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Effects of Brewers' Condensed Solubles (BCS) on the Production of Ethanol from Low-Grade Starch Materials

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ABSTRACT

Yeast fermentation was performed on grain and bakery byproducts with and without adding the same volume of brewers' condensed solubles (BCS). Starch material in the grain and bakery byproducts effectively was converted to fermentable sugars with conversion ratios of 93–97% by successive treatments of samples with bacterial α-amylase and fungal glucoamylase. The yeast fermentation of these enzyme-digested byproducts alone showed that ethanol concentrations of 16.4–42.7 mL/100 g dry solid in the broth were achieved with fermentation efficiencies of 87–96%. Addition of BCS to the grain byproducts increased ethanol concentration by 10–86% by increasing the potential glucose content of the broth. The rates of fermentation measured by CO_2 gas production demonstrated that BCS addition to bakery byproducts reduced the fermentation time from 62–72 h to 34–35 h. In bakery byproducts that were low in amino nitrogen, exhaustion of nitrogenous compounds in substrates was found to be a limiting factor for yeast growth. Because BCS is a rich source of nitrogen, adding BCS to these substrates markedly increased the fermentation rate.

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Index Entries: Ethanol; brewers' condensed solubles (BCS); grain and bakery byproducts; enzymatic hydrolysis; starch; yeast fermentation; amino nitrogen; CO_2 gas production; gasograph.

INTRODUCTION

Good quality conventional feedstocks traditionally have been used by the beverage alcohol industry. Although this industry requires a good food grade feedstock and has used mainly corn and grain sorghum to make ethanol, such is not the case with the fuel alcohol industry. Modern technology permits the use of many nonconventional feedstocks, potentially making fuel alcohol production more economically feasible (*1*). Interest in fermentation of low-grade biomass to produce alcohol for use as a liquid fuel has grown tremendously with the increasing use of oxygenated fuel to abate hydrocarbons in exhaust of petroleum-derived energy sources.

Biomass is abundantly available in nature and includes all plant and animal materials from which energy can be derived. Bassam (*2*) estimated that the earth produces 155.2 billion tons of dry biomass in a year. However, only 360 million tons are used for human food, and 13.5 billion tons, or 9% of total land mass productivity, are lost as waste from harvesting or processing operations. In many cases, these wastes are in forms and quantities that still could be considered raw materials for other purposes.

Domestic biomass waste is derived from three major sources: agricultural waste, municipal solid waste, and forestry residues (*3*). Agricultural and food processing types of waste such as grain dusts, milling byproducts, and bakery wastes contain large amounts of starch. Unlike cellulosic biomass, which requires more pretreatment prior to alcohol fermentation, the starchy biomass is relatively easily converted to alcohol and, therefore, is less energy intensive. However, the problem associated with the waste is that these byproducts are generated in relatively small amounts each time the raw materials are processed. One logical way to utilize these byproducts is to collect them in a small community base and use them as feedstocks for producing alcohol in small-sized production units.

Grain dust is always present in grain handling facilities and has the potential to cause fires, explosions, and health hazards (*4*). Grain dust consists of dirt, pieces of other plant materials, tiny fragments of grain kernels, and broken kernels resulting from abrasion during handling. The amount of dust in grain is estimated to range from 0.01–1.0% (*5*). Wheat screenings are byproducts from cleaning of wheat before milling. Screenings consist primarily of broken and shrunken kernels of wheat, wild oats, and other weed seeds.

The shelf life of most commercial white bread produced in the United States is only 2 d because of a complex phenomenon called bread staling.

Staling results in the initial return to the bakery of an average of 8% of the bread produced, which represents over a half million tons of bread per year (*6*).

Brewers' condensed solubles (BCS) is a mixture of the concentrated water-soluble (40–55%, w/w) and suspended solids (3–5%, w/w) from the manufacture of beer. Because BCS primarily comes from the dewatering of spent mash, it is a rich source of fermentable carbohydrates and contains peptide, phosphorous, calcium, trace minerals, and some water-soluble vitamins (*7*). The nutrient value and physical properties of BCS are well defined elsewhere (*7–9*).

The objectives of this study were to determine the yield of ethanol from hydrolyzed grain and bakery byproducts and to determine if BCS can be used to enhance the rate of fermentation and the yield of ethanol from them.

MATERIALS AND METHODS

Materials

The types of grain byproducts tested were grain dusts, wheat screenings (WS), and low-grade wheat flour (LGF). For the bakery byproducts, bread waste (BW) and cake waste (CW) were chosen.

Nine samples of grain dusts were collected from three commercial grain elevators in northeast Kansas at three different harvesting times. The sources of grain from which the dusts came were determined before the samples were collected. After chemical compositions were determined, the types of grain dusts for further experiment were selected as low- and high-starch wheat-corn dust (WLS and WHS, respectively); low- and high-starch corn-sorghum dust (CLS and CHS, respectively); and sorghum-soybean dust (SSD). BW was obtained from the baking science laboratory of the Department of Grain Science and Industry, Kansas State University. CW was obtained from the American Institute of Baking (Manhattan, Kansas). WS and LGF were obtained from the pilot flour mill of the Dept. of the Grain Science and Industry, Kansas State University. LGF was a mixture of 2.2 parts of 5th middling flour, 1.92 parts of 6th middling flour, 0.55 part of tailing flour, 1.92 parts of bran and shorts duster, and 1.1 parts of red dog. BCS with 48.5% solid content was obtained from Anheuser-Busch Inc. (Columbus, OH).

Distillers' active-dry yeast was obtained from Biocon (US) Inc. (Lexington, KY). The recommended usage rate was 2–4 lbs/1000 gal (5–10 million cells/mL) of mash when the sugar concentration was between 15 and 25%. The optimum pH given by the yeast supplier was between 4.0 and 5.5, and the optimum temperature was 30°C. A bacterial α-amylase

(TAKA-THERM) was obtained from Miles Laboratories, Inc. (Elkhart, IN). One gram of TAKA-TERM had a leveled activity of 170,000 Modified Wohlegemuth Units (MWU). One MWU is the amount of enzyme that dextrinizes 1 mg of soluble starch to a definite size of dextrin in 30 min under the conditions of assay. A fungal glucoamylase (Diazyme L-200) also was obtained from Miles Laboratories Inc. One milliliter of Diazyme L-200 has a leveled activity of 200 Diazyme Units (DU). One Diazyme Unit is the amount of enzyme that catalyzes the production of 1 g of glucose from starch in 1 h at 60°C and pH 4.2.

General Methods

Sun-dried BW and CW were ground in a Burrows hammermill using a 1/16 in. (1.6 mm) screen and placed in cold storage with other samples. The moisture of the samples was determined by evaporation at 95°C for 4 h under a vacuum of 4000 Pa (30 torr) using A.O.A.C. Method 7.003 (*10*). Total starch contents of the grain dusts, WS, and LGF were determined by A.A.C.C. method 76-11 (*11*), and those of BW and CW were determined using the same method after extracting sucrose with 80% hot ethanol. Crude protein, crude fat, and crude ash were determined by A.O.A.C. methods 47.021, 7.060, and 7.009, respectively (*10*). Amino nitrogen was determined by A.A.C.C. method 46-31 (*11*). Glucose, fructose, and ethanol were determined by high-performance liquid chromatography (HPLC) using a Varian Model 5000 LC (Varian Associates, Inc., Palo Alto, CA) chromatograph equipped with a loop-injection device (10 μL) and a refractometer as the detector. All separations were done using a Bio-Rad Aminex Ion-Exclusion Column (HPX-87H, 7.8 mm id × 300 mm, Bio-Rad Laboratories, Richmond, CA) operated at 45°C. Components were eluted with 0.01*M* aqueous sulfuric acid at a flow rate of 0.7 mL/min. Sucrose also was determined by HPLC using a Beckman 100A system with an Altex Model 156 refractive index detector. Sucrose was separated on an Amino Sepheri-5 column (AS-5A, 4.6 mm id × 250 mm, Brownlee Labs, Santa Clara, CA) operated at room temperature with 2.0 mL/min of acetonitrile/water (75/25, vol%) mixture. Standard curves were obtained from solutions of known concentrations of sugars and ethanol.

Enzymatic Hydrolysis

Each starchy byproduct (120 g dry solids [ds]) was dispersed in about 460 mL water to give a 20% slurry. After the pH of each slurry was adjusted to 6.2 with 2*N* NaOH, 0.3 mL of TAKA-THERM was added. The temperature was maintained at 90°C for 1 h with constant stirring to gelatinize and degrade starch to soluble dextrin. The liquefied slurry was adjusted to pH 4.2 using 5*N* HCl and saccharified with 0.9 mL Diazyme L-200 at 60°C for 4 h with stirring.

For a 1:1 mixture of starch byproduct and BCS, 300 g of slurries containing 60 g(ds) of starchy byproduct were liquefied with 0.15 mL of TAKA-THERM under the conditions described previously. Each thinned slurry was mixed with 300 g of BCS (20%, w/w) and saccharified with 0.9 mL Diazyme L-200 under the same conditions described previously. Hydrolyzed slurries were diluted to 15% solids (as solids content before hydrolysis) for fermentation.

Fermentation

The yeast (1 g) was rehydrated in 25 mL sterilized warm water (42°C) for 5–10 min prior to use. Media were sterilized at 121°C for 15 min, and fermentations were performed at 30°C using 0.2 g yeast/kg medium. The pHs were readjusted to 4.2 for grain dusts, WS, and LGF and to 4.6 for BW and CW to give the optimum conditions for fermentation. The hydrolyzed slurries (50 g) were fermented in 125-mL Erlenmeyer flasks fitted with a water seal. After fermentation, samples were centrifuged at 12,000 rpm (17,418*g*) for 10 min in a Beckman Model J2-21 centrifuge. The supernatents were collected separately, and residual sugars and ethanol concentrations were determined using HPLC.

To determine the rate of fermentation and the optimum fermentation times, CO_2 gas production was followed using a 12-channel recording gasograph. A gasograph Model 12 manufactured by D&S Instrument Ltd. (Pullman, WA) was used in this experiment. A test tube (15 mL) containing 7 g of inoculated medium was placed inside a 250-mL jar that contained 70 mL water to improve heat transfer to the test tube. The jar was plugged with a rubber stopper and connected to a channel equipped with a recording pen.

RESULTS AND DISCUSSION

Proximate Chemical Composition

All samples were analyzed for total starch, crude protein, crude fat, and crude ash (Table 1). Grain dusts contained high amounts of ash and varied widely in starch content, even though the sources of grain were the same.

Total fermentable sugars from hydrolyzed grain and bakery byproducts after successive treatments with α-amylase and glucoamylase also are presented in Table 1. Glucose, sucrose, and fructose were included in total fermentable sugars. The values were proportional to the starch content except for CW, which contained a high amount (28–30%) of sucrose. Overall, 93–97% of sugar conversions from starch were achieved during the enzymatic hydrolysis of these byproducts.

Table 1
Proximate Composition of Grain
and Bakery Byproducts and BCS (percent dry basis)

Substrates[a]	Starch	Crude protein[b]	Crude fat	Crude ash	Total fermentable sugars after enzyme digestion[c]
Grain byproducts					
WLS	27.0	10.4	4.2	20.9	28.0
WHS	41.9	8.9	3.9	17.7	43.3
CLS	29.5	10.3	4.1	19.1	30.6
CHS	41.7	9.1	4.2	15.2	43.9
SSD	39.4	9.0	3.5	17.9	40.5
LGF	61.6	16.8	3.7	2.2	65.4
WS	43.3	16.6	2.9	5.4	45.6
Bakery byproducts					
BW	66.2	12.1	2.2	2.0	68.8
CW	32.4	5.7	16.9	2.1	66.2
BCS[d]	—	8.9	1.4	2.5	74.8

[a]Definitions of acronyms: WLS = low starch wheat-corn dust; WHS = high starch wheat-corn dust; CLS = low starch corn-sorghum dust; CHS = high starch corn-sorghum dust; SSD = sorghum-soybean dust; LGF = low-grade wheat flour; WS = wheat screenings; BW = bread waste; CW = cake waste.
[b]Nitrogen factor; 5.7 for WS, LGF, BW, and CW, and 6.25 for grain dusts and BCS.
[c]Total fermentable sugars included glucose, sucrose, and fructose.
[d]Data from Sebree et al. (*1*), except for total fermentable sugars.

Fermentation of Hydrolyzed Grain and Bakery Byproducts and Their Mixtures with BCS

Effects of BCS on Ethanol Yields

The ethanol concentration in the beer produced by fermentation of grain byproducts ranged from 16–38 mL/100 g(ds) (Table 2). Addition of BCS increased ethanol production by 32–86% in grain dusts and WS and by 10% in LGF. The improvement was owing to the increased proportion of fermentable solids after addition of BCS. When BCS was added to bakery byproducts, no significant difference ($p < 0.05$) in ethanol concentration was found in BW (42.7–44.1 mL/100 g[ds]), but ethanol production was increased about 7% in CW (40.5–43.7 mL/100 g[ds]). In all cases, only trace amounts of residual sugars were detected after fermentation.

The fermentation efficiency of each sample was calculated by observed alcohol produced from the sample divided by theoretical alcohol yield from the level of fermentable sugars (Table 2). The fermentation efficiency is an index of the physiological condition of the yeast and a standard for the evaluation of all process operations. The fermentation efficiencies of grain and bakery byproducts were generally above 85% and tended to

Table 2
Yeast Fermentation of Enzyme-Digested Grain and Bakery Byproducts and Their Mixtures with Glucoamylase-Treated BCS[a]

Substrates[b]	Ethanol yields[c] mL/100 g ds	Minimum fermentation time,[c] h	Fermentation efficiency, percent
Grain byproducts			
WLS	16.4*	20*	90.4
WLS + BCS (1:1)	30.5**	25**	
WHS	26.3*	26*	93.7
WHS + BCS (1:1)	35.3**	26*	
CLS	17.7*	20*	89.4
CLS + BCS (1:1)	30.8**	25**	
CHS	26.2*	25*	92.1
CHS + BCS (1:1)	34.7**	26*	
SSD	22.9*	25*	87.3
SSD + BCS (1:1)	34.3**	26*	
LGF	38.4*	31*	90.6
LGF + BCS (1:1)	42.1**	31*	
WS	26.0*	25*	88.0
WS + BCS (1:1)	34.4**	26*	
Bakery byproducts			
BW	42.7*	62*	95.7
BW + BCS (1:1)	44.1*	34**	
CW	40.5*	76*	94.4
CW + BCS (1:1)	43.7**	35**	

[a] All fermentations were done at 15% solids in the beer.
[b] *See* Table 1 for identification of acronyms.
[c] Values represent an average of two replications. Values followed by a different number of asterisks are significantly different at $p = 0.05$.

improve as total fermentable sugar levels increased in the hydrolyzed substrates. Bakery byproducts gave the highest fermentation efficiencies (94–96%), and sorghum-soybean grain dust the lowest (87%). Therefore, the effectiveness of these byproducts in alcohol production is comparable to that of grain itself. Coble et al. (*12*) found that the average efficiency for fermentation of sugar to alcohol was 90% when corn and grain sorghum were used as feedstocks for a small-scale ethanol plant.

The effects of pH on fermentation of hydrolyzed BW and CW also were studied (data not given). The large drops in pH after fermentation (4.6–3.8 for BW and 4.6–3.5 for CW) implied that BW and CW had poor buffering capacities. Carbon dioxide production during fermentation of bakery wastes was not affected by the initial pH, which ranged from 4.2–5.0. However, fermentation times were reduced slightly when the initial pH was adjusted to 4.6.

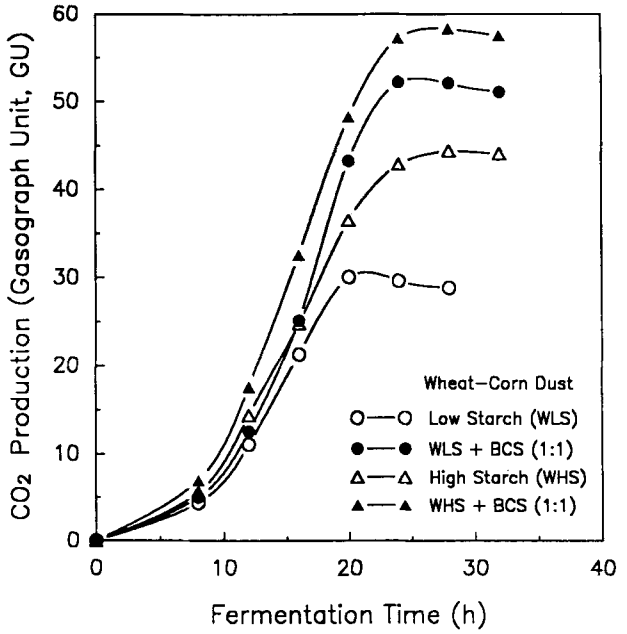

Fig. 1. Carbon dioxide production during fermentation of enzyme-digested wheat-corn dusts and their 1:1 mixtures with BCS. Fermentations were done using 15% solids with 0.2 g dry yeast/kg medium at 30°C and pH 4.2.

Effects of BCS on Fermentation Rates

The rates of fermentation were measured by CO_2 gas production during fermentation using the gasograph. The gasograph was introduced first by Rubenthaler et al. (*13*) to measure and continuously record the volume of gas produced at constant temperature and pressure in a fermenting dough. Values are recorded in gasograph units (GU). GU can be converted to mm of Hg by multiplying by the factor of 7.3. Gas production in gasograph units also may be expressed in cc by multiplying GU by 2.38.

Fermentation of grain dusts and other grain byproducts was very similar to that indicated by CO_2 gas production of wheat-corn dust (Fig. 1). Gas production during yeast fermentation leveled off in 20 h for grain dusts containing low starch and at 25–26 h for those containing high starch (Table 2). For 1:1 mixtures of BCS and grain dusts, the gas production reached its peak in a minimum time of 25–26 h. Minimum fermentation time remained the same for WS and LGF regardless of adding BCS (25–31 h). When equal amounts of BCS were added to grain byproducts, minimum fermentation times were not reduced, but CO_2 produced during fermentation was increased markedly. Addition of BCS to BW and CW did not increase CO_2 gas production, but did reduce the minimum fermentation times from 62 to 34 h and from 76 to 35 h, respectively (Fig. 2 and Table 2). This significant reduction ($p < 0.05$) of minimum fermentation time might have been owing to the presence of some nutrients in BCS.

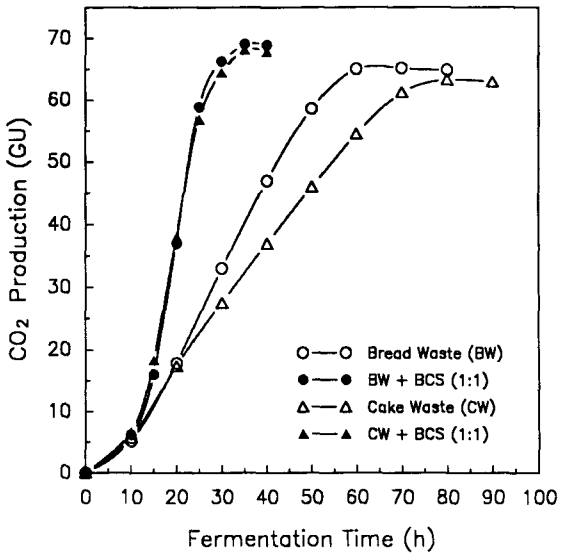

Fig. 2. Carbon dioxide production during fermentation of enzyme-digested bakery byproducts and their 1:1 mixtures with BCS. Fermentations were done using 15% solids with 0.2 dry yeast/kg medium at 30°C and pH 4.6.

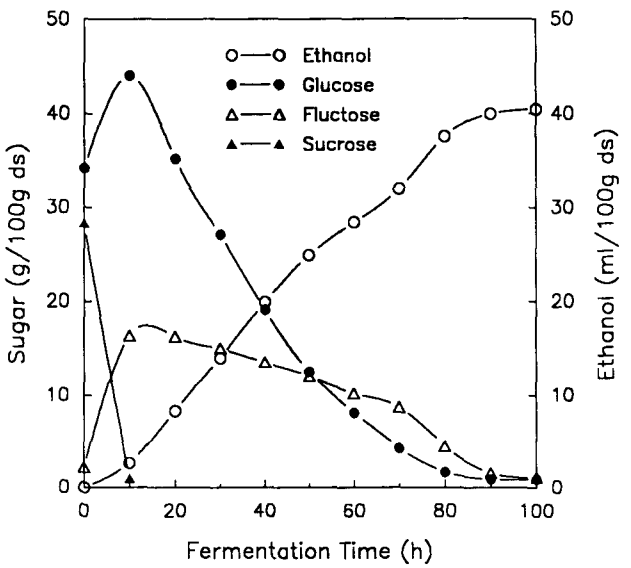

Fig. 3. Sugar consumption and ethanol production during fermentation of cake waste. Fermentations were done using 15% solids with 0.2 g dry yeast/kg medium at 30°C and pH 4.6.

The fermentation rate of CW also was followed by measurement of ethanol production and showed a trend similar to that found by the gasograph (Fig. 3). However, minimum fermentation times measured by the gasograph were 10–15 h shorter than those found by actual fermentation. This effect probably was owing to CO_2 absorption in the water and

Table 3
Amino Nitrogen in Enzyme-Digested Grain and Bakery Byproducts and BCS

Substrates[a]	Amino nitrogen[b] mg/100 g ds
Grain byproducts	
WLS	78.5 ± 1.3
WHS	67.4 ± 1.0
CLS	80.9 ± 0.7
CHS	75.8 ± 1.3
SSD	72.1 ± 2.9
LGF	72.0 ± 1.5
WS	81.0 ± 1.1
Bakery byproducts	
BW	14.8 ± 0.1
CW	11.7 ± 0.7
Grains[c]	
Corn	39.1 ± 1.1
Sorghum	32.6 ± 0.2
Wheat	30.8 ± 0.2
BCS	229.6 ± 1.6

[a] *See* Table 1 for identification of acronyms.
[b] Each value is a mean of two replications ± standard deviation.
[c] Data from Chung et al. (*15*). Minimum fermentation times are 61 h for corn, 65 h for sorghum, and 57 h for wheat.

the reduction of total volume by the increased pressure in the gasograph jar. The decline in total CO_2 after the peaks supports this explanation. Also, the pH of the water (5.7 to 3.9–4.0) in the jar decreased after fermentation, indicating probable CO_2 absorption by the water. During fermentation of CW, sucrose was hydrolyzed quickly to glucose and fructose by yeast invertase in the first 10 h of fermentation, and fructose was consumed by yeast at a significantly slower rate than glucose.

Amino Nitrogen in Hydrolyzed Grain and Bakery Byproducts and BCS

The nitrogen content of yeasts is about 10% of the dry weight, which indicates that nitrogen is an important constituent of any growth medium (*14*). With amounts of 67–81 mg/100 g(ds) of amino nitrogen, grain byproducts were about twice as rich a source of amino nitrogen compared to grain itself (Table 3). BW and CW contained low concentrations of amino nitrogen, 15 and 12 mg/100 g(ds), respectively. BCS had 3–20 times more amino nitrogen than any of the grain and bakery byproducts (Table 3).

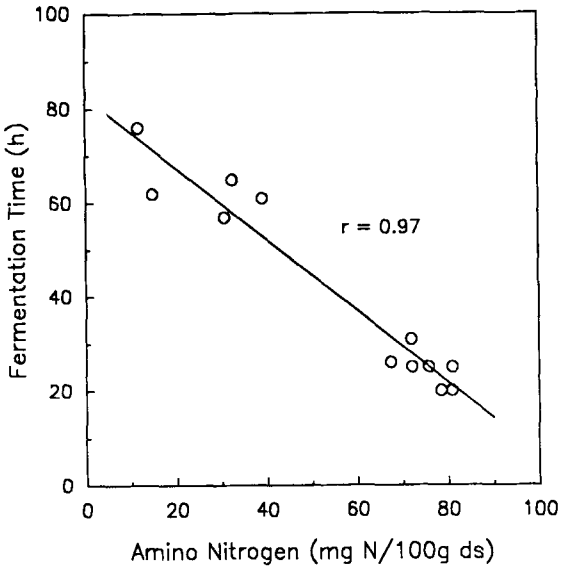

Fig. 4. Relationship between amino nitrogen in enzyme-digested substrates and their fermentation times measured by a gasograph.

Chung et al. (*15*) reported that adding vitamins and minerals to the hydrolyzed grain materials had no effect on either fermentation time or ethanol production, because grains were rich sources of these nutrients. However, addition of amino nitrogen to the hydrolyzed grains markedly increased the rate of fermentation.

Adding BCS to hydrolyzed bakery byproducts significantly reduced the fermentation times. This effect might have been owing to the high amino nitrogen content of BCS. An experimental study based on equal amounts of initial glucose was performed to find the effects of amino nitrogen in BCS on grain byproducts. The addition of equal amounts of BCS to grain byproducts did not reduce fermentation times, indicating that the grain byproducts had enough nitrogen content for yeast growth. From the data given in Tables 2 and 3, amino nitrogen contents in enzyme-digested substrates were plotted against their fermentation times (Fig. 4). A good linear relationship (r = 0.97) was found between optimum fermentation time and amino nitrogen in the hydrolyzed grains, grain byproducts, and bakery byproducts. The rate of fermentation was proportional to the values for amino nitrogen, suggesting that exhaustion of nitrogenous compounds was the limiting factor for yeast growth.

CONCLUSIONS

Grain and bakery byproducts could be used as good feedstocks for ethanol production with high fermentation efficiencies. Some biomass raw materials are low in amino nitrogen, and exhaustion of nitrogenous

compounds in a yeast fermentation medium is a limiting factor. Because BCS is a rich source of amino nitrogen, adding BCS to those substrates reduces fermentation time and increases the yield of ethanol.

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REFERENCES

1. Fahrenholz, C. H. (1983) MS Thesis, Kansas State University, Manhattan, KS.
2. Bassam, J. A. (1975), in *Cellulose as a Chemical and Energy Resource, Biotechnol. Bioeng. Symp.* No. 5, Wilke, C. R., ed., Wiley, NY, pp. 9–19.
3. Wise, D. L. and Augenstein, D. (1988), *Solar Energy* **41,** 453.
4. Martin, C. R. and Stephens, L. E. (1977), *Trans. ASAE* **20,** 168.
5. Miller, B. S. (1981), *Cereal Foods World* **26,** 239.
6. Kim, S. K., and D'Appolonia, B. L. (1977), *Bakers Digest* **51,** 38.
7. Sebree, B. R., Chung, D. S., and Seib, P. A. (1983), *Cereal Chem.* **60,** 147.
8. Sebree, B. R., Chung, D. S., and Seib, P. A. (1983), *Cereal Chem.* **60,** 151.
9. Chung, K. M., Chung, D. S., and Seib, P. A. (1986), *Cereal Chem.* **63,** 124.
10. Association of Official Analytical Chemists (1984), *Official Methods of Analysis,* 14th ed., The Association, Washington, DC.
11. American Association of Cereal Chemists (1976), *Approved Methods of the AACC,* The Association, St. Paul, MN.
12. Coble, C. G., Hilar, E. A., Sweeten, J. M., O'Neal, H. P., Reidenbach, V. C., Le Poli, W. A., et al. (1981), in *Cereals: A Renewable Resource. Theory and Practice,* Pomeranz, Y and Munck, L., eds. A. A. C. C., St. Paul, MN, pp. 611–632.
13. Rubenthaler, G. L., Finney, P. L., Demaray, D. E., and Finney, K. F. (1980), *Cereal Chem.* **57,** 212.
14. Jones, R. P., Pamment, N., and Greenfield, P. F. (1981), *Proc. Biochem.* **16,** 42.
15. Chung, K. M., Chung, D. S., and Seib, P. A. (1988), *Brewers Digest* **63,** 20.