4 g/100 mL sugarcane juice is fermented to yield 14.0% (v/v) ethanol. [d] Figures in parentheses represent FAN utilized by the yeast, expressed as a percentage of that initially present in the fermentation medium.

wheat hydrolysate- nor molasses-fortified cane juice yielded ethanol in quantities corresponding to the fermentable sugar content of these mixtures. Raising the dissolved solids content of cane juice to 36% (w/v) increased ethanol production to 16% (v/v). Although this is a significant improvement over the 12.9% (v/v) ethanol produced from VHG molasses at 30 °C, it is not as high as the 18.6% (v/v) achieved at 30 °C from the fermentation of VHG wheat mash (Jones and Ingledew, 1994b).

Addition of yeast extract failed to enhance the rate of fermentation at 30 °C of adjunct-fortified cane juice (Table 5). Although yeast extract contributed a significant amount of FAN and other nutrients to these VHG fermentations, the yeast did not take up any more FAN than in the corresponding control fermentations. These nutrient supplementations were ineffective most likely because of the high FAN content of the juice.

In all cane juice fermentations, yeast cell multiplication continued during the entire course of fermentation, reaching a maximum number at 48 h (data not shown). As was the case with VHG molasses, cells lost their viability regardless of the adjunct or nutrient supplement provided. By 48 h, only 66–74% of the cells remained viable in unsupplemented fermentations and even fewer (38–46%) in fermentations supplemented with yeast extract.

DISCUSSION AND CONCLUSIONS

Generally, under defined fermentation conditions, we did not find large variations in the amount of ethanol produced from a given amount of substrate, although the time required to complete the fermentation could vary by several hours even in duplicate fermentations. Although the reason for this is not clearly understood, it is possible that differences in mixing and the fact that variable fractions of the inocula lost viability may be the factors responsible for these variations in fermentation time. Wherever possible, fermentations were conducted in duplicate and the results analyzed statistically. We found that ethanol yield expressed as a percentage of the theoretical maximum was a good indicator of fermentation performance.

Under VHG conditions, the efficient and complete utilization of substrates is affected by sugar concentration, osmotic pressure, fermentation temperature, inoculation levels (pitching rates), and an adequate supply of nutrients for yeast growth. The most common problem encountered in high-gravity fermentations of brewers' wort, grape must, and grain mash is the low assimilable nitrogen content (Casey *et al.*, 1984; Ingledew and Kunkee, 1985; Thomas and Ingledew, 1990; Jones and Ingledew, 1994a). To prevent stuck or protracted fermentation under VHG conditions, it is necessary to supplement such media with assimilable nitrogen. In contrast, blackstrap molasses contains large amounts of FAN (1000 mg of N/kg), although the yeast used only 43–47% of this nitrogen for growth. Much of this FAN may be unusable due to its molecular weight. Supplementing molasses with yeast extract increased the rate of fermentation but did not affect the extent of fermentation.

For each sugar concentration there appears to be an optimum fermentation temperature. A temperature of 20 °C may be ideal for maximum ethanol production from diluted molasses poised at VHG concentrations. With increasing fermentation temperature the amount of sugar that can be fermented decreased. Some insight on the effects of temperature in VHG fermentation of wheat mashes has been recently published (Jones and Ingledew, 1994b; Thomas *et al.*, 1993).

The fermentable sugar contents of the adjunct and the basal medium determine how much ethanol may be obtained when all of the sugars are fermented. Our previous studies with wheat mash showed that sugar utilization was incomplete at sugar concentrations greater than 39 g/100 mL. The total dissolved solids rather than the total fermentable sugar concentration appears to restrict fermentation. Wheat hydrolysate served as an excellent adjunct to raise the gravity of wheat mash or sugarcane juice (Tables 4 and 5) perhaps in part because slowly hydrolyzed dextrin in mash is less osmotically difficult for the yeast than the lower molecular weight sugars in molasses. An ethanol yield of 19.9% (v/v) was realized when wheat mash containing wheat hydrolysate

as an adjunct was fermented at 20 °C; this yield approached the theoretical maximum.

The increase in ethanol concentration was considerably less when blackstrap molasses was used as an adjunct. This was expected because molasses added at about 13 g/100 mL to raise the gravity contributed only 6.7–7.5 g of fermentable sugars/100 mL of the basal medium. The usefulness of blackstrap molasses to raise the gravity depends to a great extent on the composition of the basal medium. If the medium already contains significant amounts of nonfermentable dissolved solids, only a small amount of molasses can be added to raise the gravity. In such cases, appreciable increases in ethanol concentration will not be achievable. In contrast, if the content of nonfermentable dissolved solids in the basal medium is low, as in the case of sugarcane juice, molasses can be used as an adjunct. Here, the ethanol production is expected to correspond to the total fermentable sugar in the medium. Sugarcane juice fortified with molasses was fermented to produce 15.8% (v/v) ethanol, although sufficient sugar was present for the production of 18.8% (v/v) ethanol. This lesser yield was due to thermal stress since a fermentation temperature of 30 °C was employed. This is supported by the fact that wheat hydrolysate-fortified wheat mash, which normally ferments completely and yields greater amounts of ethanol at 20 °C, also yielded lesser amounts of ethanol at 30 °C than at 20 °C.

Thus, this research shows that blackstrap molasses and sugarcane juice can be employed to facilitate moderately efficient batch VHG fermentation. This confirms and extends previous studies (Thomas and Ingledew, 1990, 1992; Jones and Ingledew, 1994a; Thomas *et al.*, 1993) from our laboratory that VHG technology can increase final ethanol concentrations in wheat mash fermentations. VHG fermentation technology, therefore, has potential as a process improvement to increase the efficiency, productivity, and profitability of fuel alcohol production from saccharine materials in tropical countries such as Brazil.

ACKNOWLEDGMENT

This research was supported by grants from the National Research Council of Canada's Industrial Research Assistance Program (IRAP-L), GPCP Technology and Resource Marketing Inc., the Western Grains Research Foundation, and the Natural Sciences and Engineering Research Council of Canada. We thank A. Dhas, V. Healey, S. Hynes, and W. South for technical assistance in various aspects of this study.

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Received for review October 18, 1993. Revised manuscript received February 3, 1994. Accepted March 16, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1994.