Brewers' Condensed Solubles. III. Enzymatic Hydrolysis, Viscosity Reduction, and Fermentation[1]

K. M. CHUNG,[2] D. S. CHUNG,[3] and P. A. SEIB[2]

ABSTRACT

Brewers' condensed solubles (BCS) were treated with glucoamylase, pullulanase, cellulase, and β-glucanase to increase the level of yeast-fermentable sugars. Enzymatic hydrolysis with glucoamylase alone at 60° C increased the level of fermentable sugars by a factor of 1.8. Pullulanase, cellulase, and β-glucanase did not significantly increase fermentable sugar. Glucoamylase-treated BCS (GT-BCS) was fermented at 30° C. Optimum fermentation conditions were pH 4.2, solids level 15–20% GT-BCS, and an inoculum of 0.2 g of distillers' active dry yeast/L medium. After three days of fermentation, 76–97 gal of ethanol (95%, v/v) was produced from 2,000 lb of dry BCS. Distillers' active dry yeast and bakers' compressed yeast gave comparable fermentation times and yields of ethanol on 10 and 20% GT-BCS, but brewers' spent yeast at its optimum fermentation time produced slightly more ethanol from 10% GT-BCS and slightly less ethanol from 20% GT-BCS than the other two yeasts. The viscosity of BCS at 59.1% was halved when it was treated with cellulase or β-glucanase; cellulase was the most cost-effective enzyme.

Cereal Chem. 63(2):124–130

Brewers' condensed solubles (BCS) is a mixture of the concentrated water-soluble (41.3–54.7%, w/w) and suspended (3.1–4.4%, w/w) by-products from the manufacture of beer. Sebree et al (1983a,b) studied the composition and physical properties of BCS. They found that the average composition of dry BCS was 75% carbohydrate, 8.9% protein, 1.4% fat, and 2.5% ash. These authors also reported that the viscosity of BCS at 20% solids was reduced by enzymes, which apparently depolymerized β-glucans originating from barley.

Ethanol has been produced from many agricultural by-products, such as molasses (Honig 1963), wheat straw (Detroy et al 1981), whey (Lyons and Cunningham 1980), wood (Robinson 1980), and the waste of cassava starch processing (Kunhi et al 1981). Besides these by-products, grain is a useful feedstock for ethanol production (Hunt 1981, USDA 1980). Acid or enzyme hydrolysis is necessary to convert the polysaccharide chains of starch to sugars before yeast fermentation.

Because of its high percentage of carbohydrate, BCS is a suitable raw material for ethanol production. However, half of the carbohydrates are limit dextrins. The objectives of this study were to convert these limit dextrins into fermentable sugars by enzymatic hydrolysis and to determine the optimum conditions for fermentation of enzyme-treated BCS to ethanol. In addition, the viscosity reduction of BCS was examined using cellulase, β-glucanase, and glucoamylase.

MATERIALS AND METHODS

BCS

A sample of BCS was obtained in 1982 from the Anheuser-Busch brewery in Williamsburg, VA. The sample was representive of BCS produced over a 24-hr period. The syrupy, brown BCS contained 54.7% (w/w) soluble solids and 4.4% (w/w) suspended solids, giving a total of 59.1% (w/w) solids. Two more samples of BCS were obtained from Williamsburg in 1983 and 1984. The total solids contents of these samples were 50.9 and 56.9%, respectively. Unless otherwise stated, the 1982 sample was used in this work.

General Methods

The reducing power of BCS before and after hydrolysis was determined by the Nelson colorimetric method (Nelson 1944) using glucose as a reference standard.

Moisture of BCS was determined by evaporation at 65° C under vacuum of 4,000 Nm^{-2} (30 torr) for 20 hr (method 31.008, AOAC 1980), or at 105° C for 9 hr in a forced-draft oven.

Sugars and ethanol were determined by high-performance liquid chromatography (HPLC), using a Varian model 5,000 LC (Varian Associates, Inc., Palo Alto, CA) chromatograph equipped with a loop-injection device (10 μl) and a refractometer as detector. All separations were done using a Bio-Rad Aminex ion-exclusion column (HPX-87H, 300 × 7.8 mm; Bio-Rad Laboratories, Richmond, CA) operated at 45° C with 0.005*M* aqueous sulfuric acid at a flow rate of 0.7 ml/min. Retention times for disaccharides were 6.6 min compared to 6.1 min for trisaccharides. Isomaltose and maltose coeluted in the disaccharide peak. Standard curves were obtained from solutions of known concentrations of sugars and ethanol.

Viscosity was measured with a Brookfield viscometer (LVT model, Brookfield Engineering Laboratories, Inc., Stoughton, MA) at 60° C using spindle no. 3 at 60 rpm and 180 ml of BCS in a 250-ml jar. Newtonian solutions of known viscosity were used to standardize the instrument. The pH of the BCS was adjusted with 6*M* aqueous hydrochloric acid or 12*M* sodium hydroxide solution.

Enzymatic Hydrolysis

The enzymes used, their sources and activities were as follows: glucoamylase was Diazyme L-100 from Miles Laboratories, Inc., Elkhart, IN, and had a labeled activity of 100 Diazyme Units (DU)/ml, where one DU is the amount of enzyme that liberates 1 g of glucose from starch in 1 hr at 60° C and pH 4.2; pullulanase was Novozym 247 from Novo Laboratories, Inc., Wilton, CT, and had a labeled activity of 1,415 PU/g, where one PU is the amount of enzyme that liberates 1 μmol of maltotriose from pullulan in 1 min at 60° C and pH 5.0; cellulases were Celluclast 200 L from Novo Laboratories, Inc., with a labeled activity of 200 CavU/g, where one CavU is the amount of enzyme that liberates reducing power equal to 1 μmol of glucose from cellulose in 1 min at 50° C and pH 4.8, and Cellulase Tv concentrate from Miles Laboratories, Inc., with a labeled activity of 19,400 CU/g, where one CU is the amount of enzyme that, in 5 min at 40° C and pH 4.5, produces a relative fluidity change of one in sodium carboxymethyl cellulose; β-glucanase was Finizym from Novo Laboratories, Inc., and had a labeled activity of 200 FBG/g, where one FBG unit is the amount of enzyme that in 1 min at 30° C and pH 5.0 produces reducing power equal to 1 μmol of glucose from barley β-glucan.

To increase reducing sugars, BCS (60 ml) was treated with the four different enzymes under the following conditions: 1) four levels of glucoamylase (0.02–0.4%, v/v) all at pH 4.2, three levels of solids contents (5, 20, and 59.1%, w/w), and two temperatures, 45 or 60° C; 2) a combination of glucoamylase (0.1%, v/v) and pullulanase (0.1%, w/v) on 20% solids at pH 4.5 and 60° C; and 3) a combination of glucoamylase (0.1%, v/v) and cellulase (0.1%, v/v) or β-glucanase (0.1%, v/v) on 20% solids at pH 4.2 and 60° C.

[1]Contribution 85-446-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan 66506.

[2]Graduate research assistant and professor, respectively, Department of Grain Science and Industry, Kansas State University.

[3]Professor, Department of Agricultural Engineering, Kansas State University.

Fermentation

Distillers' active dry yeast was obtained from Biocon (U.S.) Inc. Lexington, KY. Bakers' compressed yeast and brewers' spent yeast (11.5%, w/w, suspension) were obtained from Anheuser-Busch, Inc., St. Louis, MO. The amounts of yeast used are reported on a dry solids basis. Glucoamylase-treated brewers' condensed solubles (GT-BCS) were used in all fermentations. GT-BCS was obtained by diluting BCS to 20% (w/w) solids and hydrolyzing with 0.1% (v/v) glucoamylase at 60° C for 9 hr. Unless otherwise stated, fermentations were done at 30° C using 50 ml of sterilized (121° C, 15 min) medium in 125-ml Erlenmeyer flasks. The fermentation flasks were plugged with cotton, except in one experiment where the flasks were fitted with rubber stoppers containing hoses leading to a watertight seal.

To determine the optimum pH for yield of ethanol, the acidity of the medium was varied between pH 3.2 and 6.9. The fermentations were carried out for 48 hr on 20% (w/w) GT-BCS using 0.1 g of distillers' active dry yeast/L (2.1×10^6 cells/ml). The yeast (4 g) was rehydrated in 100 ml of warm (42–43° C) water for 10 min before use. Before fermentation, pH of the 20% GT-BCS was adjusted with 1 *M* and 6 *M* aqueous hydrochloric acid or with 1 *M* and 12 *M* sodium hydroxide solution.

To determine optimum solids for fermentation, GT-BCS (20%, w/w) was diluted to 10 and 15% solids by addition of water or concentrated to 26 and 32% solids by evaporation below 50° C on a rotary evaporator. Fermentations were done with 0.1 g of distillers' active dry yeast/L.

To determine optimum yeast level, GT-BCS (20%, w/w) was fermented with 0.001, 0.01, 0.05, 0.1, 0.2, and 0.4 g of distillers' active dry yeast/L.

Other fermentations were done on 10 or 20% (w/w) GT-BCS with 0.1 g (as dry weight) of yeast/L at 20 or 30° C.

In one experiment, air was excluded from the fermentation flasks using a watertight seal. In this experiment, GT-BCS (20%, w/w) was fermented at 30° C and pH 4.2 using 0.1 g distillers' active dry yeast/L.

Viscosity Versus pH of BCS

The pH of BCS (59.1%, w/w) was adjusted between 3 and 11, using 10 *M* aqueous hydrochloric acid or 12 *M* sodium hydroxide solution. The maximum amount of added acid or base was 3 ml/180 ml BCS. The viscosity of BCS was then measured by the Brookfield viscometer at 60° C as previously described.

Viscosity Reduction

BCS (59.1%, w/w, 180 ml) was treated at pH 4.2 and 60° C with glucoamylase (Diazyme L-100), β-glucanase (Finizym), and cellulase (Celluclast 200 L or Cellulase Tv concentrate). The quantity of each enzyme (except glucoamylase) was adjusted for cost to equal $2/metric ton of "as is" BCS (59.1%, w/w, solids). Those quantities were 0.048 ml of Finizym, 0.016 ml of Celluclast 200 L, and 0.0046 g of Cellulase Tv concentrate per 100 ml of BCS, respectively.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis to Increase Fermentable Sugars

Figure 1 shows the results of enzymatic hydrolysis of BCS with glucoamylase at 60° C. At the lowest enzyme level (0.02%, Fig. 1A), the reducing power of BCS at 5% solids increased from 28 to 70% during the first 9 hr of treatment; after that, it increased only slightly. At the same glucoamylase concentration but at 20 and 59.1% solids, reducing power increased from 28 to 66% in 32 hr, and from 28 to 47% in 48 hr, respectively.

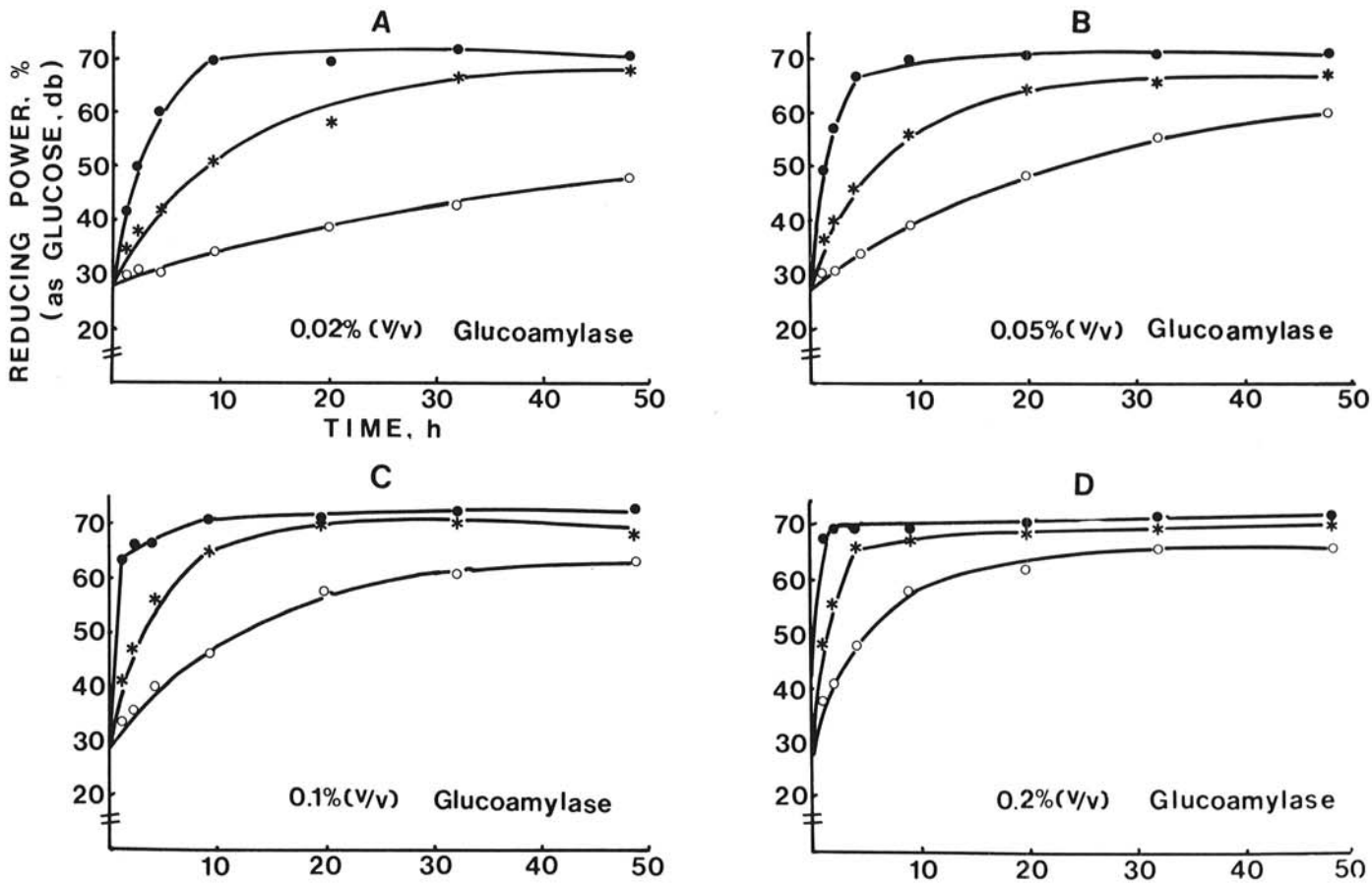

Fig. 1. Changes in reducing power of brewers' condensed solubles (BCS) treated with glucoamylase at 60° C and pH 4.2. •—•, 5% (w/w) BCS; *—*, 20% (w/w) BCS; o—o, 59.1% (w/w) or "as is" BCS. The concentration of enzyme in DU/g of dry-solids BCS is: 0.02% (v/v) glucoamylase at 5, 20, and 59.1% solids is 0.39, 0.09, and 0.03, respectively; at 0.05% (v/v) glucoamylase, 0.99, 0.23, and 0.07, respectively; at 0.1% (v/v) glucoamylase, 1.97, 0.46, and 0.14, respectively; and at 0.2% (v/v) glucoamylase, 3.94, 0.92, and 0.28, respectively.

When the amount of enzyme was increased, the rate of hydrolysis increased, as expected. At 0.1% enzyme, the maximum reducing power was reached in 5 hr at 5% BCS, in 20 hr at 20% BCS, and in 48 hr at 59.1% BCS. At the highest enzyme level (0.2%), the maximum was reached in 2 hr at 5% BCS, 9 hr at 20% BCS, and 32 hr at 59.1% BCS.

We also examined the hydrolysis of BCS with glucoamylase at 45° C instead of 60° C. The results shown in Figure 2 indicate that enzymatic hydrolysis at 60° C is preferred over 45° C. The rate of hydrolysis was faster at 60° C than at 45° C, and the yield of reducing power at the two temperatures was the same. Furthermore, at 60° C growth of most microorganisms is less likely than at 45° C.

When the hydrolysis reaction approached equilibrium, the highest conversion to reducing power (71–73%) was observed at 5% solids (Fig. 1). As the solids content of BCS increased, the rate of hydrolysis decreased.

We attempted to increase the rate of enzymatic hydrolysis and the yield of reducing sugars using a combination of pullulanase and glucoamylase. In recent years, Novo Laboratories introduced a new pullulanase with a pH and temperature optimum nearly equal to those of glucoamylase. It is well known that glucoamylase is an exo-acting enzyme, which cleaves α-1,4 D-glucosidic bonds more rapidly than α-1,6 D-glucosidic bonds. Pullulanase, which debranches α- and β-limit dextrins, might be expected to rapidly hydrolyze the α-1,6 bonds in BCS, thereby accelerating the release of reducing sugar by glucoamylase.

When 0.1% w/v (6.6 PU/g dry solids [ds] of BCS) pullulanase was added with 0.1% v/v (0.46 DU/g ds) of glucoamylase at pH 4.5 on 20% solids BCS at 60° C, the reducing power of the BCS increased to 70% after 48 hr. Without pullulanase, the reducing power increased to 65%. These preliminary results are encouraging, and a detailed investigation on the use of a mixture of glucoamylase/pullulanase on BCS should be undertaken.

β-Glucanase was used in combination with glucoamylase to try to increase the fermentables in BCS. β-Glucanase is an endo-acting carbohydrase that hydrolyzes β-glucan. β-Glucan is a constituent of cell walls in barley; it contains β-1,4 and 1,3 D-glucose units at a ratio of about 7:3 (Bathgate and Dalgliesh 1975). When BCS was hydrolyzed at pH 4.2 and 60° C for 48 hr with 0.1% v/v (0.46 DU/g ds) glucoamylase or with a mixture of 0.1% v/v (0.46 DU/g ds) glucoamylase and 0.1% v/v (1.21 FBG units/g ds) β-glucanase, the hydrolyzed solids gave 69.3% and 71.5% of reducing sugar, respectively. The slight increase in reducing power cannot justify the expense of adding β-glucanase. Cellulase also gave no significant increase in reducing power when it was used in combination with glucoamylase.

The hydrolysis data in Figures 1 and 2 were based on measurement of reducing power. We also used HPLC to measure the content of glucose and disaccharides present in enzyme-treated BCS. Table I shows the amount of fermentable sugars in 5, 20, and 59.1% BCS before and after treatment with glucoamylase (0.02–0.2%). Fermentable sugars could be increased approximately 75% over their initial levels. As the solids in the digests were decreased or the amount of enzyme increased, the reaction time decreased. The increase of fermentable sugars leveled off at almost the same times as those for reducing power measurements (Fig. 1). At the lowest solids content (5%), the fermentable sugars of BCS treated with 0.02% enzyme (0.39 DU/g ds) increased from 37.2% to 65% in 20 hr, whereas the same increase in fermentables was produced in approximately 1 hr at 0.2% glucoamylase (3.94 DU/g ds). At 20% solids, the fermentable sugars did not increase significantly beyond 9 hr for 0.1% enzyme (0.46 DU/g ds) or 4 hr for 0.2% enzyme (0.92 DU/g ds). Production of fermentable sugars at the highest solids content (59.1%) was slow and required two days to reach the maximum level with 0.2% enzyme (0.28 DU/g ds).

As shown in Figure 1 and Table I, hydrolysis with glucoamylase can be done over a reasonable length of time even at a high solids level in BCS. This may be important to some application of BCS. However, when fermenting BCS to ethanol, the optimum solids for fermentation is 20% BCS. Enzymatic hydrolysis at 20% solids is reasonably rapid (~ 9 hr) and complete using 0.1% (0.46 DU/g ds)

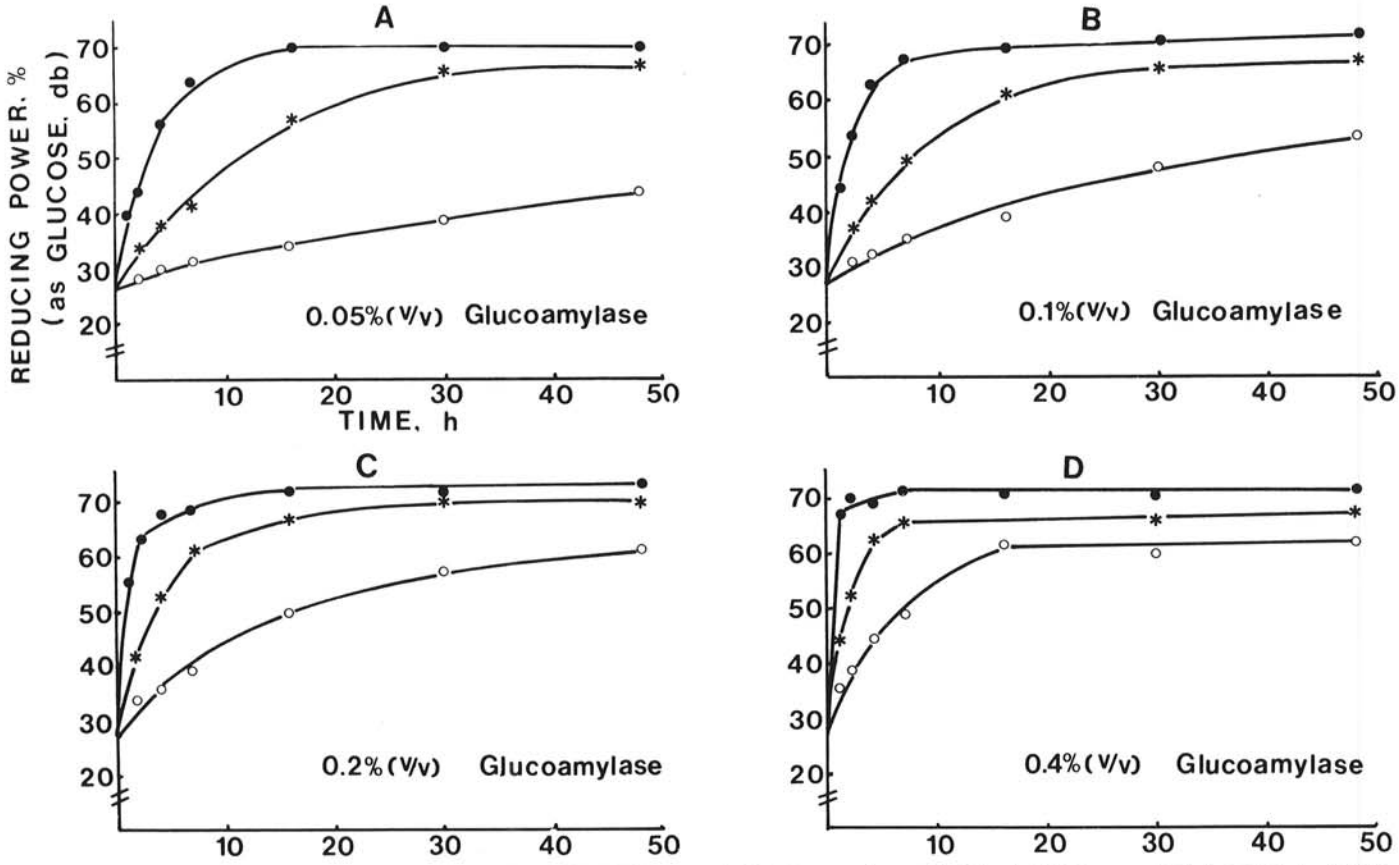

Fig. 2. Changes in reducing power of brewers' condensed solubles (BCS) treated with glucoamylase at 45° C and pH 4.2. •—•, 5% (w/w) BCS; *—*, 20% (w/w) BCS; o—o, 59.1% (w/w) or "as is" BCS. See caption to Fig. 1 for units of enzymes/g dry solids BCS.

glucoamylase. If enzymatic hydrolysis is done on 59.1% solids BCS, then 0.2% (0.28 DU/g ds) glucoamylase is recommended (Table I).

Fermentation in Cotton-Plugged Flasks

The results of fermentation done at various initial pH values (3.2–6.9) are presented in Figure 3. Yeast gave relatively high yields of ethanol above pH 3.5. The maximum yield occurred at pH 4.2, which is almost the same as the original pH of BCS. Thus, fermentation can be done without adjusting the pH of BCS. When the initial pH was 4.6 or less, the final pH after fermentation of GT-BCS was almost the same as its initial pH. But when the initial pH was between 4.6 and 6.9, the final pH decreased by 0.4–1.0 units.

GT-BCS was fermented at five different solids levels using 0.1 g distillers' active dry yeast/L or 0.83 lb/1,000 gal; the results are shown in Figure 4. When GT-BCS was fermented at a solids content of 10%, ethanol concentration in the beer as determined by HPLC rose to a maximum of 3.5% (v/v) in 36 hr, after which time the concentration of ethanol declined slightly. At 15% and 20% GT-BCS, the concentration of ethanol peaked at 5.0% after two days and 6.6% after three days, respectively. Fermentation of GT-BCS at high solids required long reaction times to reach maximum ethanol production. At 26% and 32% GT-BCS, ethanol concentration peaked after five days at 7.9% and 9.1%, respectively (Fig. 4).

The maximum ethanol yields at the various solids levels are given in Table II. As the concentration of substrate increased, the ethanol yield decreased. At 10% solids, the yield was 339 ml of 95% ethanol/kg ds (81 gal/2,000 lb ds). As the solids content increased up to 20%, the yield declined slightly to 316 ml/kg ds. But at 32% solids, the yield was only 266 ml/kg ds.

Figure 5 shows the effect of the level of yeast inoculum and fermentation time on the production of ethanol in 20% GT-BCS. As the inoculum increased, fermentation rates increased markedly, and ethanol yields increased slightly. The optimum fermentation times were one and a half days with an inoculum of 0.2–0.4 g of distillers' active dry yeast/L ($4.2–8.3 \times 10^6$ cells/ml), three days with 0.05–0.1 g/L, four days with 0.01 g/L, and five days with 0.001 g/L.

Besides the 1982 sample of BCS, a 1983 and a 1984 sample of BCS were treated with glucoamylase, and the hydrolyzed samples

TABLE I
Sugars[a] in Brewers' Condensed Solubles Determined by High-Performance Liquid Chromatography after Enzymatic Hydrolysis with Glucoamylase at 60°C and pH 4.2

Dry Solids Level (% w/w)	Hydrolysis Time (hr)	Glucoamylase (% v/v) 0.02	0.05	0.1	0.2
5	0	37.2% (w/w)	37.2	37.2	37.2
	1	50.4	59.2	61.6	65.4
	2	55.2	62.4	62.4	65.8
	4	62.6	66.0	66.2	66.8
	9	62.2	65.8	66.2	68.0
	20	65.0	67.6	66.4	66.0
20	0	37.2	37.2	37.2	37.2
	2	49.1	55.1	54.9	59.0
	4	51.8	55.6	60.7	64.8
	9	54.4	59.9	64.9	65.5
	20	59.6	64.1	67.0	65.2
59.1	0	37.2	37.2	37.2	37.2
	9	45.5	48.5	52.6	55.8
	20	47.8	52.8	55.1	60.2
	32	50.4	56.5	58.9	62.6
	48	52.4	57.5	58.8	65.1
	96	56.3	61.1	62.6	65.3

[a]Sugar equals the sum of glucose and disaccharides, which were assumed to be predominantly maltose plus some isomaltose. Sugars given as weight per 100 parts of dry solids. Towards the end of enzymatic hydrolysis, the disaccharide fraction accounted for ~10% of the mixture of glucose plus disaccharides in the hydrolyzates.

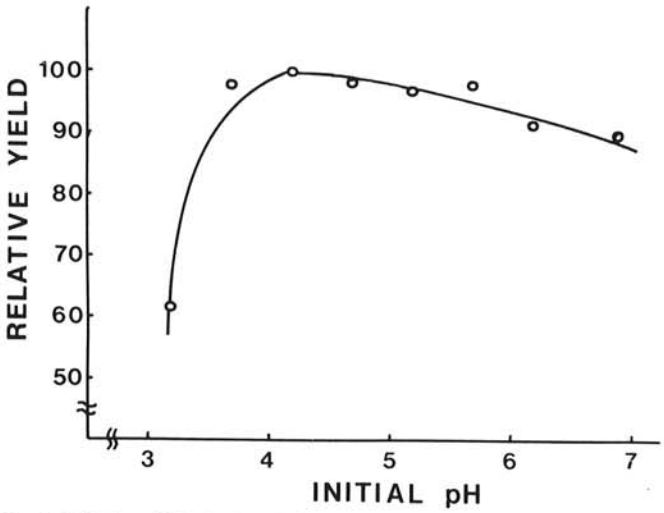

Fig. 3. Relative yield of ethanol after 48 hr fermentation of glucoamylase-treated brewers' condensed solubles (GT-BCS). GT-BCS was prepared by treating BCS (20% solids) with 0.1% Diazyme at 60°C for 9 hr. Fermentations were done with 0.1 g yeast/L at 30°C in cotton-plugged flasks.

TABLE II
Ethanol Yield Versus Concentration of Dry Solids in Glucoamylase-Treated Brewers' Condensed Solubles (GT-BCS)[a]

Dry Solids in Broth (% w/w)	Absolute Ethanol in Ferment (% v/v)[b]	Ethanol (95% v/v) Yield ml/kg of Dry BCS	gal/2,000 lb of Dry BCS
10	3.5	339	81
15	5.0	324	78
20	6.6	316	76
26	7.9	285	68
32	9.1	266	64

[a]GT-BCS at different solids content was prepared and fermented as described in caption to Fig. 4.
[b]Maximum ethanol concentration, Fig. 4.

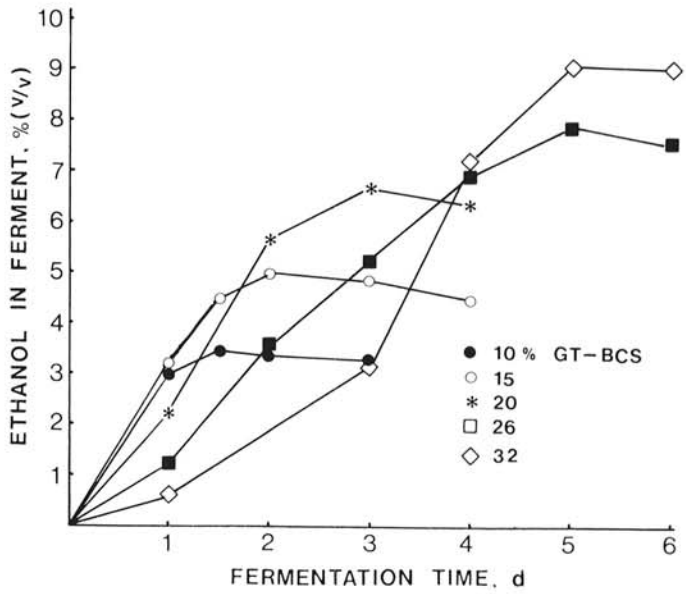

Fig. 4. Ethanol concentration in glucoamylase-treated brewers' condensed solubles (GT-BCS) ferments at various solids concentrations. GT-BCS, prepared as described in the caption to Fig. 3, was diluted with water or concentrated under vacuum to obtain different solids contents. Fermentations were done with 0.1 g yeast/L at 30°C and pH 4.2 in cotton-plugged lasks.

were fermented at 10 and 20% solids. Table III shows that the yield of ethanol (95%) varied between 81 and 97 gal/ton ds when the fermentation was done at 10% solids on nonsterilized medium. The ethanol yield increased with decreasing solids in the BCS from the brewery and with no sterilizing of the GT-BCS in the laboratory. Furthermore, the yield of ethanol expected from a ton of solids depended on the method used to determine the dry solids in BCS. When BCS was dried from about 20% solids to dryness at 105° C in a forced-draft oven, it lost more moisture (three percentage points) than when it was dried at 65° C under vacuum. When yields were calculated based on drying at 105° C rather than 65° C, they increased from 81–97 to 90–109 gal of ethanol (95%) per ton of BCS solids (Table III).

The Maillard browning reaction may account for the variable yield of ethanol from fermented GT-BCS. BCS contained 8.9% protein (db), much of which is probably low-molecular-weight peptides resulting from the action of protease during malting. The peptides react with reducing sugars during evaporation or sterilization to give products that resist the hydrolytic action of glucoamylase and are nonfermentable. To obtain high yields of ethanol from yeast fermentation of GT-BCS, evaporation of BCS should be done at low temperature, and the diluted GT-BCS should be pasteurized instead of sterilized.

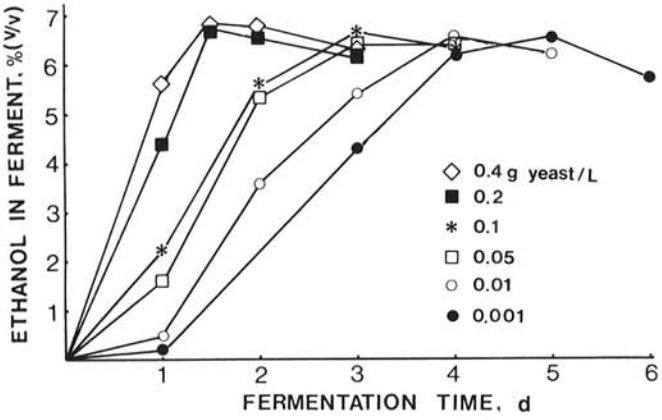

Fig. 5. Ethanol concentration in glucoamylase-treated brewers' condensed solubles (GT-BCS) ferments with various levels of yeast inoculum/L. GT-BCS was prepared as described in the caption to Fig. 3. Fermentations were done using 20% (w/w) GT-BCS at 30° C and pH 4.2 in cotton-plugged flasks.

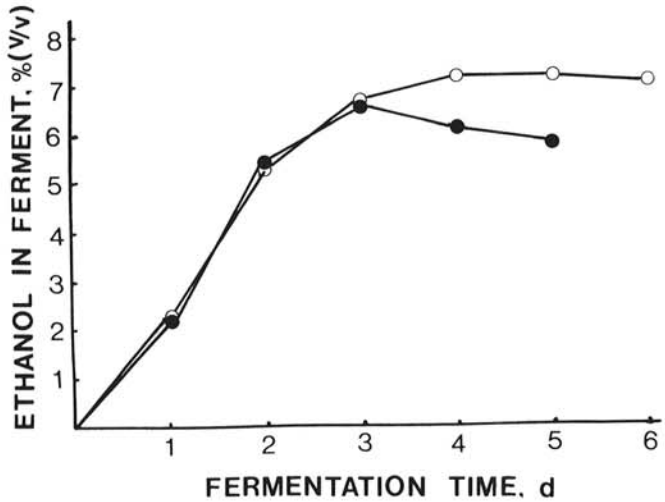

Fig. 6. Ethanol concentration in glucoamylase-treated-brewers' condensed solubles (GT-BCS) ferments when fermentation flasks were plugged with cotton (•—•) or fitted with a watertight seal (o—o). GT-BCS was prepared as described in the caption to Fig. 3. Fermentations were done using 20% solids GT-BCS with 0.1 g yeast/L at 30° C and pH 4.2.

Distillers' active dry yeast, bakers' compressed yeast, and brewers' spent yeast were compared for their ability to ferment GT-BCS (Table IV). When fermentations were done on 10% and 20% (w/w) GT-BCS at 20 and 30° C, there were no significant differences in the rate and time of fermentation for distillers' active dry yeast and bakers' compressed yeast, and they yielded comparable amounts of ethanol. When fermenting 20% GT-BCS at 30° C, the two yeasts gave more ethanol (76–77 gal/2,000 lb of dry BCS) than did brewers' spent yeast (74 gal). However, when fermenting 10% GT-BCS at 30° C, brewers' spent yeast gave 82 gal of ethanol in two days compared to 78–79 gal of ethanol in two days

TABLE III
Ethanol Yield from Three Samples of Brewers' Condensed Solubles (BCS)[a]

Sample	Glucoamylase Treated BCS Solids in Broth (%)	Sterilized (S) or Nonsterilized (NS)[b]	Ethanol (95% v/v), Yield, gal/2,000 lb of dry BCS 65°C[c]	105°C[d]
1982	10	S	78	86
		NS	81	90
	20	S	76	84
		NS	77	86
1983	10	S	95	107
		NS	97	109
	20	S	89	100
		NS	93	104
1984	10	S	86	99
		NS	88	101
	20	S	85	98
		NS	84	97

[a] The dry solids contents of the samples from the brewery were: 1982, 59.1%; 1983, 50.9%, and 1984, 56.9%. Glucoamylase-treated BCS was fermented with 0.1 g yeast/L at 30° C and pH 4.2 for two days (10% solids) or three days (20% solids) in cotton-plugged flasks.
[b] Broth was sterilized at 121° C for 15 min or not sterilized prior to use.
[c] Ethanol yield when solids content was determined at 65° C under vacuum for 20 hr.
[d] Ethanol yield when solids content was determined at 105° C for 9 hr.

TABLE IV
Fermentation of Brewers' Condensed Solubles (BCS) with Three Yeasts; Ethanol Yield and Fermentation Time[a]

Yeast	Glucoamylase-Treated Solids in Broth (%)	Fermentation Temp. (° C)	Ethanol (95% v/v) Yield gal/2,000 lb of Dry BCS	Fermentation Time[b] (days)
Distillers' active dry yeast	10	20	82	3
		30	78	2
	20	20	80	5
		30	77	3
Bakers' compressed yeast	10	20	83	2
		30	79	2
	20	20	80	5
		30	76	3
Brewers' spent yeast[c]	10	20	86	3
		30	82	2
	20	30	82	2
		30	74	3

[a] Fermentations were done at pH 4.2 in cotton-plugged flasks.
[b] Time at maximum ethanol yield.
[c] Obtained in viable form at 11.5% solids.

from the other two yeasts. These results might be expected, because brewers' spent yeast is usually used at a low substrate concentration to produce beer.

Fermentation in Flasks Fitted with a Watertight Seal

When GT-BCS was fermented in cotton-plugged flasks, the ethanol concentration peaked with fermentation time, then declined (Figs. 4 and 5). The decline in ethanol concentration was likely caused by aerobic oxidation of ethanol by yeast. When air was excluded from the fermentation flasks with a watertight seal, the maximum ethanol concentration in the ferment of 20% GT-BCS at 30° C and pH 4.2 was 7.2% (v/v) instead of the 6.6% obtained with cotton plugs (Fig. 6). Furthermore, ethanol concentration did not decline after it peaked in rigorously anaerobic fermentations. The yield of ethanol obtained by fermenting 20% GT-BCS increased from 76 gal per ton of BCS (ds) using cotton plugs (Table II) to 83 gal using a watertight seal. It is anticipated that maximum yields of ethanol reported in Tables III and IV would increase about 9%, and the fermentation times required to achieve maximum yields would increase approximately one day based on data in Figure 6.

Viscosity Reduction

As shown in Figure 7, the viscosity of BCS (59.1% solids) decreased about 18% for each pH unit below 8.4. It is not known why the viscosity of BCS decreased with pH.

BCS at 59.1% solids was treated with several enzymes to reduce its viscosity. Figure 8 shows that a commercial glucoamylase is not efficient at lowering the viscosity of BCS. Even at a high concentration of glucoamylase (0.4%, v/v, 0.56 DU/g ds), a reaction period of four days was needed to halve the viscosity at 60° C and pH 4.2 (Fig. 8). On the other hand, cellulase (0.016%, v/v [0.05 CavU/g ds] Celluclast, or 0.0046%, w/v [1.22 CU/g ds] Cellulase Tv concentrate) halved the viscosity within 12 hr, and 0.048% v/v (0.17 FBG units/g ds) β-glucanase did so within one day (Fig. 9). The data in Figure 9 show that the viscosity of BCS (59.1% solids) can be reduced more than 50% at an enzyme cost of $2/metric ton at 1983 prices. BCS with low viscosity can be pumped at low cost and mixed easily with other materials.

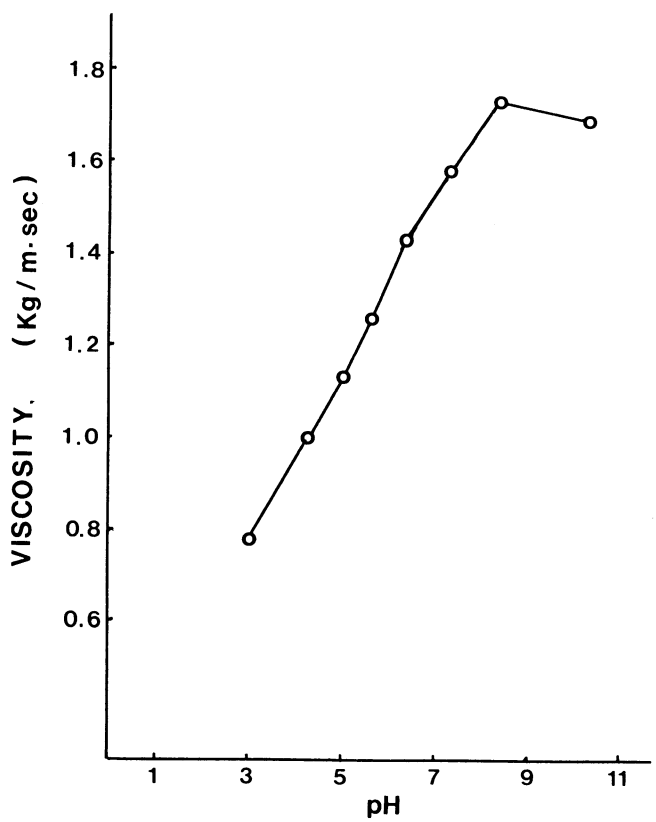

Fig. 7. Viscosity of brewers' condensed solubles (59.1% solids) at 60° C vs. pH. (1 kg/m·s = 1,000 centipoise.)

CONCLUSIONS

For ethanol production from BCS, we recommend enzymatic hydrolysis at 60° C and pH 4.2 using either 0.1% (v/v) glucoamylase on 20% (w/w) BCS (0.46 DU/g ds BCS) for 10–20 hr, or 0.2% (v/v) glucoamylase on 59.1% (w/w) BCS (0.28 DU/g ds BCS) for 32–48 hr. Optimum conditions for fermentation at 30° C appear to be pH 4.2, 15–20% (w/w) GT-BCS, and inoculum of 0.2 g of distillers' active dry yeast/L. By pretreating BCS with glucoamylase (0.46 DU/g ds, $12 for enzyme/metric ton ds, 1983

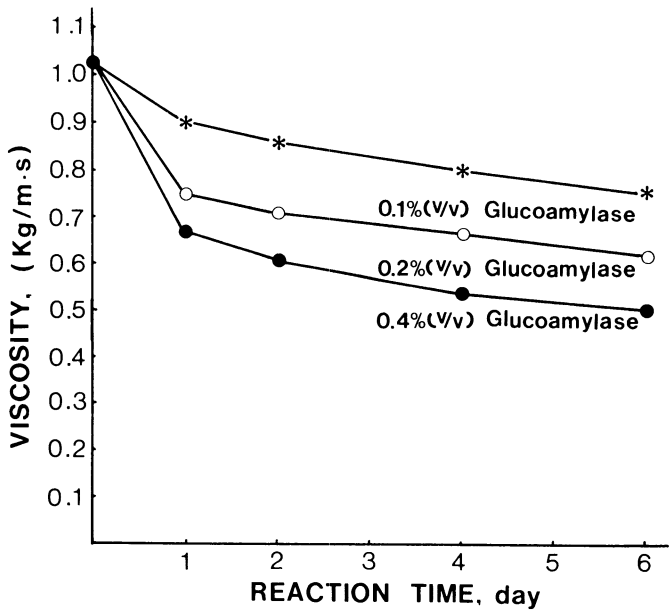

Fig. 8. Change in viscosity of brewers' condensed solubles (BCS) (59.1% solids) treated with glucoamylase (Diazyme L-100) at 60° C and pH 4.2. A level of 0.1% (v/v) glucoamylase costs $2/metric ton (2,200 lb) of BCS (as is weight basis) for enzyme. (1 kg/m·s = 1,000 centipoise.)

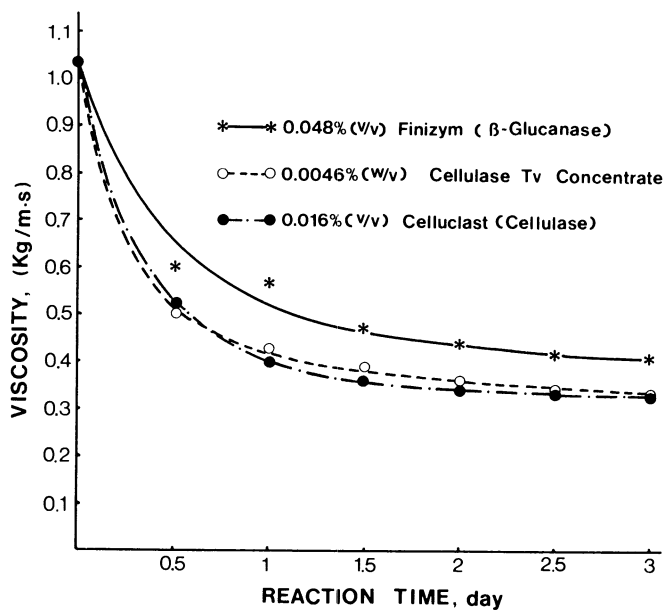

Fig. 9. Change in viscosity of brewers' condensed solubles (BCS) (100 ml, 59.1% solids) treated with cellulase (0.016 ml Celluclast 200 L, or 0.0046 g Cellulase Tv concentrate) or β-glucanase (0.048 ml Finizym) at 60° C and pH 4.2. The quantity of enzyme used was adjusted to equal a cost of $2/metric ton (2,200 lb) of BCS (as is weight basis). (1 kg/m·s = 1,000 centipoise.)

price), ethanol yield increased from 13.6 to 23.7% (w/w) based on dry solids when fermentation was done at optimum conditions. To obtain high yields of ethanol from fermentation of GT-BCS, BCS should be evaporated at low temperature, diluted GT-BCS should not be sterilized, and air should be excluded from the fermentor. Distillers' active dry yeast and bakers' compressed yeast gave comparable yields of ethanol and fermentation time on 10 and 20% GT-BCS, but brewers' spent yeast at its optimum fermentation time produced slightly more ethanol from 10% GT-BCS and slightly less from 20% GT-BCS than the other two yeasts. To reduce the viscosity of BCS, the use of cellulase on high-solids BCS is recommended. After 12 hr at 60° C and pH 4.2, cellulase halved the viscosity of BCS at an enzyme cost of $2/metric ton (1983 prices, as is weight basis).

ACKNOWLEDGMENTS

We thank Anheuser-Busch, Inc., for financial support. We also thank Robert W. Sucher and Lawrence W. Long of the company for helpful discussions, as well as John Iandolo of Kansas State University. We thank the following companies for samples of enzymes and yeast: Miles Laboratories, Inc., Elkhart, IN; Novo Laboratories, Inc., Wilton, CT; and Biocon (U.S.) Inc., Lexington, KY.

LITERATURE CITED

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1980. Official Methods of Analysis. 13th ed. The Association: Washington, DC.

BATHGATE, G. N., and DALGLIESH, C. E. 1975. The diversity of barley and malt β-glucans. Proc. Am. Soc. Brew. Chem. 33:32.

DETROY, R. W., LINDENFELSER, L. A., SOMMER, S., and ORTON, W. L. 1981. Bioconversaion of wheat straw to ethanol: chemical modification, enzymatic hydrolysis, and fermentation. Biotechnology and Bioengineering 23:1527.

HONIG, P. 1963. Utilization of molasses. Page 580 in: Principles of Sugar Technology. Vol. III. Elsevier Publishing Company: New York.

HUNT, V. D. 1981. Fundamentals of feedstocks. Page 139 in: The Gasohol Handbook. Industrial Press Inc.: New York.

KUNHI, A. A., GHILDYAL, N. P., LONSANE, B. K., AHMED, S. Y., and NATARAJAN, C. P. 1981. Studies on production of alcohol from saccharified wasted residue from cassava starch processing industries. Staerke 33:275.

LYONS, T. P., and CUNNINGHAM, J. D. 1980. Fuel alcohol from whey. Am. Dairy Rev. 42:42A.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375.

ROBINSON, J. S. 1980. Secondary conversion process. Page 187 in: Fuels from Biomass, Technology and Feasibility. Noyes Data Corp.: Park Ridge, NJ.

SEBREE, B. R., CHUNG, D. S., and SEIB, P. A. 1983a. Brewers condensed solubles I. Composition and physical properties. Cereal Chem. 60:147.

SEBREE, B. R., CHUNG, D. S., and SEIB, P. A. 1983b. Brewers condensed solubles II. Viscosity and viscosity reduction of brewers condensed solubles by cellulase and β-glucanase. Cereal Chem. 60:151.

USDA (with assistance of Development Planning and Research Associates Inc., Manhattan, KS). 1980. Small-scale fuel alcohol production. USDA: Washington, DC.

[Received June 3, 1985. Revision received September 11, 1985. Accepted September 11, 1985]